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DIPLOMARBEIT

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„Potential effects of selected medicinal plant extracts and natural products on vascular smooth muscle cell proliferation“

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ABSTRACT

The chronic progressive arterial disease atherosclerosis is declared to be the leading cause of death worldwide. The most serious complications that can occur are myocardial infarction, cerebral infarction, aortic aneurysm and peripheral artery disease. The high prevalence of this disease results in a strong research interest in it, particularly considering that there is still a need for an effective treatment. A promising approach therefore seems to be the inhibition of the pathologically increased vascular smooth muscle cell (VSMC) proliferation, which is one of the key events in the development of atherosclerosis.

In the framework of this work about 120 compounds were tested aiming to identify substances with an antiproliferative activity on VSMCs. The majority of the test substances originate from the DNTI (Drugs from Nature targeting Inflammation) project, which has the objective to identify and characterize natural compounds that are able to prevent inflammation. The remaining substances, that include various olive oil components, cortisol, dexamethasone, indirubin-3´-monoxime, and others, were chosen based on a literature research revealing relevant anti-atherosclerotic action or known antiproliferative effects against VSMCs. All selected substances were tested for antiproliferative action *in vitro* with a fluorometric assay based on the conversion of the dye resazurin to a fluorescent product as a result of the metabolic activity of living cells.

This diploma work could confirm the literature findings that the glucocorticoids, such as cortisol and dexamethasone, inhibit VSMC proliferation. In addition the indirubin derivate indirubin-3´-monoxime (I3MO), which has already been tested in multiple models in the “Molecular targets” group at the University of Vienna, could be shown to inhibit serum-induced VSMC proliferation at a 10 µM concentration.

Semecarpus anacardium crude extract exhibited a very strong antiproliferative effect on VSMCs. Characteristic changes under the light microscope lead to the presumption that this extract may be able to trigger apoptosis of VSMCs.

Furthermore it could be demonstrated that baicalein, a pure compound from *Oroxylum indicum*, and erythrodiol, originating from olive oil, are in contrast able to increase the number of metabolic active VSMCs, indicating a possible proliferation-promoting effect.

ZUSAMMENFASSUNG

Atherosklerose, eine chronisch progressive Erkrankung der arteriellen Blutgefäße, gilt als die Haupttodesursache weltweit. Die schlimmsten Komplikationen, die auftreten können sind Myokardinfarkt, Schlaganfall, Aortenaneurysma und periphere arterielle Verschlusskrankheit. Die hohe Prävalenz dieser Erkrankung weckt starkes wissenschaftliches Interesse, besonders da noch immer der Bedarf besteht eine effektive Therapie zu entwickeln. Eine Hemmung der pathologisch gesteigerten Proliferation von vaskulären glatten Muskelzellen, welche eine entscheidende Rolle in der Entstehung von Atherosklerose spielt, scheint ein viel versprechender Ansatz dafür zu sein.

Im Rahmen dieser Arbeit wurden circa 120 Verbindungen getestet mit dem Ziel Substanzen zu identifizieren, die die Proliferation von vaskulären glatten Muskelzellen hemmen. Die Mehrheit der Testsubstanzen stammt aus dem DNTI (Drugs from Nature targeting Inflammation) Projekt, das sich damit beschäftigt natürliche Substanzen zu identifizieren, die in der Lage sind Entzündungen zu bekämpfen. Weitere Substanzen, wie zum Beispiel Olivenölkomponenten, Cortisol, Dexamethason, Indirubin-3´-monoxime etc., wurden auf Basis einer Literaturrecherche ausgewählt, die deren Potenzial in der Atherosklerosebehandlung aufdeckt. All diese Substanzen wurden hinsichtlich ihrer antiproliferativen Aktivität mit Hilfe eines fluorometrischen Assays, der auf der Umwandlung des Farbstoffes Resazurin zu einem fluroeszierenden Produkt, als Resultat der metabolischen Aktivität von lebenden Zellen beruht, *in vitro* getestet.

Diese Diplomarbeit konnte die in der Literatur niedergeschriebene Aussage bestätigen, dass die beiden Glukokortikoide Cortisol und Dexamethason in der Lage sind die Proliferation von vaskulären glatten Muskelzellen zu hemmen. Außerdem konnte gezeigt werden, dass das Indirubinderivat I3MO, das in der „Molecular targets“ Gruppe der Universität Wien bereits in mehreren Modellen getestet wurde, in einer Konzentration von 10 µM in der Lage ist die Serum-induzierte Proliferation von vaskulären glatten Muskelzellen zu hemmen.

Der Rohextrakt von *Semecarpus anacardium* war in der Lage einen sehr starken antiproliferativen Effekt auf vaskuläre glatte Gefäßmuskelzellen auszuüben. Die mikroskopische Analyse der Zellen führt zu der Annahme, dass der Extrakt möglicherweise in der Lage ist bei diesen Zellen Apoptose auszulösen.

Weiters konnte im Gegensatz dazu demonstriert werden, dass die Reinsubstanz Baicalein aus der Pflanze *Oroxylum indicum* und Erythrodiol, ein Inhaltsstoff von

Olivenöl, die Anzahl an metabolisch aktiven Zellen erhöhen können, was auf einen möglichen proliferationsfördernden Effekt dieser Substanzen schließen lässt.

A) TABLE OF CONTENTS

B) INTRODUCTION

1. Aim of this diploma thesis

Atherosclerosis is a serious disease threatening human health worldwide[1] and being responsible for about half of all events of death. As discussed in details in the following chapters, VSMCs play an important role in the development of atherosclerosis by their migration from the medial into the intimal layer of the arterial blood vessel, as well as their proliferation and production of matrix proteins.[2] Consequently VSMCs are an interesting target for the development of an effective atherosclerosis treatment, which may be able to delay the complex process of atherogenesis and to prevent the development of in-stent restenosis..

As a result it is very important to identify compounds, which may be able to act as antiproliferative agents. Previous work in the “Molecular targets" group at the University of Vienna has already revealed some interesting findings in this area. For example in his diploma thesis Mag. Thomas Nakel describes several extracts, which exert an antiproliferative effect on VSMCs. These include plant extracts *of Sideritis hyssopifolia, Aristolochia debilis, Stephania tetrandra, Melia toosendan* and *Peucedanum ostruthium*.[4]

To further follow this successful approach, the aim of this diploma work was to test selected plant extracts and natural products with the objective of identifying further compounds, which may prevent VSMC hyperplasia.

2. Atherosclerosis

2.1 Background

Atherosclerosis is defined as an asymmetric focal thickening of the intima,[5] a normally very thin region of the arterial blood vessel.[6] Including its complications, it represents a leading cause of morbidity and mortality in the western world. Furthermore, atherosclerosis becomes increasingly frequent all over the world because of a lifestyle change that resembles the western one and it may even be likely to reach epidemic proportion in the near future.[7]

The most serious complications of atherosclerosis that may occur are myocardial infarction (MI), cerebral infarction, aortic aneurysms and peripheral artery disease,[1] consequently being responsible for about 50 % of all deaths.

The development of atherosclerosis already starts from the fetal life, as a response to an injury of the endothelium,[2] and it can generally be divided into two phases: Firstly there is an asymptomatic period, characterised by non-obstructive lesions. Although complications can also occur in this period due to plaque rupture, the majority of clinical manifestations occur in the following symptomatic phase at middle age.[49]

2.2 Risk factors for atherosclerosis

Several risk factors for atherosclerosis have been identified by epidemiologic studies (**table 1**). These can be divided into environmental risk factors and risk factors with strong genetic correlations.[8]

Risk factors, including a significant genetic component	Environmental risk factors
Elevated levels of LDL, VLDL	Smoking
Elevated lipoprotein (a)	Lack of exercise
Elevated levels of homocysteine	High fat diet
Elevated levels of haemostatic factors like fibrinogen	Infectious agents like cytomegalovirus and *Chlamydia pneumoniae*
Low levels of HDL	
Hypertension	
Diabetes Mellitus	
Metabolic syndrome	
Obesity	
Family history	
Gender (male)	
Hyperurcemia	

Table 1: Genetic and environmental risk factors for atherosclerosis
Adapted from Glass, C.K. & Witztum, J.L. (2001)[8], Kumar, V., Fausto, N. & Abbas, A.K (2004)[2] and Krishnan, et.al. (2011)[47]

2.3 Architecture of the arterial blood vessel

As shown in **figure 1**, an arterial blood vessel mainly consists of three different concentrically arranged layers: intima, media and adventitia. The internal layer, called intima is separated from the blood flow by the endothelium, a monolayer of endothelial cells (ECs). The intima is very thin in comparison with the other two layers and it is composed of extracellular connective tissue matrix and only a few VSMCs. As a border to the next region, the media, serves an elastic membrane, referred to as the internal elastic lamina. The media is composed of VSMCs and also sparse macrophages and fibroblasts. The external layer, the adventitia, mainly consists of connective tissue with nerve fibres and small blood vessels, referred to as vasa vasorum, as well as fat cells, fibroblasts and smooth muscle cells (SMCs). As a separation between media and adventitia there is a further membrane, the so called external elastic lamina.[2, 9, 10]

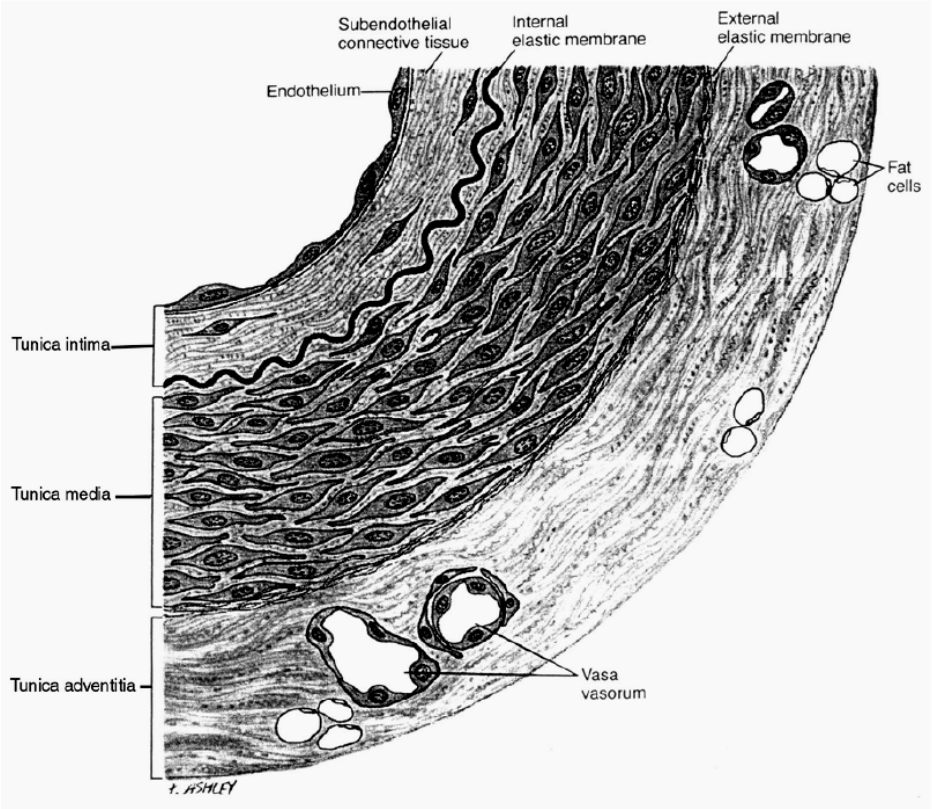

Figure 1: Cross section of an arterial blood vessel wall
Picture taken from http://dc245.4shared.com/doc/t2GACyXa/preview.html[46]

2.4 Pathogenesis of atherosclerosis

Atherosclerosis is defined as a chronic immunoinflammatory disease.[7] This disease preferentially affects the intima of elastic arteries. Coronary, carotid, cerebral and renal arteries and also the aorta are most prone to develop atherosclerotic lesions.[11] The pathogenesis of atherosclerosis is characterized by multiple processes, to which count amongst others endothelial dysfunction, inflammation, vascular smooth muscle cell proliferation and matrix alteration.[12]

Figure 2 gives a short overview of the complex process of atherogenesis.

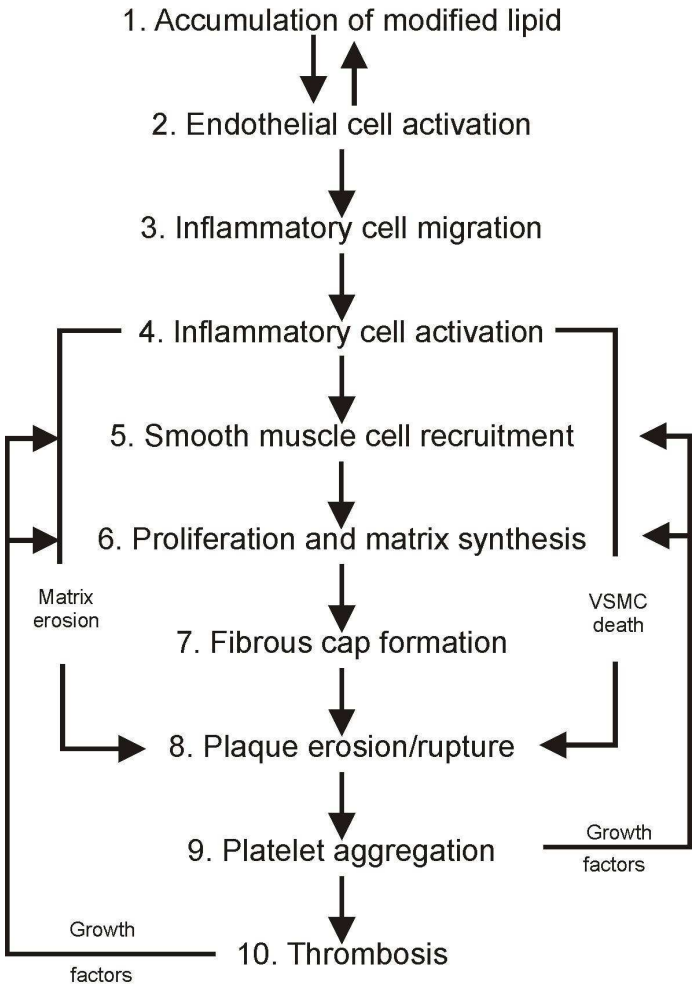

Figure 2: Overview of the different steps in atherogenesis
Picture taken from Topol, J.T. (2007)[11]

Atherogenesis starts with an injury of the endothelium and the subsequent infiltration of monocytes.[13]

The duties of the endothelium comprise regulation of vascular wall homeostasis and regulation of vascular permeability. Furthermore the endothelium is involved in the regulatory process of platelet and leukocyte adhesion, platelet aggregation as well as thrombosis. Various factors,[15] like for example modified low density lipoproteins, hypertension, hyperglycemia and others,[10] can disturb the important regulation balance. This annoyance leads to a phenotypical modulation, to a so called non-adaptive stage of the endothelium, also referred to as endothelial dysfunction.[15] As a result the production of nitric oxide (NO) is decreased. Further consequences are an enhancement of adhesiveness for leukocytes and thrombocytes and an enhanced permeability of the endothelium for lipoproteins and leukocytes.

Subsequently ECs start to express various adhesion molecules.[10] Whilst selectins like E-selectin and P-selectin trigger a loose rolling interaction between ECs and leukocytes, integrins lead to a firm attachment.[16] Intracellular adhesion molecule-1 (ICAM-1) binds various classes of leukocytes, whilst vascular cell adhesion molecule-1 (VCAM-1) is able to bind monocytes and T-lymphocytes.[10] The binding of lymphocytes to adhesion molecules is carried out through a linkage to very late antigen-4 (VLA-4).[17] An overexpression of ICAM-1, VCAM-1 and P-selectin can function as potential markers for atherosclerosis.[18] The following step is mediated by the expression of monocyte chemotactic protein-1 (MCP-1) by ECs, leading to the migration of monocytes into the intima, whilst the expression of T cell chemoattractants leads to a lymphocyte migration.[19]

The interaction of monocytes with ECs leads to a trigger of moncyte matrix metalloproteinase 9 production, in turn giving rise to an enhanced infiltration of leukocytes.[16] Monocyte recruitment may initially have a protective role being able to remove cytotoxic and pro-inflammatory oxidized low density lipoprotein (oxLDL) particles or apoptotic cells, but further accumulation and lipoprotein uptake contribute to lesion development.[8]

As a next step macrophage colony-stimulating factor (M-CSF) triggers the differentiation of monocytes into macrophages and leads to an up-regulation of scavenger receptors and toll like receptors.[5] There are two major types of macrophages: The M1 macrophages, termed as classically activated, which promote inflammation and on the other hand the M2 type, referred to as alternatively activated, being able to promote resolution. An imbalance between these two types may lead to impaired resolution.[20]

Macrophages can initiate additional recruitment of leukocytes by producing interleukin-1 (IL-1), tumor necrosis factor (TNF) and various chemokines like MCP-1.[10]

In further consequence macrophages take up lipoproteins and form foam cells.[9] Isolated foam cells characterize the first type of the six atherosclerotic lesions, called the initial lesion (A complete list of all atherosclerotic lesion types can be viewed in **table 2**).[2] The receptors involved in the LDL up-take process are called scavenger receptors with scavenger receptor A (SR-A) and cluster of differentiation 36 (CD36) appearing to be of primary importance. Their expression is regulated by peroxisome proliferator-activated receptor γ (PPARγ) and cytokines like TNFα and interferon γ (IFN-γ).

Low density lipoprotein (LDL) particles, lipoprotein(a) and remnants can infiltrate the intima by a passive diffusion through EC junctions. Once the lipoproteins have entered the intima they are trapped in the vessel wall by an interaction between apolipoprotein B (apoB) and matrixproteoglycans. To be taken up by macrophages the LDL particles need to be highly oxidized. The oxidation is generated by reactive oxygen species (ROS) and various enzymes like myeolperoxidase, sphingomyelinase, secretory phospholipase,[9] nitric oxide synthase and 15-lipoxygenase.[8] The oxidized particles are seen as exogenous by the immune system, which need to be removed by leukocytes, leading to the view that atherosclerosis is an autoimmune disease.[18] The binding of oxLDL to scavenger receptors leads to a release of proinflammatory cytokines by macrophages.[7] OxLDL particles are able to inhibit macrophage mobility and therefore prevent macrophages and foam cells from returning to the circulating blood.[21]

Moreover macrophages and also ECs express toll-like receptors, which can bind heat shock protein 60, oxLDL and other ligands. The stimulation of these receptors triggers the production of many pro-inflammatory molecules.[17]

The next step during atherogenesis is the fatty streak formation. Fatty streaks are visible as yellow streaks. These are composed of macrophage foam cells, arranged in multiple layers in comparison to type I lesions, which consist of isolated groups of a few foam cells. Moreover T-lymphocytes and a small amount of mast cells could be identified to be components of type II lesions.[22] Fatty streaks were already discovered in the aortas of children younger than one year.[2] Post mortem examinations could demonstrate this lesion type in the aorta at the end of the first decade, in the coronary arteries in the second decade and in the cerebral circulation in the third decade of life. Fatty streaks are nonstenotic and they do not cause any symptoms.[11] Type II lesions can be seen as precursors of plaques, but not all fatty

streaks automatically develop to advanced lesions,[2] which can be characterized by the presence of VSMCs and a lipid rich necrotic core.

As the disease progresses macrophages and T-cells produce cytokines and growth factors[9] like platelet derived growth factor (PDGF), which trigger VSMC migration from the media into the intima.[16] This migratory process is followed by a phenotype change of VSMCs. As a result VSMCs are able to produce growth factors like PDGF and vascular endothelial growth factor (VEGF), allowing them to stimulate their proliferation in an autocrine process.[11] Lipoprotein lipase, which is secreted by the endothelium, can further trigger the proliferation of VSMCs.[18] As a result VSMCs take up lipoproteins, form foam cells and synthesize extracellular matrix (ECM), thereby triggering the formation of a fibrous cap.[8] This process can be seen as a local wound healing response. Especially in the shoulder region of the cap, also inflammatory cells can be identified, like T-cells, mast cells and particularly macrophages.[11]

The so called necrotic core is formed when foam cells and smooth muscle cells die by apoptosis or necrosis and leave the engorged lipids behind.[14] The apoptotic structures are rich in tissue factors (TFs), which are able to trigger thrombosis, if they get in contact with circulating platelets. The necrotic core consists of cellular debris, inflammatory cells, most of all foam cells and crystalline cholesterol. In later stages of atherogenesis calcification, ulceration and new microvessel formation can promote the structure of a plaque to become more complex.[11]

Clinical complications can occur when the plaque becomes large enough to limit blood flow and leads to a stenosis. Much severer complications follow plaque rupture, which can induce a thrombotic occlusion.[17] Another reason for thrombus formation, which may lead to a complete occlusion of the affected vessel, can be the erosion of endothelial cells, that cover the fibrous cap. This process seems to occur more often in women and is probably responsible for about 30% of all acute coronary syndroms.[11]

Dependent on the affected tissue, plaque rupture can trigger the following clinical outcomes: If thrombosis affects the heart, myocardial infarction and heart failure can occur, if the arteries that perfuse the brain are affected, ischaemic stroke and transient ischaemic attacks can be caused and if atherosclerosis has an impact on arterial branches, renal impairment, hypertension, abdominal aortic aneurysms and critical limb ischaemia can be triggered.[17]

However not every plaque rupture or endothelial erosion automatically leads to an occlusion of the affected vessel, but rather a silent healing process may be established, leading to the formation of a new fibrous cap, which covers the

thrombus, due to a migration and proliferation of VSMCs. Though this process causes restabilization of the plaque, with every healing process the lesion is increasing in size and as a result is potentially able to block blood flow and cause ischemic symptoms.[11]

Histological classification and terms used for atherosclerotic lesions		Additional terms used and often based on appearance with the unaided eye		Earliest onset	Clinical correlation
Type I lesion	Initial change	Fatty dot, fatty streak	Early lesion, minimal lesion	From first decade	Clinically silent
Type II lesion	IIa: Progression rupture IIb: Progression resistant				
Type III lesion	Preatheroma	Intermediate lesion, transitional lesion		From third decade	
Type IV lesion	Atheroma	Fibrolipid plaque, fibrous plaque, plaque	Advanced lesion, raised lesion		Clinically silent or overt, depending on the degree of obstruction
Type V lesion	Va: Fibroatheroma Vb: Calcific lesion Vc: fibrotic lesion			From fourth decade	
Type VI lesion	VIa: Surface disruption VIb: Hematoma or Hemorraghe VIc: Thrombosis VIabc: Presence of all three features				
Alternatively:					
Type VI lesion	Fissured lesion, ulcerated lesion, hemorrhagic lesion, thrombotic lesion	Complicated lesion			
Type VII lesion	Calcific lesion	Calcified plaque			
Type VIII lesion	Fibrotic lesion	Fibrous plaque, plaque			

Table 2: Classification of atherosclerotic lesions, their onset and clinical correlation
Table adapted from Stary, H.C. (2000)[23] and Stary, H.C. (1995)[24]

3. The role of VSMCs in atherogenesis

First of all it is important to emphasize that the role of vascular SMCs is not a constant during the development of atherosclerosis, instead it varies according to the stage of the disease. On the one hand it has a proatherogenic role in lesion development, but on the other hand it carries out rather a protective role within advanced lesions.[25]

3.1 The different phenotypes of VSMCs

VSMCs exist in different phenotypes. In contrast to other muscle cells they never reach a state of terminal differentiation and consequently they can undergo a phenotype switch in answer to changing environmental conditions.[26]
The two important phenotypes are on the one hand the contractile or differentiated type, and on the other hand the synthetic or proliferative type.[10] The switch between these two different VSMC phenotypes is a very complex process, involving various important regulatory factors like mechanical forces, contractile agonists, extracellular matrix components, reactive oxygen species, thrombin or transforming growth factor β1 (TGF-β1), which were identified in studies on cultured SMCs.[25]

3.1.1 The contractile phenotype

Contractile SMCs, which are present in adult blood vessels regulate blood vessel diameter, blood pressure and also blood flow distribution.[25] This phenotype is characterized by a high proportion of contractile proteins, which represent 80-90% of the cell volume,[27] and also by various ion channels and signalling molecules, that are essential for the cell to exert its contractile function.[25] The proportion of synthetic organelles i.e. Golgi apparatus and rough endoplasmatic reticulum is low.[27] The contractile phenotype can be typified by special markers like smooth muscle α-actin, h-caldesmon etc.[25] A detailed list of specific markers both for the contractile and the synthetic phenotype can be seen in **table 3**.

SM phenotype-dependent markers	Contractile phenotype	Non-contractile phenotype
Smoothelin	Up-regulated	Down-regulated
SM MLC1 and 2	Up-regulated	Down-regulated
α8 Integrin	Up-regulated	Down-regulated
α1 Integrin	Up-regulated	Down-regulated
α7 Integrin	Up-regulated	Down-regulated
Calponin	Up-regulated	Down-regulated
SM α-tropomyosin	Up-regulated	Down-regulated
h-Caldesmon	Up-regulated	Down-regulated
SM α-actin	Up-regulated	Down-regulated
Myocardin	Up-regulated	Down-regulated
Fibroblast-like tropomyosin	Down-regulated	Up-regulated
Kruppel-like factor 4	Down-regulated	Up-regulated
α5 Integrin	Down-regulated	Up-regulated
α2 Integrin	Down-regulated	Up-regulated
αv Integrin	Down-regulated	Up-regulated
SMemb	Down-regulated	Up-regulated
I-Caldesmon	Down-regulated	Up-regulated

Table 3: Overview of different SM phenotype-dependent markers and their up- and respectively down-regulation dependent on the phenotype.
Picture taken from Zargham, R. (2008)[28]

3.1.2 The synthetic phenotype

Synthetic SMCs show a higher rate of synthetic organelles, but in contrast a lower amount of contractile filaments.[27] Furthermore a switch to this phenotype also leads to an increased production of proteases, as well as to elevated production of inflammatory cytokines and expression of inflammatory cell markers. The synthetic phenotype is not only present during vascular development, when cells need to synthesise a large amount of extracellular matrix components to build up the cardiovascular system, but it also plays an important role in vascular injury.

As a reaction to changes in local environmental cues VSMCs can re-enter the cell cycle and change their phenotype to the proliferative state with the objective of

generating an effective repair of the damaged region by their migration, proliferation and synthesis of extracellular matrix components. The phenotypic change can be reversed after the healing process is terminated.
In response to a disregulation of this process different diseases, like atherosclerosis or cancer and hypertension may occur.[25]

3.2 The role of VSMCs in plaque development

VSMCs play an outstanding role in the initiation and early progression of atherosclerosis by their migration from the media into the intima, as well as their proliferation and synthesis of matrix proteins.[2] The involvement of VSMCs in the development of an atherosclerotic plaque is shown in **figure 3**.

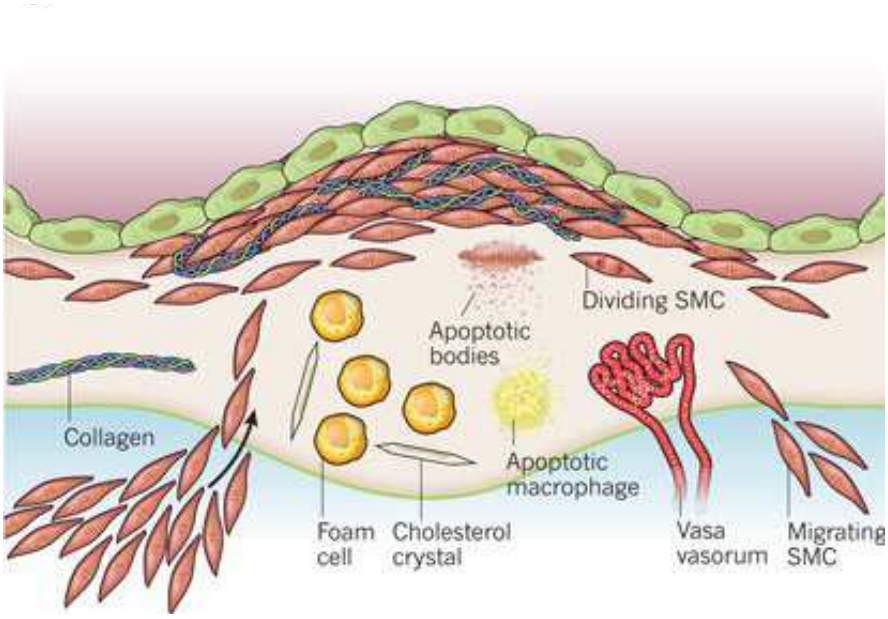

Figure 3: The role of VSMCs in the development of an atherosclerotic plaque
At an advanced stage of atherosclerosis development, VSMCs start to migrate from the media into the intima. Once they have reached the intima, they start to proliferate and synthesize ECM components. The death of macrophages and VSMCs leads to a liberation of incorporated lipids, which accumulate in the centrum of the plaque and form the necrotic core. In this stage of the disease, the plaque also contains microvessels and cholesterol crystals.
Picture taken from Libby, P., Ridker, P.M. & Hansson, G.K. (2011)[29]

3.2.1. The migratory process of VSMCs

PDGF, which is released by activated macrophages and endothelial cells, stimulates VSMCs to migrate from the media into the intima.[16] Other promoters of VSMC migration are endothelin-1 (ET-1), thrombin, fibroblast growth factor (FGF), IFN-γ and IL-1. Contrariwise heparin sulphate, NO and TGF-β inhibit VSMC migration.[2]
Matrix metalloproteinases (MMPs) may also be involved in the migratory process by removing the basement membrane around VSMCs. This enables contact between VSMCs and the interstitial matrix, causing a phenotypic switch to the synthetic state. This change in phenotype allows VSMCs to migrate and proliferate. Especially MMP-2 and membrane-type MMP seem to be involved in this process. MMPs can be activated by inflammatory cytokines like IL-1, IL-4 and TNF-α, and by growth factors, like PDGF and FGF-2.[10]
Studies could demonstrate that the majority of intimal SMCs come from the media. However there is evidence that bone marrow-derived SMCs out of the circulating blood are also recruited in the intima after vascular injury.[25] In studies bone marrow mesenchymal stem cells, which were stimulated to differentiate into VSMCs, express typical VSMC markers like α-smooth muscle actin and smooth muscle myosin heavy chain and have similar biological characteristics like VSMCs derived from the media. Like intimal SMCs they seem to be able to proliferate, take up lipoproteins, form foam cells and consequently play a role in the development of atherosclerosis.[30] The proportion of these cells seems to vary, depending on the severity of medial damage. Mouse arterial injury models could show that following a severe injury, a high amount of bone marrow-derived SMCs resides in the intima,[25] contributing to arterial remodelling.
In addition experimental data indicates that VSMCs may also come from the adventitia.[12]

3.2.2. The role of VSMCs in ECM production

VSMCs can also promote development of atherosclerosis by the production of ECM, converting a fatty streak into a mature fibrofatty atheroma.[10] The synthetic SMCs are able to synthesise up to 46 times more collagen than the contractile phenotype. Furthermore the constitution of the ECM in an atherosclerotic lesion differs from that of a healthy artery. Whilst in the healthy artery type I and type III fibrillar collagen are

predominant, proteoglycans with scattered type I collagen fibrils and fibronectin, are mainly found in atherosclerotic vessels.

With progression of the disease ECM synthesis, as well as fibronectin and proteoglycan production accelerates, as a result of cytokine stimulus[3] (e.g. IFN-γ released from activated macrophages).[26] Proteoglycans can capture additional LDL particles and thereby facilitate LDL oxidation. The oxidized LDL particles can be taken up by macrophages and also VSMCs, resulting in foam cell formation. Furthermore the oxidized lipoproteins can stimulate VSMCs to produce larger and more highly sulphated proteoglycans, which in turn have a higher affinity to oxLDL.[3]

The resulting fibrous cap exerts a protective role by bordering the highly thrombogenic core of the plaque from the blood, which contains platelets and proteins of the coagulation cascade.[11]

3.2.3. The role of VSMCs in foam cell formation

In vitro and in vivo studies could demonstrate that in addition to macrophages, also VSMCs are able to take up lipids, form foam cells and consequently lead to a further promotion of atherogenesis. To facilitate this process VSMCs can express various receptors like the LDL receptor, very low density lipoprotein (VLDL) receptor, CD36, type I and type II scavenger receptors and CXCL16/SR-PSOX. The CXCL16/SR-PSOX receptor is only present in atherosclerotic vessels.[3]

3.2.4. The role of VSMCs in the retention of monocytes and macrophages

VSMCs can support endothelial cells in the attraction of monocytes into the intima by expressing adhesion molecules like ICAM-1, VCAM-1 and chemokine ligand 1 (CX3CL1). These molecules are only expressed by intimal SMCs in atherosclerotic lesions, but not by SMCs of healthy aortas. Monocytes can, stimulated by oxidized lipids, provoke an overexpression of chemokine receptor 1 (CX3CR1), the receptor for CX3CL1. This process can enhance monocyte binding on VSMCs and accumulation of monocytes in the vessel wall.

Moreover *in vitro* studies could demonstrate that VSMCs can perform an anti-apoptotic effect on monocytes, resulting from an increased expression of B-cell lymphoma-2 (Bcl-2) and increased activity of the protein kinase B (Akt) and the mitogen-activated protein kinase (MAPK) pathways.[3]

3.3 The role of VSMCs in plaque rupture

3.3.1 VSMC apoptosis

In contrast to their promoting effect on lesion formation, VSMCs play a beneficial role in advanced lesions. VSMCs lead to the secretion of collagen and extracellular matrix and thus to the construction of a thick fibrous cap,[31] the part of the plaque, that is able to separate the thrombogenic plaque interior from blood.[32] An atherosclerotic plaque generally is composed of VSMCs, inflammatory cells like macrophages, T-lymphocytes and mast cells, as wells as intracellular and extracellular lipids.[31] **Figure 4** visualizes the distinct regions of an atherosclerotic plaque.

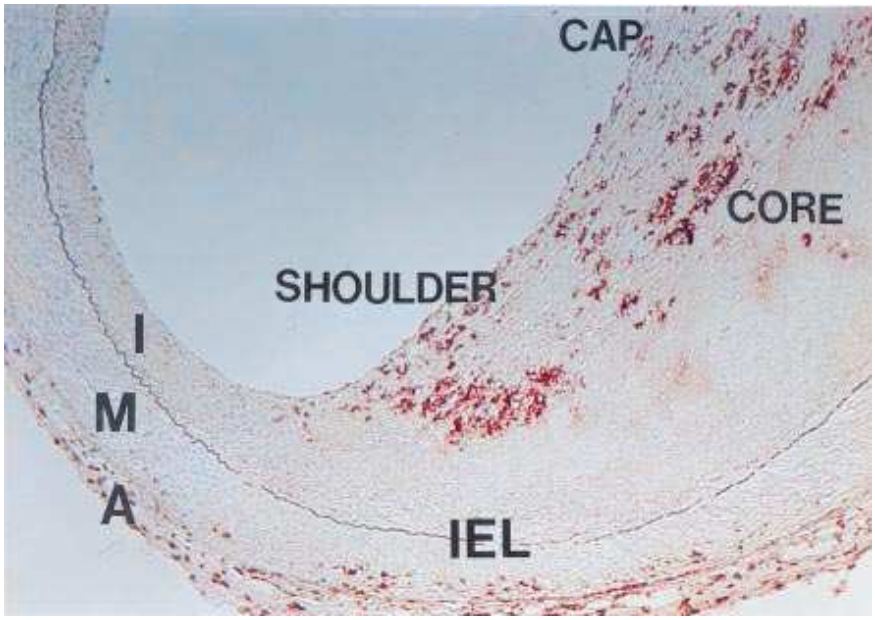

Figure 4: Light micrograph (x 400) of a section of an advanced human coronary atherosclerotic lesion
I: Intima; M: Media; A: Adventitia; IEL: Internal elastic lamina
Picture taken from Lusis, A.J. (2000)[9]

Plaque rupture, normally occurring at the shoulder region, leads to the liberation of lipids and TFs, which get in contact with blood components, resulting in an initiation of the coagulation cascade, platelet adherence and thus thrombosis.[8] Morphologically, plaques can be divided into two groups - the stable plaques and the unstable or vulnerable plaques.[21] Plaques likely to rupture are not characterized by their size but rather by their composition,[33] more precisely by the relative ratio of

VSMCs to lipids and macrophages. The unstable plaques show a thin fibrous cap with a low proportion of VSMCs,[34] as well as collagen and ECM components.[35] In contrast there is a high amount of lipid filled macrophages within the shoulder region and also a large necrotic core,[8] consisting of oxidized lipids and infiltrated inflammatory cells.[35] If the cap`s gauge is smaller than 65 µm and the lipid core accounts for 50% of the plaque`s volume the plaque is defined to be particularly at risk to rupture.[11] These observations lead to the assumption that VSMC apoptosis may give rise to plaque rupture. Actually a trigger of plaque rupture and thrombosis of plaques could be demonstrated by a direct induction of VSMC apoptosis in the fibrous cap of mice lesions.[36] Furthermore it could be demonstrated that plaques from patients, which are prone to rupture show higher levels of apoptosis than stable plaques. The programmed cell death leads to a thinning of the fibrous cap and to an accumulation of apoptotic bodies because of a defect in phagocytic clearance. The apoptotic bodies become necrotic and enlarge the necrotic core. This inflammation process probably leads to an increased release of MCP-1, IL-6, [34] IL-1 and IL- 8.[32] As a result more macrophages are recruited and activated.[34] These are in turn able to trigger the apoptosis of VSMCs by a direct cell-cell contact. One of the underlying modes of action is the interaction of Fas, a protein which is increasingly expressed on the surface of VSMCs, with Fas ligand on the surface of macrophages.[44]

Furthermore activated T-lymphocytes are stimulated to produce proinflammatory cytokines like CD 154 [37] and IL-1.[16] The interaction of CD 154 with CD 40 leads to the production of TFs and MMPs,[37] like MMP-1, -8, -9,-13, Moreover CD 154 [37] and IL-1 [16] indirectly lead to a degradation of ECM by stimulating macrophages to release MMPs[37] and other proteolytic enzymes like stromelysins, collagenases or elastases.[10] These enzymes carry out a further destabilising effect on plaques by degrading ECM and by promoting macrophage invasion, resulting in further plaque inflammation.[38]

Especially MMP-9 seems to play an important role in plaque rupture because of an increased expression of tissue factors and a tissue factor mediated activation of the coagulation cascade.[16]

Contrariwise it must be mentioned that other studies point out that some further types of MMPs rather execute a stabilising effect on advanced plaques by promoting migration and proliferation of VSMCs. The determining factors which effect is triggered may therefore be dependent on the spectrum of MMPs expressed, their level of activity and may also be controlled by the stage of plaque development.[38]

In addition activated T-lymphocytes also produce INF-γ. This factor inhibits VSMC proliferation directly,[11] and in combination with IL-1β and TNF-α,[18] produced by macrophages,[29] it prevents VSMCs from collagen production.[18]
Further results of VSMC apoptosis are the calcification of plaques, the expansion and degeneration of the media,[31] as well as thrombin generation.[32]

3.3.2 VSMC senescence

Another factor, which may contribute to the development of unstable plaques, is VSMC senescence. This term defines a cell cycle arrest, which is irreversible, in contrast to quiescence. Consequently VSMCs in advanced plaques show only a low rate of proliferation.[35] Thus they have a reduced ability to compensate their own loss by apoptosis.[11] However although more cells die, than can be renewed by proliferation, paradoxically plaques may grow further, because as a result more cells are recruited.[14]
Senescent cells show an overexpression of the adhesion molecule ICAM-1, plasminogen activator inhibitor-1 (PAI-1) and MMPs. These factors can lead to the promotion of plaque instability. As markers for cell senescence senescence-associated β-galactosidase (SAβG), short telomers, cell cycle regulators and the shape of the affected cells, which is enlarged, flattened and stellar, can be taken into consideration.[35]

3.4 The role of VSMCs in restenosis

Restenosis is the main adverse effect, which can follow a percutaneous coronary intervention (PCI),[39] normally occurring 3-6 months after the intervention.[40] It can lead to clinical symptoms like angina or myocardial ischaemia.[39] Neointima formation, as a result of VSMC migration and proliferation in combination with vascular remodelling are the main causes of restenosis.[28] This process plays an important role in the normal healing process of the vessel after stenting, but an excessive neointima formation leads to a stenosis of the vessel. Experimental angioplasty of rat carotid artery shows that VSMC proliferation always occurs after the intervention, but the extent is proportional to the injury caused. During these experiments it could also be shown, that the scale of proliferation correlates with the

rupture of the internal elastic lamina. When this structure was intact only a slight induction of proliferation resulted.[41]

The PCI leads to an injury and removal of the endothelial layer. As a result the thrombogenic platelet activating factors of the subendothelial matrix can get in contact with circulating blood cells, immediately leading to thrombus formation.[28] During the following inflammatory phase, inflammatory cells aggregate at the site of vascular injury and contribute to the healing process.[40] Leukocytes and monocytes are recruited by macrophage-1 antigen (MAC-1) and further chemokines like MCP-1.[39] Activated platelets and macrophages release cytokines and growth factors, triggering the phenotype modulation of VSMCs, which is a precondition for VSMC migration and proliferation.[28] Leukocytes produce tissue-digesting enzymes, which allow SMCs to migrate to the thrombus and by time the neointima, composed of proliferating VSMCs and extracellular matrix, replaces the thrombus.[39,40]

After neointima formation a remodelling process of the vessel takes place.[28] This process represents a compensatory mechanism as a response to the expansion of the media and the external elastic membrane,[39] either leading to enlargement (positive remodelling) or shrinkage (negative remodelling) of the vessel. Beside from neointima formation late negative remodelling also contributes to the process of lumen loss, leading to a restenosis.[28]

4. Diagnosis and treatment of atherosclerosis

4.1 Diagnosis of atherosclerosis

The gold standard for the diagnosis of atherosclerosis is coronary angiography. It allows detection of severely stenotic lesions that are already voluminous enough to limit blood flow. However a major disadvantage of this method is its inability to identify early, silent stages of atherosclerosis, which are also able to cause severe complications as well. To prevent these outcomes atherosclerosis needs to be diagnosed and treated years before a severe stenosis occurs.

Another invasive method is the intravascular ultrasonography (IVUS). IVUS can be applied as a supportive method together with coronary angiography and provide information about plaque volume and morphology.

Magnetic resonance imaging and computed tomography belong to the non-invasive imaging methods. These methods have great potential, they provide information

about stenotic and also non-stenotic plaques,[45] they can be applied easily and are experienced as more comfortable by patients. Further non-invasive methods, which may play a future role, are pulse wave velocity, intima-media thickness measurement,[48] as well as fluorodeoxyglucose positron emission tomography.[11]
As already discussed in the previous chapters, the role of VSMCs is dependent on the disease´s progression.[25] Consequently the establishment of diagnosing methods, which are able to provide detailed information about the stage of disease, has an important role because only then an appropriate treatment of the disease can be applied.

4.2 Treatment of atherosclerosis

There are four usual strategies in the treatment of atherosclerosis: life style modification, drug treatment, percutaneous coronary interventions and coronary surgery. Whilst percutaneous coronary interventions and coronary surgery play an important role in the treatment of atherosclerosis complications, life style modification and drug treatment have the potential to prevent atherosclerosis development and are as a result able to prevent the occurrence of severe complications.[49]

4.2.1. Life style modification

The great potential of a life style modification is its inexpensiveness, safety and efficacy. In comparison drugs can be very expensive, show side effects and often they are characterised by an absence of effectiveness. Although the switch to a healthier lifestyle may not be easy, regular exercise, non smoking, a healthy diet, an intake of low amounts of alcohol and a body mass index ≤25 are all associated with a reduction in cardiac risk.[49]

4.2.2. Drug treatment

Statins are used both as primary and secondary prevention of cardiovascular events. These drugs, representing HMG CoA reductase inhibitors, lead to a reduction of cholesterol[49] by inhibiting the formation of mevalonic acid, a precursor of cholesterol.[5] In addition statins enhance the number of LDL receptors, leading to a

reduction of plasma LDL. Further effects of these drugs are a slight reduction of triglyceride levels and an increase of HDL levels. For patients with coronary artery disease or other atherosclerotic disease the National Cholesterol Education Program recommends a reduction of LDL cholesterol ≤100 mg/dl as a goal.
In addition to their lipid lowering effect, statins also perform further pleiotropic effects on the process of atherogenesis. They directly up-regulate the endothelial nitric oxide synthase, leading to an increased production of NO, which can improve endothelial function. Inhibition of LDL oxidation, reduction of LDL uptake by macrophages and reduction of adhesion molecule expression are other beneficial effects. An inhibition of VSMC migration and proliferation may also be an anti-atherogenic property of the HMG CoA reductase inhibitors.[50] Moreover statins show anti-inflammatory properties, enhance fibrinolysis, reduce platelet activity[5], improve vascular relaxation, stabilize vulnerable plaques and promote the formation of new vessels.[51]

Angiotensin converting enzyme (ACE) inhibitors, a group of antihypertensive drugs are used for secondary prevention of cardiovasculare diseases.[52] Angiotensin converting enzyme is responsible for the conversion of angiotensin I to angiotensin II. The major receptor for angiotensin II is the angiotensin II receptor, type 1 (AT_1 receptor), a G-protein-coupled receptor, which is particularly expressed on VSMCs. Atherosclerotic plaques are characterised by an over-expression of ACE.[53] Especially in the shoulder region, where a plaque normally ruptures, an extensive activity of ACE could be shown.
Angiotensin II plays an important role in the pathogenesis of atherosclerosis, both by leading to an increase of blood pressure, and by exerting direct effects on all the phases of atherogenesis.[54] Via formation of ROS, Angiotensin II leads amongst others to an activation of Nuclear factor 'kappa-light-chain-enhancer' of activated B-cells (NFκB), which in turn enhances the expression of adhesion molecules, chemoattractant proteins and production of IL-6.[53] Moreover Angiotensin II is able to enhance LDL oxidation and its uptake by endothelial cells and macrophages, as well as VSMC migration and proliferation.[54] Furthermore Angiotensin II leads to an enhanced activity and expression of MMP-2 and contributes to thrombosis by inhibition of the fibrinolytic system, increased production of PAI-1 and TF and increased platelet activation and aggregation. Hence both ACE inhibitors and AT_1 receptor blockers can be used in the treatment of atherosclerosis because of their various beneficial effects.[53]

Aspirin and other antiplatelet drugs like clopidogrel, prasugrel or ticagrelor are used as secondary prevention for patients with acute coronary syndrome.[52] The application of a low dose of aspirin leads to an inhibition of thromboxan $A_{2,}$ whilst higher doses may also be able to affect atherosclerosis development because of their anti-inflammatory properties. Studies could demonstrate that high doses of aspirin can lead to a decrease of macrophage and cyclooxygenase-2 (COX-2) expression in the plaque.[55]

Another possible approach to treat atherosclerosis are NO donors. Atherosclerosis is characterised by a reduction of the endothelial relaxing factor NO. The lack of NO contributes to the development of endothelial dysfunction. However, within the plaque inducible nitric oxide synthase (iNOS) leads to an over expression of NO, which is interacting with oxygen-derived radicals, generating peroxynitrite. Thus the NO level needs to be well controlled to benefit from the use of NO donors. Although further studies are needed, the treatment with NO donors seems to be a promising strategy.[51]

4.2.3. Percutaneous coronary interventions

Percutaneous coronary intervention, or also called percutaneous transluminal coronary angioplasty, was first performed in 1977. Nowadays this method plays a major role in the treatment of acute coronary syndromes. Especially for older patients, which show a higher rate of operative morbidity and mortality, the PCI represents a promising alternative to coronary artery bypass surgery (CABG).[11]
The procedure is started with the introduction of a guiding catheter into the brachial artery in the arm or into the femoral artery in the groin.[39] The guiding catheter is positioned in the stenotic area and a contrast agent is injected. Subsequently an angioplasty catheter is inserted and the contained balloon is inflated several times. Following the removal of the initial angioplasty catheter, another catheter, which is carrying a stent is introduced and inflated for 10-20 seconds. After the inflation the delivery catheter and the guiding catheter are removed.[57] In addition to balloon-expandable stents, also self-expandable stents are available.[40]
The leading disadvantage of this method is the development of restenosis, which occurs in 25 up to 50 % of all cases. Another complication that can occur is acute vessel closure, which develops during the first 24 hours after the intervention in 3 up to 5 % of all patients.[39] The inflation of the balloon leads to a stretching of the vessel

wall, which may cause vascular injury. The responses are intimal hyperplasia and restenosis. The use of a stent to keep the vessel open leads to a considerable reduction in restenosis rate, in comparison with angioplasty alone. However stents cannot totally abolish the risk of restenosis because the stent causes a permanent strain on the vessel wall, which also leads to injuries.

A breakthrough seemed to be drug eluting stents. (DES) These stents contain drugs, which are able to prevent multiple biological processes, occurring as a response to the injury.[39] Especially VSMCs are an important target because these cells are the main contributors of restenosis.[40] For this purpose stents can be coated for example with Sirolimus, Tacrolimus, Paclitaxel, Heparin or Abciximab.[11] However studies could not really prove yet that DES can significantly reduce the risk of in stent thrombosis and some studies even point out that, especially the first generation of DES, leads to a higher risk of death and MI than bare metal stents,[39] by increasing the risk of late in stent thrombosis.[41] One of the reasons for this adverse effect is an excessive inflammatory response and a neointimal hyperplasia as a reaction to the synthetic polymers matrix, in which the drugs are embedded.[40]

Therefore the second generation of DES contains polymers, which are more biocompatible and are able to exert a better effect on re-endothelialisation than does the first generation.[41]

Another promising invention seems to be the biodegradable drug eluting stent. This stent only remains in the vessel as long as it is necessary to prevent the remodelling process that leads to a restenosis, and is afterwards absorbed and metabolised by the vessel.[39] Although there is a lot of data about animal studies with biodegradable drug eluting stents, only a few patients have undergone this sort of intervention until now.[41]

4.2.4. Coronary surgery

In comparison with percutaneous coronary interventions, coronary artery bypass graft is a surgical intervention. This method uses a saphenous vein graft or an arterial graft to bypass an obstruction in the coronary arteries, which is caused by an atherosclerotic lesion.[39]

C) MATERIALS AND METHODS

1. Materials

1.1 Samples

1.1.1 Plant extracts and pure compounds from the DNTI project

The following samples, that were tested using the resazurin conversion assay, are all part of the DNTI project. The abbreviation DNTI stands for Drugs from Nature Targeting Inflammation. This project deals with the identification and characterisation of natural compounds, which may be able to be applied against inflammation, especially targeting the cardiovascular system.[58] Inflammation is known to play an outstanding role in the pathogenesis of atherosclerosis.[5] As the inflammatory process progresses, it leads to a stimulation of VSMC migration and proliferation. The linkage between these processes leads to the presumption that plant extracts and natural products, that are able to exert anti-inflammatory properties may also have an inhibitory effect on the proliferation of VSMCs and thus on the development of atherosclerosis.

- **Extracts from plants, used in the traditional Vietnamese medicine**

The table on the following two pages shows a list of extracts from plants, used in the traditional Vietnamese medicine (TVM), where they are applied to medicate inflammatory diseases. The extracts were obtained from the Department of Pharmacognosy at the University of Innsbruck.

Latin Plant name	Internal identification number	Used part of the plant	Solvent
Abutilon indicum	1599	Aerial part	DCM
Abutilon indidum	1600	Aerial part	MeOH
Achyranthes aspera	1601	Aerial part	DCM
Achyranthes aspera	1602	Aerial part	MeOH
Achyranthes aspera	1603	root	DCM
Achyranthes aspera	1604	Root	MeOH
Barleria lupina	1605	Aerial part	DCM
Barleria lupina	1606	Aerial part	MeOH
Coptosapelta tomentosa	1607	stem	DCM
Coptosapelta tomentosa	1608	stem	MeOH
Coptosapelta tomentosa	1609	root	DCM
Coptosapelta tomentosa	1610	root	MeOH
Dischidia rafflesiana	1611	leaf	DCM
Dischidia rafflesiana	1612	leaf	MeOH
Drynaria quercifolia	1613	rhizome	DCM
Drynaria quercifolia	1614	rhizome	MeOH
Eupatorium odoratum	1615	leaf	DCM
Eupatorium odoratum	1616	Leaf	MeOH
Eupatorium odoratum	1617	stem	DCM
Eupatorium odoratum	1618	stem	MeOH
Eurycoma longifolia	1619	wood	DCM
Eurycoma longifolia	1620	wood	MeOH
Ficus pumila	1621	Leaf and stem	DCM
Ficus pumila	1622	Leaf and stem	MeOH
Gleditchia fera	1623	thorn	DCM
Gleditchia fera	1624	thorn	MeOH
Ipomoea biloba	1625	Aerial part	DCM
Ipomoea biloba	1626	Aerial part	MeOH
Justicia gendarussa	1627	Aerial part	DCM
Justicia gendarussa	1628	Aerial part	MeOH
Leea rubra	1629	root	DCM

Leea rubra	1630	root	MeOH
Momordica cochinchinensis	1631	seed	DCM
Momordica cochinchinensis	1632	seed	MeOH
Oroxylum indicum	1633	Stem bark	DCM
Oroxylum indicum	1634	Stem bark	MeoH
Parabarium micranthum	1635	stem	DCM
Parabarium micranthum	1636	stem	MeOH
Scoparia dulcis	1637	Aerial part	DCM
Scoparia dulcis	1638	Aerial part	MeOH
Smilax glabra	1639	rhizome	DCM
Smilax glabra	1640	rhizome	MeOH

Table 4: Extracts from Vietnamese plants

- ***Oroxylum indicum* (Bignoniaceae)**

The compounds 1950-1959 were obtained from the Department of Pharmacognosy, University of Innsbruck. These samples are the ten most prominent pure compounds of *Oroxylum indicum*, a plant known to be used in the Vietnamese medicine against inflammation.

Oroxylum indicum, also called Shivnak, Sonopatha, Shyonaka or Midnight horror belongs to the family of Bignoniaceae. The plant, which is on the list of endangered species, is native in Sri Lanka, India, South China, Malaysia, Celebes and Philippines. It is a tree with a height of up to 12 metres. Every part of this tree has a special medicinal property. *Oroxylum indicum* is used for example in the Ayurvedic medicine[59] and it also plays a role in the Bangladeshian folk medicine. There it is reported to be effective against various gastrointestinal disorders like diarrhea, colic, constipation, stomachache, lack of appetite and others. Furthermore it is used against different disorders of the respiratory tract, rheumatoid arthritis, skin disease, epilepsy,[60] urinary tract infections, Leucorrhoea, snake bites and cholera.[61] Many of the therapeutic properties of *Oroxylum indicum* may be seen as a result of its antioxidant activity due to the contained phenolic compounds. The antioxidant activity, which was tested in different assays like in the DPPH radical scavenging assay, nitric oxide scavenging assay, superoxide scavenging assay, hydroxyl radical scavenging assay and reducing power assay, was especially high for leave extracts, root bark extracts and stem bark extracts.[62]

Name of the pure compound	Internal identification number
Oroxylin A	1950
Chrysin	1951
Baicalein	1952
Hispidulin	1953
Oroxylin A-7-O-β-D-glucuronide methyl ester	1954
Baicalein-7-O-β-D-glucuronide methyl ester	1955
Oroxylin A-7-O-β-D-glucuronid	1956
Chrysin-7-O-β-D-glucuronide	1957
Bacalein-7-O-β-D-glucoside	1958
Baicalein	1959

Table 5: Pure compounds from the plant *Oroxylum indicum*

- ***Eupatorium odoratum* (Asteraceae)**

The samples 1970-1981 from the Pharmacognosy Institute of the University in Innsbruck are extracts and subfractions of *Eupatorium odoratum*

Declaration	Internal identification number
Diethyl ether extract	1970
A1+2+3 fraction	1971
A4+5 fraction	1972
A7 fraction	1973
A8 fraction	1974
A9 fraction	1975
A10 fraction	1976
A11 fraction	1977
A12 fraction	1978
A13 fraction	1979
A14 fraction	1980
A15 fraction	1981

Table 6: Extracts and subfraction from the plant *Eupatorium odoratum*

Eupatorium odoratum is a shrub, counting to the family of Asteraceae.[63] The plant, which is able to reach a height up to 3 meters,[64] can be found in North America, West India, tropical Asia, Western Africa and parts of Australia.[63] The active compounds of the plant seem to be phenols, terpenoids, alkaloids and flavonoids.[64] Despite its toxicity the plant is widely used in the folk medicine.[63] For example in the Vietnamese medicine different formulations of the leaves are used to treat soft wounds, burn wounds and skin infections.[64] In the literature various effects of different parts of the plant are reported like an immunmodulatory, anti-inflammatory, antipyretic, antidiabetic, antihypertensive, antiprotozoal, antispasmodic effect and others.[63]

The effectiveness of the ethno medicinal use of this plant could be shown in rat studies as an aqueous leave extract was able to reduce carrageenin- and formaldehyde-induced oedemas because of its significant anti-inflammatory activity. Furthermore the aqueous extract showed a significant membrane stabilizing effect,

being able to protect rat red blood cell membranes against lysis induced by hypotonic medium.[64]

In addition the aqueous and the ethanolic leave extracts of *Eupatorium odoratum* were able to exert significant antioxidant activity in different assays, including the DPPH assay, the nitric oxide scavenging assay and the hydroxyl radical scavenging assay. As the presence of ROS is associated with the pathogenetic process of atherosclerosis, *Eupatorium odoratum* may be able to positively affect this disease.[63]

- **Lichen species**

The following samples 1932-1940 and 2006-2019 are crude extracts from different lichen species. The samples were obtained from the Pharmacognosy Department, University of Innsbruck.

Latin Plant name	Internal identification number	Preparation
Xanthoparmelia somloensis	1932	Crude ethanol extract
Cladonia arbuscula	1933	Crude ethanol extract
Cladonia sp.	1934	Crude ethanol extract
Lobaria linita	1935	Crude ethanol extract
Stereocaulon alpinum	1936	Crude ethanol extract
Cetraria nivalis	1937	Crude ethanol extract
Umbilicaria cylindrica	1938	Crude ethanol extract
Peltigera rufescens	1939	Crude ethanol extract
Xanthoria elegans	1940	Crude ethanol extract

Table 7: Crude extracts from different lichen species

Latin plant name	Internal identification number	Solvent
Peltigera polydactylon	2006	Ethanol
Lepraria incana	2007	Ethanol
Nephroma resupinatum	2008	Ethanol
Platismatica glauca	2009	Ethanol
Pseudevernia furfuracea	2010	Ethanol
Cetraria pinastri	2011	Ethanol
Lobaria pulmonaria	2012	Ethanol
Parmeliopsis hyperopta	2013	Ethanol
Cetrelia monachorum	2014	Ethanol
Peltigera leucophlebia	2015	Ethanol
Parmelia caperata	2016	Ethanol
Thamnolia vermicularis	2017	Ethanol
Cetrelia monachorum	2018	Ethanol
Cetraria islandica	2019	Acetone

Table 8: Crude extracts from different lichen species

- **Sesame**

The following samples, obtained from the Pharmacognosy Institute of the University in Innsbruck are all extracted from sesame seeds. Sample 1761 is a methanolic extract, whilst sample 1762 is a diethyl ether fraction of the methanolic extract. The remaining samples are pure compounds, isolated from 1761 and 1762.

Sesame oil contains a high proportion of both, mono- and polyunsaturated fatty acids. Studies showed that LDL receptor deficient mice, which receive a diet rich in sesame oil, show a reduction in atherosclerotic lesion formation in comparison to mice receiving an atherogenic diet, containing a high proportion of saturated fatty acids. The anti-atherosclerotic effect seems to result from a reduction of plasma cholesterol, triglyceride and LDL cholesterol levels and an increase of HDL levels. Finally also an antioxidant effect and an inducing effect on PPARγ could be reported.[68]

Declaration	Internal identification number
Methanolic extract of sesame seed after defatting with petroleum ether	1761
Diethyl ether fraction of the methanolic extract Sesame extract	1762
(+)-Pinoresinol	1763
(+)-Sesamin	1764
(+)-Sesamolin	1765
(-)-75-hydroxy-matairesinol	1766
(+)-Lariciresinol	1767

Table 9: Extracts and pure compounds from sesame oil

- ***Echinacea pallida* (Asteraceae)**

Sample number 1424 and 1425 represent two different CO_2 extracts, which contain polyacetylenes from the plant *Echinacea pallida*. They originate from the Pharmacognosy Department, University of Graz.

Declaration	Internal identification number
E.pallida CO_2 extract NATEX	1424
E.pallida CO_2 extract Dr. Mele	1425

Table 10: Extracts from the plant *Echinacea pallida*

The genus Echinacea, belonging to the family of Asteraceae, counts 9 species. Amongst them 3 are medically used: *Echinacea purpurea* (L.) Moench, E. angustifolia (DC.) Hell. and *E. pallida* (Nutt.) Nutt.[69] *Echinacea* products are very common for their use in the prevention and treatment of the common cold. They seem to be able to reduce the incidence as well as the duration of an infection. At the moment an impressing quantity of about 800 products are available, containing this drug. Important ingredients are polyacetylenes, alkamides, polysaccharides, echinacosides, chicoric acid, caffeic acid, flavonoids,[70] polyenes and glycoproteins.[69] The underlying mode of action of *Echinacea pallida* is based on the stimulation of non specific innate immune processes. Mice studies could demonstrate an increase in natural killer (NK) cell cytotoxicity, an increase of splenic lymphocyte proliferation, increased IL-2 production and a marginally significant increase of IL-10 production.[71] Furthermore polyacetylens and polyenes of *Echniacea pallida* could be shown to exert a cytotoxic effect on the cancer cell lines pancreatic MIA PaCa-2 and the colonic COLO320 human cancer cell line, making these components interesting for further investigation with regard to their anticancer activity.[69]

1.1.2 Plant extracts and pure compounds, which are not part of the DNTI project

- ***Dryopteris filix-mas* (Dryopteridaceae)**

Samples number 1565-1570 are fractions and pure compounds of *Dryopteris-filix mas*, also called male fern. The plant material originates from the Ebenwaldhöhe at Lower Austria

The fern, which is used as a liquid extract is one of the oldest plants know to have an anthelminitic effect, especially on tape worms. The plant was used on men and animals and it has even been officinal in the British Pharmacopoeia.[65]

However because of its high toxicity, characterised by diarrhoea, vomiting, tremor, headache, dyspnea, mental disturbances, even blindness etc., it is not used anymore against worms.[66]

Furthermore the plant is stated to have an antiamebic, antiparasitic and also antiprotozoal effect.[67]

Declaration	Internal identification number	Used part of the plant	Plant source
Pure compound P1/Luna	1565	herb	Ebenwaldhöhe NÖ
Pure compound P2/Luna	1566	herb	Ebenwaldhöhe NÖ
Pure compound P3/Luna	1567	herb	Ebenwaldhöhe NÖ
HPLC fraction P4/Luna	1568	herb	Ebenwaldhöhe NÖ
Pure compound P5/Luna	1569	herb	Ebenwaldhöhe NÖ
HPLC fraction A/Luna	1570	herb	Ebenwaldhöhe NÖ

Table 11: Fractions and pure compounds from the plant *Dryopteris filix-mas*

- ***Semecarpus anacardium* (Anacardiceae)**

Sample number 1839 is a crude extract of *Semecarpus anacardium*, a plant traditionally used in the Ayurveda medicine to treat infections with parasitic worms and fungis. Furthermore it is also applied in the treatment of cancer, as well as nervous debilities and arthritis. *In vitro* studies have revealed an anti-inflammatory effect by the inhibition of IL-1β and IL-12p40 production. Furthermore an inhibition of NF-κB and AP-1, as well as a suppression of NO production could be demonstrated. The mentioned pro-inflammatory cytokines and transcription factors play an important role in the pathogenesis of rheumatoid arthritis.[72] The extract was provided by Pharm.D. Hamid-Reza Adhami, a PhD student at the Department of Pharmacognosy, University of Vienna.

Latin plant name	Internal identification number	Used part of the plant
Semecarpus anacardium	1839	resin

Table 12: Crude extract from the plant *Semecarpus anacardium*

The following samples, which were tested with regard to an antiproliferative effect on VSMCs, by the resazurin conversion assay, were not part of the DNTI project. These natural compounds and also the two synthetic compounds T0901317 and dexamethasone were chosen because they are stated to exert an antiatherosclerotic effect in the literature, preferentially by an inhibition of VSMC proliferation. To investigate if there is inhibitory effect in the resazurine conversion assay utilized in this work, the following samples were tested:

- **T0901317**

T0901317 is a synthetic agonist of the liver x receptor (LXR). This group of nuclear hormone receptors can be divided into two isoforms: LXRα and LXRβ. The α isoform shows a high expression in liver, macrophages and intestine, while the β isoform was shown to be ubiquitous, also being present in the central nervous system. Binding of a natural ligand, like oxysterols, or a synthetic ligand like T0901317 leads to heterodimer formation with the retinoid X receptor and to subsequent binding to promoter regions on the DNA. These receptors are regulating the expression of proteins involved in processes like lipid metabolism and reverse cholesterol transport and they affect lipoprotein plasma profile. Because of these mechanisms, the LXR receptors, especially the α isoform are stated to be atheroprotective. Mice studies could demonstrate that T0901317 is able to increase HDL levels. HDL is an acceptor molecule for cholesterol, which is able to promote cholesterol efflux and cholesterol transport to the liver. HDL molecules of mice that were treated with T0901317 are large and show a high proportion of cholesterol and phospholipids.[73]

Other effects of LXR agonists seem to be a regulatory function in immune processes and an inhibitory effect on inflammatory gene expression in macrophages.

A further important discovery was the presence of liver X receptors in human coronary artery vascular smooth muscle cells (CASMCs). The activation of this receptor inhibited CASMC proliferation that was stimulated by PDGF/insulin, by blocking the G_1/S phase progression. The mechanism behind this effect is an inhibition of Retinoblastomaprotein phosphorylation. The phosphorylation of this protein leads to the release of E2F, a transcription factor of the S phase, which is able to activate target genes leading to DNA synthesis.[74]

Despite these beneficial effects a use of LXR agonists as a therapeutic agent is limited by a possible trigger of a hypertriglyceridemic state and hepatic stenosis.[73]

- **Cortisol & Dexamethasone**

In addition to their anti-inflammatory effect, glucocorticoids are declared in literature to carry out an antiproliferative effect on VSMCs, as well as on monocytes. Furthermore VSMC and monocyte infiltration is inhibited by glucocorticoid administration. Therefore glucocorticoids are tested as anti-restenotic drugs at the moment. However apart from this local antiatherosclerotic effect it must be mentioned, that glucocorticoids may rather exert a negative effect on atherosclerosis. Glucocorticoids are known to raise blood pressure, increase lipid levels and are also able to increase the levels of various clotting factors, triggering a hypercoagulable state, whilst fibrinolysis is reduced. These facts seem to be amongst others responsible for an enhanced risk for cardiovascular diseases under glucocorticoid administration.[75]
Based on these findings, the natural glucocorticoid cortisol and the synthetic product dexamethasone were tested in this work, regarding their inhibitory effect on serum-induced VSMC proliferation.

- **Parthenolide**

Parthenolide is a sesquiterpene lactone, which can be found in *Tanacetum parthenium*, also called feverfew, a herb traditionally used in the Mexican-Indian medicine against diseases connected with infections or inflammation, like asthma, arthritis and migraine. In addition this natural compound is probably able to inhibit tumor growth[76] by triggering apoptosis of cancer cells, making it interesting as an antitumor agent.[77]
Interestingly, studies with rat aortic smooth muscle cells could show that parthenolide is able to induce a cell cycle arrest at G_0/G_1 phase, leading to an inhibition of VSMC proliferation. Parthenolide was able to upregulate p21 and p27, inhibiting G_1/S phase transition, which also triggers inhibition of cellular proliferation.[76]
Furthermore *in vitro* studies with murine and human VSMCs could demonstrate that parthenolide inhibits NF-κB activation, by preventing IκBα from degradation and also a reduction in the number of moncytes as well as a reduction in the serum levels of MCP-1 in plaques could be shown.[77] NF-κB functions as a transcription factor being involved in the regulation of various important processes like cell differentiation, proliferation, inflammation and apoptosis. Thus it also plays an important role in the

pathogenesis of atherosclerosis. Because of these beneficial effects further investigations in studies with the perspective for using parthenolide as a therapeutic agent in the treatment of atherosclerosis and also restenosis could be promising.[76]

- **IKK inhibitor IV**

IKK inhibitors are able to prevent the activation of NFκB by an inhibition of the IκB kinase (IKK). The nuclear transcription factor NFκB consists of two subunits, p50 and p65. In its inactivated form the dimer forms a complex with IκB, the inhibitor of NFκB. Activated by different signals, the IκB kinase phosphorylates IκB, which leads to its degradation by proteasomes. As a result NFκB is liberated and activated.[78]

- **Leoligin**

The lignan leoligin originates from the root of the alpine plant Edelweiss with the Latin name *Leontopodium nivalis*. This plant has a long tradition in folk medicine, where it is used to treat diseases like diarrhoea, fever and abdominal pain. So far there are only a few studies available, which deal with the possible beneficial effect of lignans on the cardiovascular system.

Leoligin may be used to prevent complications following invasive interventions to treat coronary artery diseases. A big advantage of this compound is that it does not exert a toxic effect on ECs, like other substances currently used. The endothelium plays an important role in the healing process and in the prevention of thrombosis after PCI or CABG. Leoligin was shown to inhibit intimal hyperplasia by increasing the protein levels of p27/KIP, which results in a G_1 phase arrest. P27/KIP is able to bind the cyclinE/cdk2 complex, resulting in its inactivation. This complex is responsible for the phosphorylation of retinoblastoma protein. The phosphorylated form of this protein releases the transcription factor E2F, which in turn activates genes that promote proliferation. Moreover leoligin was demonstrated to inhibit VCAM-1 expression on endothelial cells, that is triggered by TNF-α.[79]

Further *in vitro* studies could discover an inhibitory effect on the leukotriene biosynthesis and an influence on cholesteryl ester transfer protein (CETP). This protein plays an emerging role in lipoprotein metabolism by exchanging cholesteryl esters for triglycerids. Rodent studies could demonstrate that an inactivation of CETP is able to raise HDL levels, resulting in a resistance to atherosclerosis

induced by diet. In human plasma leoligin was shown to influence CETP activity in a dose dependent manner. While low doses increase CETP activity, the application of higher doses can significantly inhibit protein activity.[80]

- **Indirubin-3´-monoxime**

Indirubin-3´-monoxime (I3MO) a derivate of indirubin, was already stated to be active against inflammation and cancer in *in vitro* studies. Previous studies at the Department of Pharmacognosy in Vienna could show that the proliferation of PDGF-BB stimulated rat VSMCs was inhibited by Indirubin-3´-monoxime.[81]
Within this work, it was further investigated if I3MO would inhibit the proliferation of rat VSMCs, which were stimulated by new-born bovine serum (NBS) instead of PDGF-BB in the resazurin conversion assay.

- **Pure compounds from olive oil**

Olive oil is rich in triacylglycerols, furthermore it contains 0.5-1% nonglyceridic constituents, which include at least 30 phenolic compounds. These are very important ingredients, amongst other properties also being responsible for the oxidative stability of the oil. The major phenols are oleuropein, hydroxytyrosol and tyrosol. Olive oil components, especially phenolic components have been shown to exert strong antioxidant and radical scavenging activities. Furthermore hydroxytyrosol and oleuropein were demonstrated to act antimicrobial on various bacterial strains.[82]
Antioxidant polyphenols of olive oil, especially oleuropein aglycone and hydroxytyrosol were shown to inhibit the expression of VCAM-1 and the adhesion of monocytes to the endothelium of human vascular endothelial cells, thus possibly being able to prevent early atherogenesis.[83] Therefore the following selected olive oil ingredients hydroxytyrosol, oleuropein, maslinic acid, erythrodiol, uvaol, all trans retinoic acid, 9-cis retinoic acid and oleocanthal were tested, regarding a potential inhibition of VSMC proliferation that could contribute to these beneficial effects. The olive oil ingredients tested in this work were supplied from commercial sources, with exception of oleocanthal, which was provided from Prof. Liselotte Krenn from the Department of Pharmacognosy, University of Vienna.

1.2 Cell culture

1.2.1 Cells

For the experiments VSMCs from male Sprawgue Dawley rat thoracic aortas were used. These were obtained from the Emory University in Atlanta.

1.2.2 Cell culture media and buffers

Name	Components	Volume or concentration
Growth Medium	Dulbecco`s Modified Eagle Medium (DMEM) NBS Penicillin/ Streptomycin L- glutamine	500 ml 10 % 100 U/ml / 100 µg/ml 2 mM
Starvation Medium	DMEM NBS Penicillin/ Streptomycin L- glutamine	500 ml 0.1 % 100 U/ml / 100 µg/ml 2 mM
PBS pH 7,4	NaCl Na_2HPO_4 KH_2PO_4 Aqua dest.	36,0 g 7,4 g 2,15 g Ad 5000 ml

Table 13: Cell culture media and buffers

1.2.3 Cell culture reagents

Name	Provider
DMEM 4,5 g/l glucose, without phenol red and L-glutamin	Lonza Group Ltd. (Basel, Switzerland)
Dimethyl sulfoxide (DMSO)	Fluka (Buchs, Switzerland)
L- glutamine 200 mM	Lonza Group Ltd. (Basel, Switzerland)
Penicillin/Streptomycin mixture (10 000 U/ml potassium penicillin/10 000 µg/ml streptomycin sulphate)	Lonza Group Ltd. (Basel, Switzerland)
Trypsin (1:250)	Invitrogen (Carlsbad, CA, USA)
New-born bovine serum (NBS)	Lonza Group Ltd. (Basel, Switzerland)
Resazurin	Sigma-Aldrich (St.Louis, MO, USA)

Table 14: Cell culture reagents

1.2.4 Technical equipment

Name	Provider
HERAsafe™ KS Workbench	Thermo Fisher Scientific Inc. (Waltham, CA, USA)
HERAcell™ 150 Incubator	Thermo Fisher Scientific Inc. (Waltham, CA, USA)
Heraeus™ Multifuge™ 1 S-R Centrifuge	Thermo Fisher Scientific Inc. (Waltham, CA, USA)
Galaxy Mini Microcentrifuge	VWR International Inc. (West Chester, PA, USA)
Tecan GENios™ Pro	Tecan (Mannedorf, Switzerland)
Light Microscope Olympus CKX 31	Olympus Europe GmbH (Hamburg, Germany)
Vi-Cell™ XR Cell Viability Analyzer	Beckmann Coulter (Fullerton, CA, USA)
Vortex Shaker	VWR International Inc. (West Chester, PA, USA)

Table 15: Technical equipment

1.2.5 Software

Name	Provider
Microsoft® Office Excel 2007	Microsoft
Vi-Cell™ XR 2.03	Beckmann Coulter (Fullerton, CA, USA)
Tecan GENios™ Pro, version 4.63	Tecan (Mannedorf, Switzerland)
GraphPad PRISM™ version 5	GraphPad Software Inc. (San Diego, CA, USA)

Table 16: Software

1.2.6 Miscellaneous

Name	Provider
96-well Microplates	Greiner bio-one (Frickenhausen, Germany)
Tissue Culture Flasks (75 m^2, 175 m^2)	Greiner bio-one (Frickenhausen, Germany)
Serological Pipettes (10 ml, 25 ml)	Sarstedt GmbH (Nümbrecht, Germany)
Boxes with sterile tips for Eppendorf® pipettes (10 µl, 200 µl, 1000 µl)	Eppendorf GmbH (Hamburg, Germany)
Eppendorf® 8-channel pipettors	Eppendorf GmbH (Hamburg, Germany)
Eppendorf® 1-channel pipettors	Eppendorf GmbH (Hamburg, Germany)
Eppendorf® tubes (2 ml, 1.5 ml, 0.5 ml)	Eppendorf GmbH (Hamburg, Germany)

Table 17: Miscellaneous

2. Methods

2.1 Resazurin conversion assay

To measure the metabolic activity of VSMCs, the resazurin conversion assay was used. The assay involves treatment of the cells with a buffered solution, which contains highly purified resazurin. Resazurin is an indicator dye with a dark blue colour and little fluorescence activity. In the presence of metabolic active cells the indicator is reduced to resorufin, which is pink in colour and has high fluorescence activity (**figure 5 and 6**).[87] Mitochondrial enzymes like flavin, mononucleotide dehydrogenase, nicotinamide adenine dehydrogenase and cytochromes or cytosolic and microsomal enzymes are able to conduct the reduction.[43] The intensity of the fluorescence signal can be used to estimate the number of metabolic active cells[87] and consequently it can give initial information about potential antiproliferative activites of the samples. The lower the fluorescence signal, the lower is the number of metabolic active cells and the higher is the potential antiproliferative activity of a sample, provided that it does not deploy a cytotoxic effect on VSMCs. However outcomes obtained from this assay must only be seen as a piece of information and further experiments are always necessary to confirm the results.

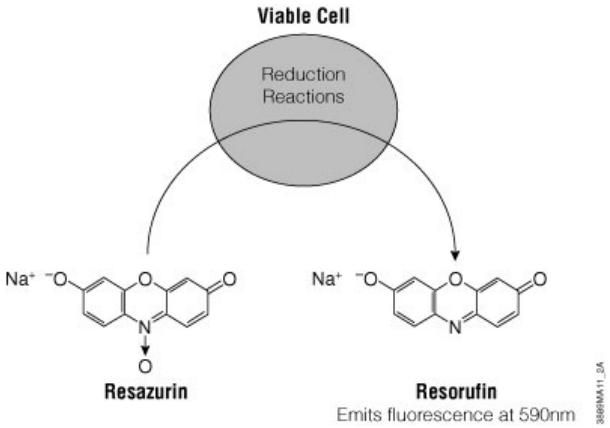

Figure 5 and 6: Resazurin assay
Pictures taken from http://www.sciencedirect.com/science/article/pii/S1046202307000096[88] and http://www.promega.com/~/media/Files/Resources/Protocols/Technical%20Bulletins/101/CellTiter-Blue%20Cell%20Viability%20Assay%20Protocol.pdf[87]

2.2 Cell culture

All cell culture steps were performed with sterile materials under aseptic conditions, which were obtained by using a laminar air flow workbench.
Before every work step all the liquids needed were warmed in a water bath to 37°C.

2.2.1 Cell storage and thawing of cells

VSMCs, isolated from aortas of Sprague-Dawley rats, were provided from Emory University (Atlanta, USA). After the isolation cells were transferred into frozen liquid nitrogen.
For the experiments one cryovial of cells was defrosted in a water bath with 37°C. The cell suspension was filled into a falcon, already containing 10 ml of growth medium. After 10 minutes of centrifugation at 1.000 rpm, the supernatant fraction was removed and the remaining cell pellet was re-suspended in 10 ml growth medium.

2.2.2 Cell cultivation

Cells were cultivated in 75 cm^2 or 175 cm^2 culture flasks at 37°C and 5% CO_2, using a HERAcell™ 150 Incubator. The used cell culture medium consisted of DMEM with 10% NBS, 2 mM glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin.
Except from an initial adaption period at the beginning of the cultivation, the used VSMCs showed an average doubling time of approximately 24 hours. During the growth period cells were observed via light microscopy and growth medium was changed twice a week. This step is necessary because while cells are cultivated nutrients diminish and metabolic products increase, possibly being able to carry out a toxic effect on the cells.[84] Once a week the cells were passaged.

2.2.3 Passaging of cells

Passaging is a term, which stands for a transfer of cells between two culture flasks that may include a dilution step. The frequency of this transfer is dependent on the growth rate of the cell line and the quantity of the cells seeded.[84] During the experiments cells were cultivated until they became nearly confluent. Confluency

describes a stage where cells completely cover the surface of the culture flask. Cells are normally passaged before they reach confluency because the biochemistry of confluent cells can differ from cells, which are exponentially growing.[84] For the experiments cells between passage 7 and 14 were used. At passage 7 cells have already reached a state of dedifferentiation, which means that cells are now able to proliferate, imitating similar conditions like they show during disease progression of atherosclerosis. Passage 14 was the last one to be used because from that time on cells start to alter and show different characteristics.

Cell passaging was started by removing the growth medium, in which cells were cultivated. Then cells were washed with 10 ml PBS. Afterwards cells were detached from the cell culture flask surface by using 3 ml trypsin/EDTA. After a short incubation time of 2 until 3 minutes, 7 ml growth medium were added and the resulting cell suspension was filled into a falcon. The culture flask was once again rinsed with 5 ml growth medium and the liquid was also filled into the prepared falcon. After this step the suspension was centrifuged for 4 minutes at 1.400 rpm. The supernatant fraction was removed, and the cell pellet was re-suspended in 10 ml growth medium. 500 µl of the cell suspension were used to determine the proportion of viable cells via ViCell XR®. The ViCell® system uses the trypan blue dye exclusion method: dead cells have a membrane, which shows higher permeability than viable cells. Thus they are able to take up the trypan blue dye resulting in a darker cell staining. This contrast allows to determine the proportion of viable cells in percent.[85] The percentage of viable cells should be more than 95% to be suitable for the following experiments.

For further cultivation cells were once again seeded into culture flasks. When a 175 cm^2 flask was used, 500.000 cells were seeded. In a 75 cm^2 flask 250.000 cells needed to be seeded. As a following step 20 and 10 ml of growth medium were added, respectively.

2.2.4 Preparation of cells for the experiments

For the experiments VSMCs were seeded into 96 well plates. For every experiment three plates were prepared in parallel. 1×10^4 cells were pipetted into every well and cultivated in growth medium for 24 hours.

On the next day cells were washed with 200 µl/well starvation medium and then 100 µl/well fresh starvation medium was added to the cells. Another 24 h later cells were treated with the samples.

2.2.5 Treatment of cells with the samples

On each 96-well plate 14 samples could be tested in quadruplicate. The stock solutions of the extracts were prepared with dimethyl sulfoxide (DMSO). To exclude a possible effect of a fluorescence signal of DMSO, which could falsify the results of the tested samples, four wells of a DMSO control (starvation medium + 0.1% DMSO, starvation medium added instead of NBS) were included into every experiment. Furthermore in each case four wells of a serum control (starvation medium + 0.1% DMSO, stimulated with NBS), of untreated cells (cells only with starvation medium added) and of starvation medium stimulated with NBS were added and 8 wells were left empty. The mean of the fluorescence signal of these empty wells was subtracted from all the other signals to exclude a background signal.

Example for a pipetting protocol:

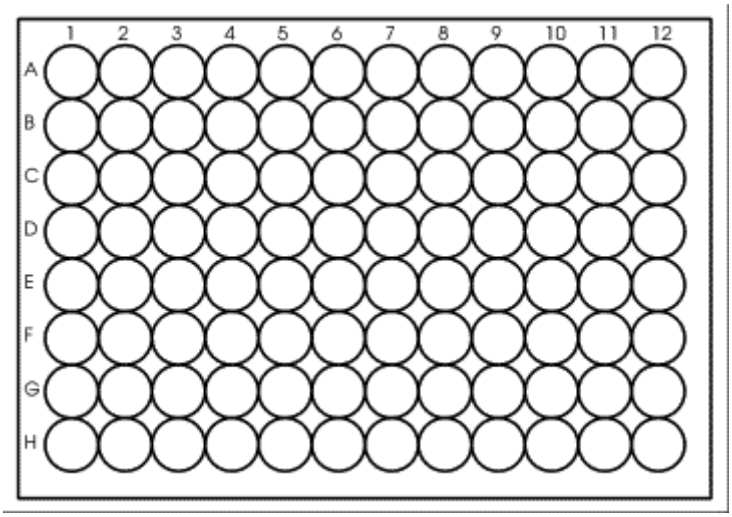

Figure 7: Schema of a pipetting protocol
Picture taken from http://cellbiology.med.unsw.edu.au/units/lab/cblmethod_elisa.htm[86]

A1-D1 and E11-H11: untreated cells

A2-D2 and E10-H10: DMSO control

A3-D3 and E9-H9: serum control

A4-D4 and E8-H8: starvation medium stimulated with serum

A5-D5: Sample number one

A6-D6: Sample number two...

...E7-H7: Sample number 14

A12-H12: empty wells

Plant extracts, which were part of the DNTI database, were always tested in a final concentration of 10 µg/ml, natural compounds in 1-30 µM concentrations. Natural compounds, which were not part of the DNTI project were tested in 0.1-30 µM concentrations.
Cells were treated with 80 µl of the samples per well for 30 minutes. Subsequently the treated cells were stimulated with 20 µl NBS (equal to 10% final concentration) per well for 48 h. Serum contains various mitogenic growth factors like PDGF, FGF, VEGF, epidermal growth factor (EGF) and others. To the untreated cells and to the DMSO control 20 µl of starvation medium was added per well instead of NBS.

2.2.6 Measurement

Before measuring the fluorescence, cells were once washed with PBS, and 200 µl of starvation medium containing 10 µg/ml resazurin were added per well.
After an incubation time of two hours, the proportion of metabolic active cells was measured, using the Tecan GENios™ Pro at an excitation wavelength of 535 nm and an emission wavelength of 590 nm.
The gained data were analyzed with Excel and GraphPad PRISM™ to demonstrate a potential antiproliferative activity of the examined samples.

2.3 Statistics

Experiments were analyzed with GraphPad PRISM™, using one-way ANOVA/Tukey statistical test. Results are shown as means ± standard error of the mean. A result was indicated as statistically significant when the p value was less than 0.05. The smaller the p value the greater the resulting effect of a sample. One star represents a p value of less than 0.05, two stars a p value of less than 0.01 and three stars represent a p value, which is smaller than 0.001.

D) RESULTS

The following chapters summarize the results received from investigation of various samples. Detailed information about the samples can be found in the Material and Methods section. All extracts and subfractions, which were selected from the DNTI project, were tested in a final concentration of 10 µg/ml and pure compounds at 1-30 µM. The pure compounds, which are not from the DNTI project, were tested in different concentrations, as described below.

Each bar in the illustrated figures represents the mean of three individual experiments. The results are expressed in percent, normalized to the serum control, which has a value of 100%.

1. Compounds, with a low inhibitory effect on VSMC proliferation

Based on the findings in literature, which declare glucocorticoids as antiproliferative agents, cortisol and the synthetic cortisol-derivative dexamethasone, were tested with regard to a possible antiproliferative effect on serum-stimulated VSMCs. For the experiments dexamethasone was used in a concentration of 2.5 µM whilst cortisol was tested in a concentration of 10 µM. Like shown in **figure 8 and 9** a reduction in serum-stimulated VSMC proliferation resulted from the treatment of cells with these agents.

The antiproliferative activity was not very strong, but statistically significant. Cells treated with cortisol showed an average proliferative activity of 54%, while dexamethasone treatment led to a proliferative activity of 56% in comparison with the serum control. Consequently the compounds were nearly able to reduce proliferation by half. No cytotoxic effects could be observed using the light microscope.

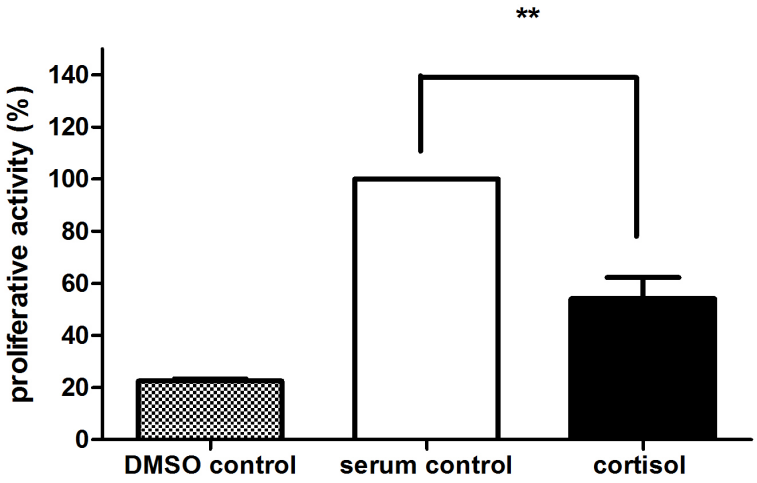

Figure 8: Inhibitory effect of cortisol on VSMC proliferation
Results obtained from the resazurin conversion assay of cortisol tested in a concentration of 10 µM and performed as described in detail in the chapter “Materials and Methods”. Final DMSO concentration in all samples was 0.1%. Each bar represents the mean of 3 experiments. All values were normalized to the serum control. Results are shown as mean with standard error of the mean; DMSO control: untreated, unstimulated cells; serum control: untreated cells stimulated with NBS; **: statistically significant with p<0.01 (one-way ANOVA/Tukey)

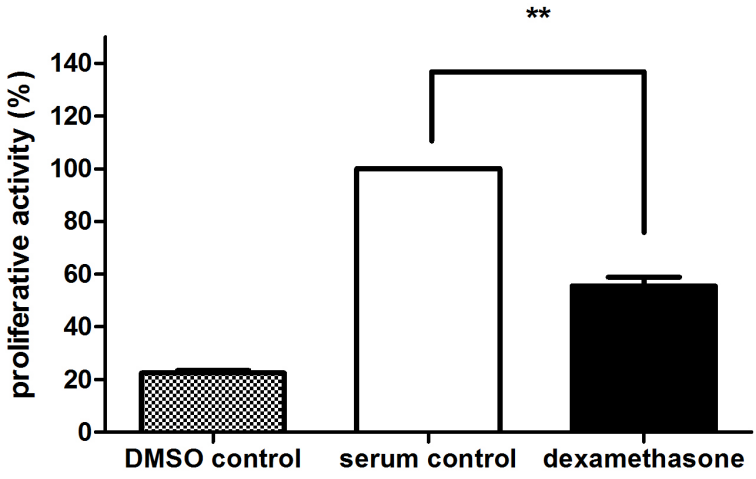

Figure 9: Inhibitory effect of dexamethasone on VSMC proliferation
Results obtained from the resazurin conversion assay of dexamethasone, performed as described in detail in the chapter “Materials and Methods”. The compound was tested in a concentration of 2.5 µM. Final DMSO concentration in all samples was 0.1%. Each bar represents the mean of 3 experiments. All values were normalized to the serum control. Results are shown as mean with standard error of the mean; DMSO control: untreated, unstimulated cells; serum control: untreated cells stimulated with NBS; **: statistically significant with p<0.01 (one-way ANOVA/Tukey)

Prior experiments in the "Molecular targets" group at the Department of Pharmacognosy, University of Vienna could demonstrate an antiproliferative effect of I3MO on PDGF-stimulated VSMCs. In the context of this work I3MO was tested in a concentration of 5 µM and 10 µM, to investigate if the compound could also block serum-induced VSMC proliferation. Whilst the lower concentration of the indirubin derivate could not inhibit serum-stimulated VSMC proliferation, the application of the higher concentration led to a reduction of proliferative activity, like shown in **figure 10**. The average inhibitory activity of the compound was about 39%, in comparison with the serum control.

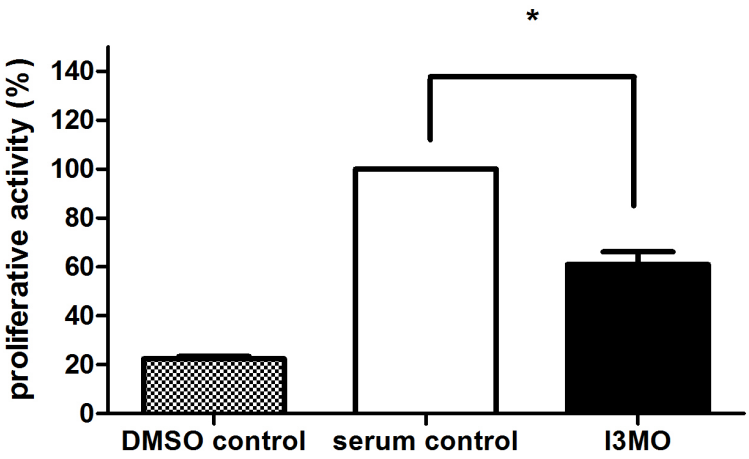

Figure 10: Inhibitory effect of indirubin-3´-monoxime on VSMC proliferation
Results obtained from the resazurin conversion assay of I3MO, performed as described in detail in the chapter "Materials and Methods". The compound was tested in a concentration of 10 µM. Final DMSO concentration in all samples was 0.1%. Each bar represents the mean of 3 experiments. All values were normalized to the serum control. Results are shown as mean with standard error of the mean; DMSO control: untreated, unstimulated cells; serum control: untreated cells stimulated with NBS; *: statistically significant with $p<0.05$ (one-way ANOVA/Tukey)

2. Samples with a possible cytotoxic effect on VSMCs

Sample number 1839 represents an extract of *Semecarpus anacardium*, which was tested in a concentration of 10 µg/ml. In this concentration the extract shows a very strong inhibitory effect on VSMC proliferation (**Figure 11**). This inhibition is so distinct that it is even below the DMSO control. The proliferative activity of the cells, treated with extract number 1839 is only 14% on average in comparison with the serum control.

Nearly none of the cells were able to convert the resazurin dye into resorufin. So only a low proportion of the seeded cells was metabolic active and thus able to conduct the reduction, leading to a fluorescence signal. This finding and also the observed changes in cell morphology under the light microscope (the cells were detaching from the bottom of the wells and becoming round as visualized in **figure 13**; healthy cells can be seen in **figure 12**), makes it likely that extract number 1839 carries out a cytotoxic or pro-apoptotic effect on VSMCs. However, further experiments utilizing different methods are needed to confirm such effects.

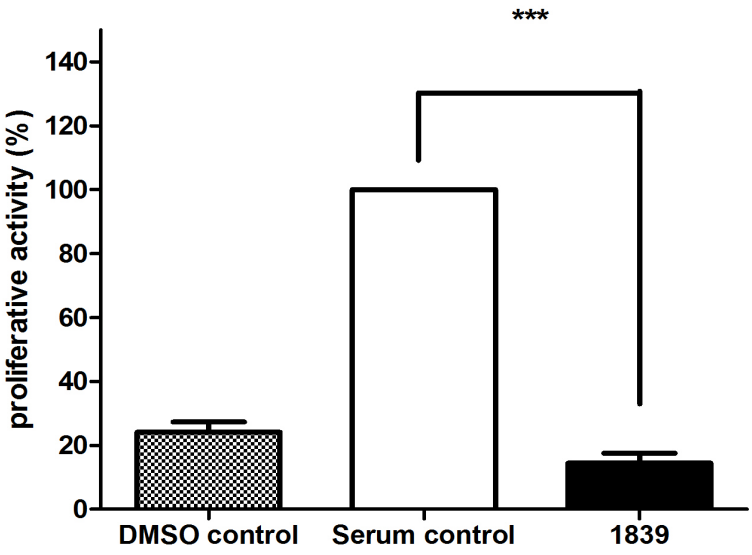

Figure 11: Cytotoxic effect of extract number 1839 on VSMCs
Results obtained from the resazurin conversion assay of extract number 1839, performed as described in detail in the chapter "Materials and Methods". The extract was tested in a concentration of 10 µg/ml. Final DMSO concentration in all samples was 0.1%. Each bar represents the mean of 3 experiments. All values were normalized to the serum control. Results are shown as mean with standard error of the mean; DMSO control: untreated, unstimulated cells; serum control: untreated cells stimulated with NBS; ***: statistically significant with $p<0.001$ (one-way ANOVA/Tukey)

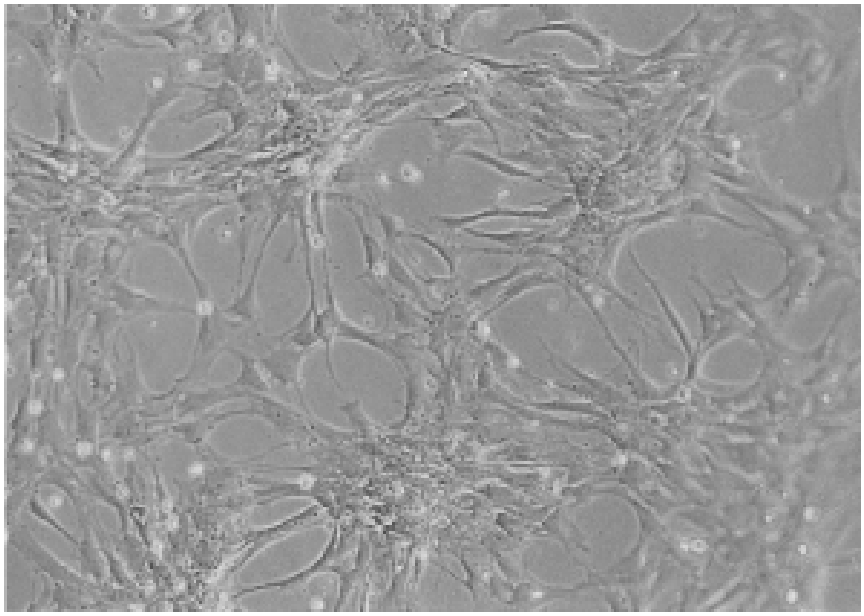

Figure 12: Illustration of healthy VSMCs
Picture taken from MacBeath, J.R.E., Kielty,C.M. & Adrian, C (1996)[89]

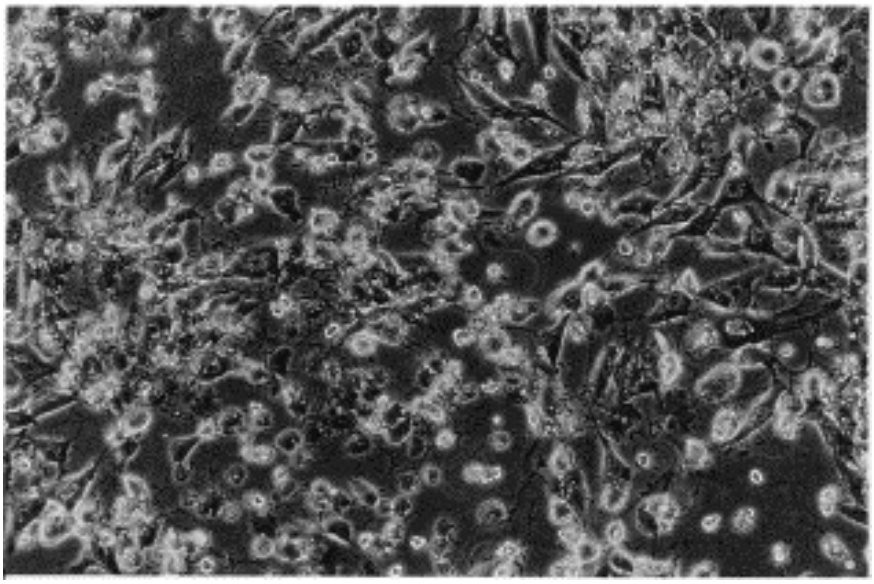

Figure 13: Illustration of apoptotic VSMCs
Picture taken from Okura, T. (1998)[90]

3. Compounds, which led to a slight increase in VSMC proliferation

As shown in **figure 14 and 15**, sample number 1952, a natural compound of *Oroxylum indicum*, and erythrodiol an ingredient of olive oil both were able to increase the number of metabolic active vascular smooth muscle cells significantly, which may lead to the presumption that these substances might promote VSMC proliferation. Both compounds were tested in a concentration of 10 µM. The application of baicalein led to a proliferative activity of 158% on average, compared to sole serum stimulation, whilst the application of erythrodiol resulted in a proliferative activity of 132%.

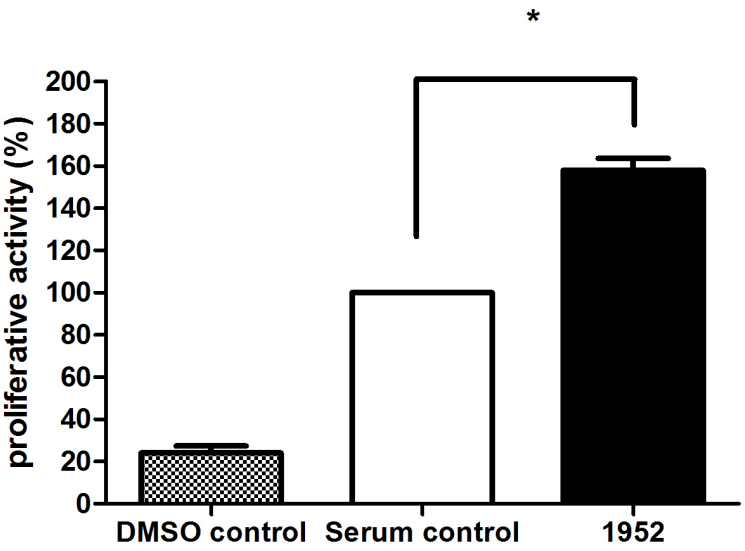

Figure 14: Induction of VSMC proliferation by compound number 1952
Results obtained from the resazurin conversion assay of compound number 1952, performed as described in detail in the chapter "Materials and Methods". The compound was tested in a concentration of 10 µM. Final DMSO concentration in all samples was 0.1%. Each bar represents the mean of 3 experiments. All values were normalized to the serum control. Results are shown as mean with standard error of the mean; DMSO control: untreated, unstimulated cells; serum control: untreated cells stimulated with NBS; *: statistically significant with $p<0.05$ (one-way ANOVA/Tukey)

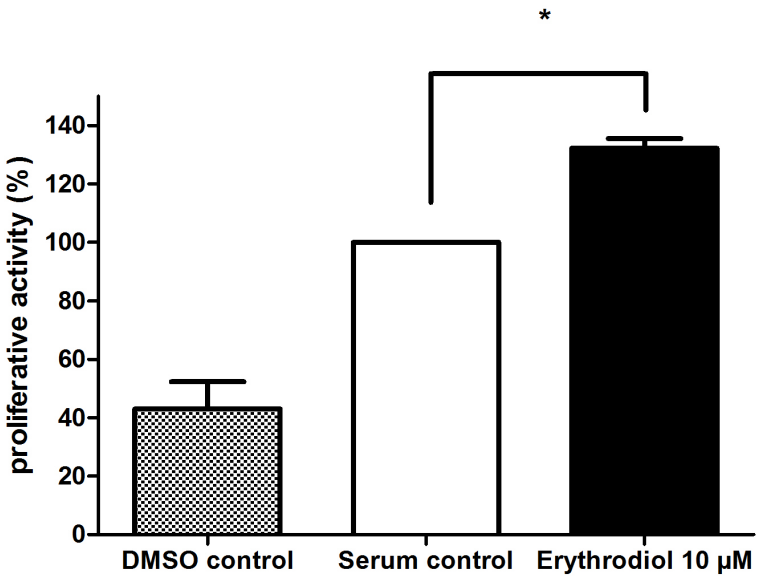

Figure 15: Induction of VSMC proliferation by erythrodiol
Results obtained from the resazurin conversion assay of the olive oil compound erythrodiol, performed as described in detail in the chapter “Materials and Methods”. The pure compound was tested in a concentration of 10 µM. Final DMSO concentration in all samples was 0.1%. Each bar represents the mean of 3 experiments. All values were normalized to the serum control. Results are shown as mean with standard error of the mean; DMSO control: untreated, unstimulated cells; serum control: untreated cells stimulated with NBS; *: statistically significant with $p<0.05$ (one-way ANOVA/Tukey)

4. Samples, which did not show any effect on VSMC proliferation

In addition the following samples were tested but unfortunately none of them was able to exert a statistically significant effect on VSMC proliferation. Neither an inhibitory, nor a promoting effect on proliferation could be observed, which was considered to be statistically significant.

4.1 *Echinacea pallida* CO_2 extracts

The *Echinacea pallida* CO_2 extracts 1424 and 1425 were not able to influence VSMC proliferation significantly in the applied concentration of 10 µg/ml (**figure 16**). Average proliferative activity was demonstrated to be 112% for VSMCs treated with extract 1424 and 119% for cells treated with extract 1425.

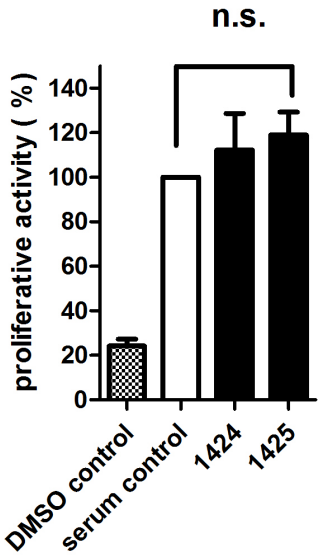

Figure 16: Proliferative activity of extract 1424 and 1425
Results obtained from the resazurin conversion assay of extract number 1424 and 1425, performed as described in detail in the chapter “Materials and Methods”. The extracts were tested in a concentration of 10 µg/ml. Final DMSO concentration in all samples was 0.1%. Each bar represents the mean of 3 experiments. All values were normalized to the serum control. Results are shown as mean with standard error of the mean; DMSO control: untreated, unstimulated cells; serum control: untreated cells stimulated with NBS; n.s. not significant (one-way ANOVA/Tukey)

4.2 *Dryopteris filix-mas* fractions and pure compounds

As shown in **figure 17** the application of fractions and pure compounds originating from the plant *Dryopteris filix-mas* led to an average proliferative activity of VSMCs ranging from 102% up to 120%. None of these effects reached statistical significance.

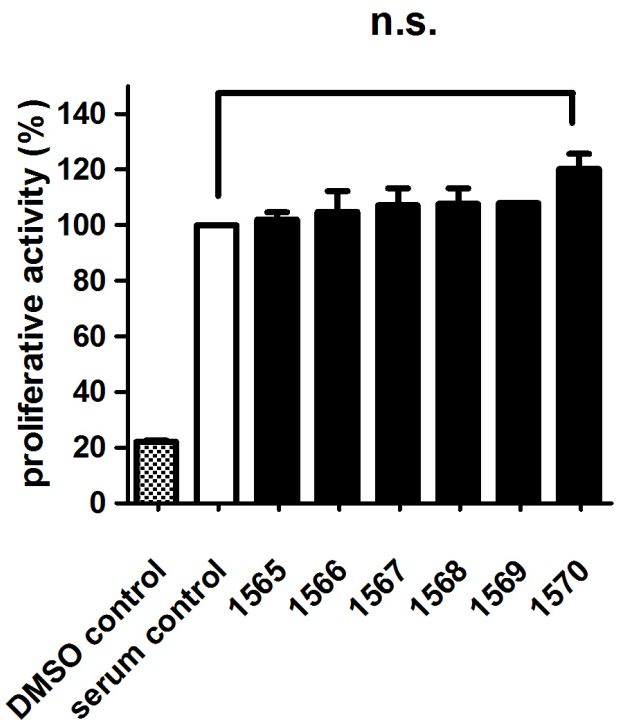

Figure 17: Proliferative activity of sample 1565-1570
Results obtained from the resazurin conversion assay of sample number 1565-1570, performed as described in detail in the chapter "Materials and Methods". The pure compounds (1565-1567) were tested in a concentration of 30 µM and the frations (1568-1570) at 10 µg/ml. Final DMSO concentration in all samples was 0.1%. Each bar represents the mean of 3 experiments. All values were normalized to the serum control. Results are shown as mean with standard error of the mean; DMSO control: untreated, unstimulated cells; serum control: untreated cells stimulated with NBS; n.s. not significant (one-way ANOVA/Tukey)

4.3 Traditional Vietnamese medicine (TVM) plant extracts

Figure 18-20 show the results from a testing of extracts from various plants used in the TVM. All these extracts were tested in a concentration of 10 µg/ml. None of the testings resulted in a considerable effect, which may raise the demand for further investigation. The lowest proliferative activity of 77% was demonstrated for cells treated with extract 1609, the highest for cells treated with extract 1613 with 127% on average.

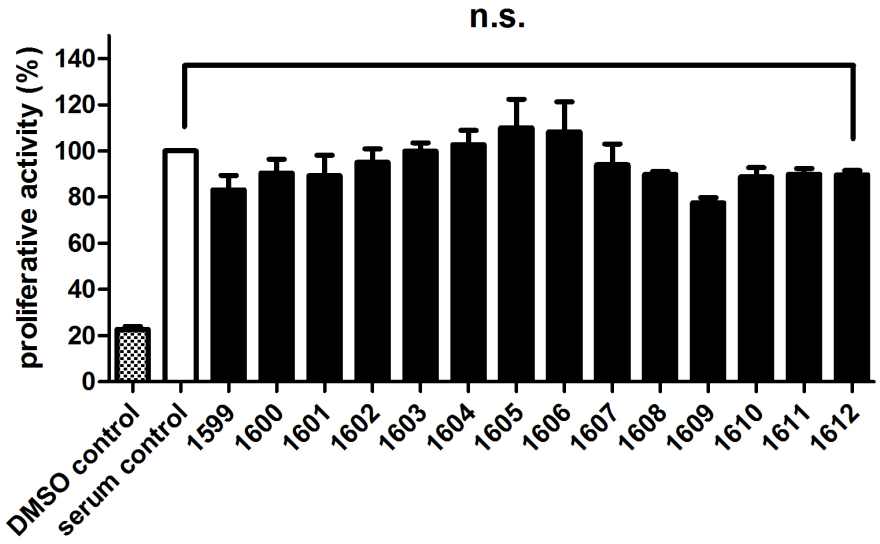

Figure 18: Proliferative activity of extract 1599-1612
Results obtained from the resazurin conversion assay of extract number 1599-1612, performed as described in detail in the chapter “Materials and Methods”. The samples were tested in a concentration of 10 µg/ml. Final DMSO concentration in all samples was 0.1%. Each bar represents the mean of 3 experiments. All values were normalized to the serum control. Results are shown as mean with standard error of the mean; DMSO control: untreated, unstimulated cells; serum control: untreated cells stimulated with NBS; n.s. not significant (one-way ANOVA/Tukey)

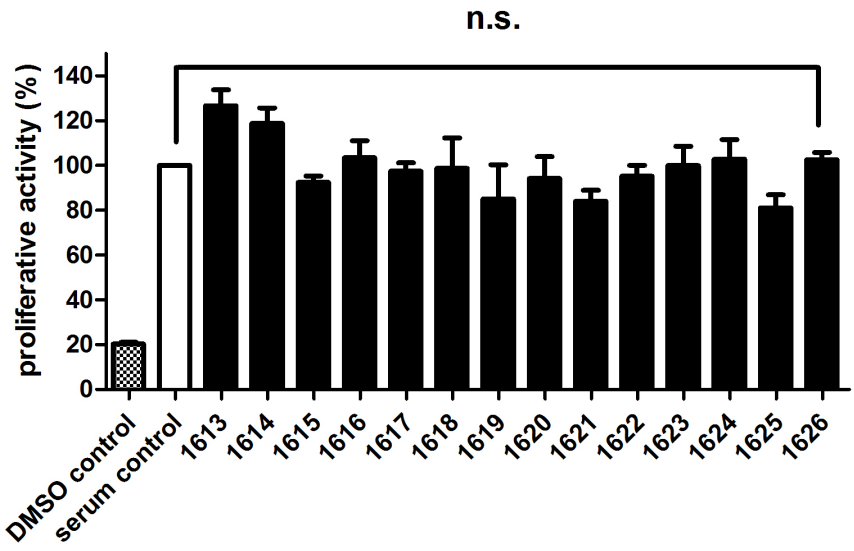

Figure 19: Proliferative activity of extract 1613-1626
Results obtained from the resazurin conversion assay of extract number 1613-1626, performed as described in detail in the chapter “Materials and Methods”. The samples were tested in a concentration of 10 µg/ml. Final DMSO concentration in all samples was 0.1%. Each bar represents the mean of 3 experiments. All values were normalized to the serum control. Results are shown as mean with standard error of the mean; DMSO control: untreated, unstimulated cells; serum control: untreated cells stimulated with NBS; n.s. not significant (one-way ANOVA/Tukey)

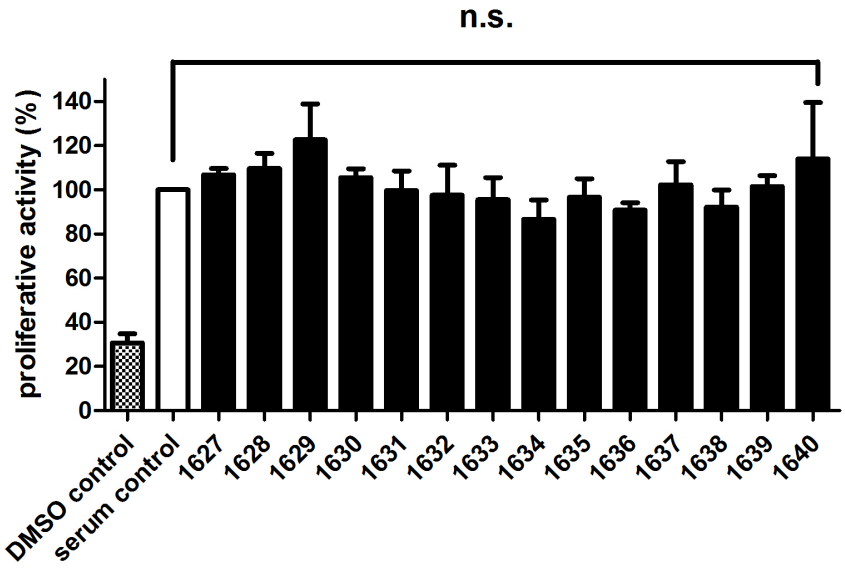

Figure 20: Proliferativ activity of extract 1627-1640
Results obtained from the resazurin conversion assay of extract number 1627-1640, performed as described in detail in the chapter “Materials and Methods”. The samples were tested in a concentration of 10 µg/ml. Final DMSO concentration in all samples was 0.1%. Each bar represents the mean of 3 experiments. All values were normalized to the serum control. Results are shown as mean with standard error of the mean; DMSO control: untreated, unstimulated cells; serum control: untreated cells stimulated with NBS; n.s. not significant (one-way ANOVA/Tukey)

4.4 *Oroxylum indicum* pure compounds

The Oroxylum indicum compounds 1950, 1951 and 1953-1959, all tested in a concentration of 10 µM, could not influence serum-stimulated VSMC proliferation strong enough to reach statistical significance (**figure 21**). The application of the compounds 1950 and 1951 led to a proliferative activity of 140% and 143% respectively, however only the application of compound number 1952 led to a statistically significant effect, which can be seen in **figure 14**, chapter three. The lowest proliferative activity was reached after a treatment of VSMCs with compound 1955 (proliferative activity 69%). However this effect did not reach statistical significance.

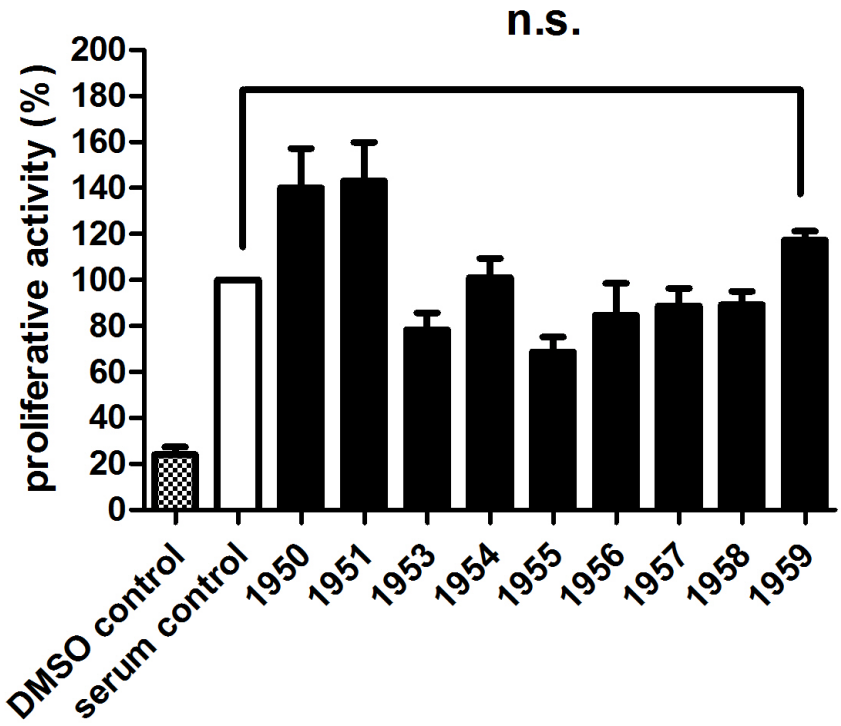

Figure 21: Proliferative activity of compound 1950, 1951, 1953-1959
Results obtained from the resazurin conversion assay of compound number 1950, 1951 and 1953-1959, performed as described in detail in the chapter "Materials and Methods". The compounds were tested in a concentration of 10 µM. Final DMSO concentration in all samples was 0.1%. Each bar represents the mean of 3 experiments. All values were normalized to the serum control. Results are shown as mean with standard error of the mean; DMSO control: untreated, unstimulated cells; serum control: untreated cells stimulated with NBS; n.s. not significant (one-way ANOVA/Tukey)

4.5 *Eupatorium odoratum* extracts and subfractions

The Eupatorium odoratum extract 1970 and the subfractions 1971-1981 were found to be inactive in a concentration of 10 µg/ml, like demonstrated in **figure 22**. Proliferative activity for cells treated with these samples ranged from 80% up to 127%.

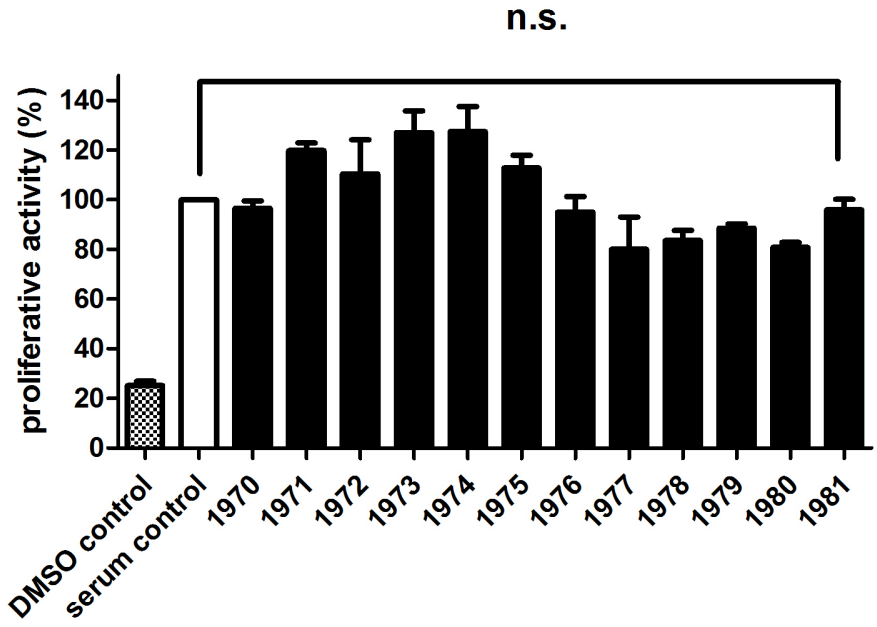

Figure 22: Proliferative activity of sample 1970-1981
Results obtained from the resazurin conversion assay of sample number 1970-1981, performed as described in detail in the chapter "Materials and Methods". The samples were tested in a concentration of 10 µg/ml. Final DMSO concentration in all samples was 0.1%. Each bar represents the mean of 3 experiments. All values were normalized to the serum control. Results are shown as mean with standard error of the mean; DMSO control: untreated, unstimulated cells; serum control: untreated cells stimulated with NBS; n.s. not significant (one-way ANOVA/Tukey)

4.6 Sesame extracts and pure compounds

Figure 23 and 24 show the results of the sesame pure compounds 1763 and 1764, tested in a concentration of 1, 5, 10 and 30 µM. Sample 1761 represents a methanolic extract of sesame seeds and sample 1762 is a diethyl ether fraction of the methanolic extract. These two extracts were tested in a concentration of 10 µg/ml.

Proliferative activity of cells treated with these samples was shown to range from 84% up to 104% on average. Consequently none of the compounds could influence serum-stimulated VSMC proliferation strong enough to be of special importance for further research.

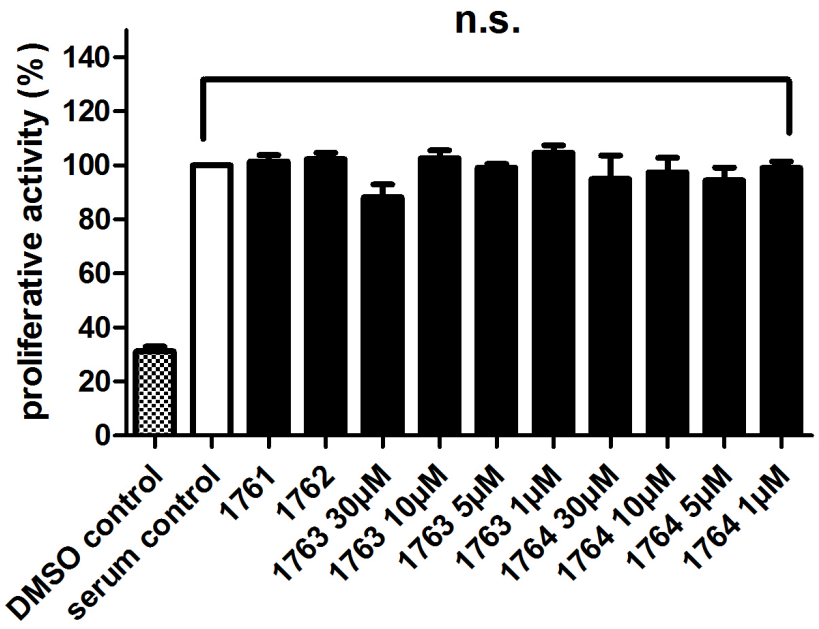

Figure 23: Proliferative activity of sample 1761-1764
Results obtained from the resazurin conversion assay of sample number 1761-1764, performed as described in detail in the chapter "Materials and Methods". Compound 1763 and 1764 were tested in a concentration of 30, 10, 5 and 1 µM, and the extracts 1761 and 1762 at 10 µg/ml.. Final DMSO concentration in all samples was 0.1%. Each bar represents the mean of 3 experiments. All values were normalized to the serum control. Results are shown as mean with standard error of the mean; DMSO control: untreated, unstimulated cells; serum control: untreated cells stimulated with NBS; n.s. not significant (one-way ANOVA/Tukey)

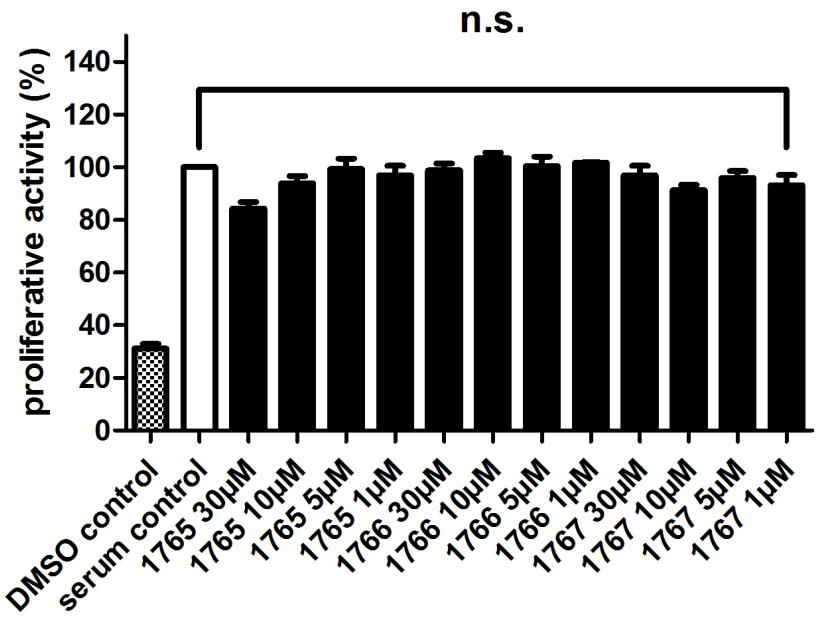

Figure 24: Proliferative activity of sample 1765-1767
Results obtained from the resazurin conversion assay of sample number 1765-1767, performed as described in detail in the chapter “Materials and Methods”. The samples were tested in a concentration of 30, 10, 5 and 1 µM.. Final DMSO concentration in all samples was 0.1%. Each bar represents the mean of 3 experiments. All values were normalized to the serum control. Results are shown as mean with standard error of the mean; DMSO control: untreated, unstimulated cells; serum control: untreated cells stimulated with NBS; n.s. not significant (one-way ANOVA/Tukey)

4.7 Lichen species extracts

The treatment of VSMCs with various lichen species extracts in a concentration of 10 µg/ml did not reveal any statistically significant effects on the cell growth (**figure 25 and 26**). Application of extract 2019 was demonstrated to result in the lowest proliferative activity of all lichen species extracts tested. However proliferative activity was with 81% still too high to make the extract interesting for advanced studies. The highest proliferative activity resulted from the application of extract 2006 with 124%.

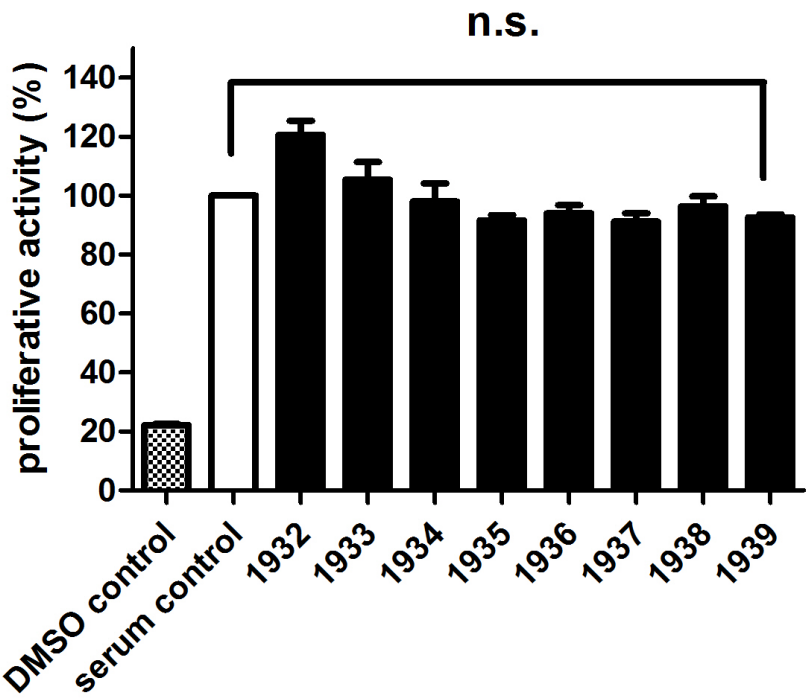

Figure 25: Proliferative activity of extract 1932-1939
Results obtained from the resazurin conversion assay of extract number 1932-1939, performed as described in detail in the chapter "Materials and Methods". The extracts were tested in a concentration of 10 µg/ml. Final DMSO concentration in all samples was 0.1%. Each bar represents the mean of 3 experiments. All values were normalized to the serum control. Results are shown as mean with standard error of the mean; DMSO control: untreated, unstimulated cells; serum control: untreated cells stimulated with NBS; n.s. not significant (one-way ANOVA/Tukey)

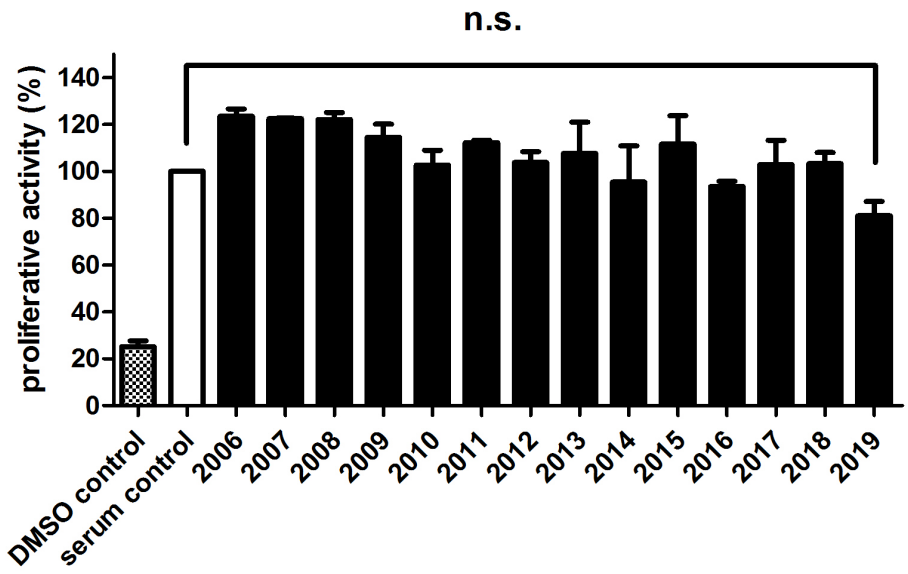

Figure 26: Proliferative activity of extract 2006-2019
Results obtained from the resazurin conversion assay of extract number 2006-2019, performed as described in detail in the chapter “Materials and Methods”. The extracts were tested in a concentration of 10 µg/ml. Final DMSO concentration in all samples was 0.1%. Each bar represents the mean of 3 experiments. All values were normalized to the serum control. Results are shown as mean with standard error of the mean; DMSO control: untreated, unstimulated cells; serum control: untreated cells stimulated with NBS; n.s. not significant (one-way ANOVA/Tukey)

4.8 Olive oil pure compounds

The following olive oil compounds were all tested in a concentration of 10 and 30 µM, except from all trans retinoic acid and 9-cis retinoic acid, which were only tested in a 10 µM concentration. The results of these tests, all of them not significant, can be seen in **figure 27**.

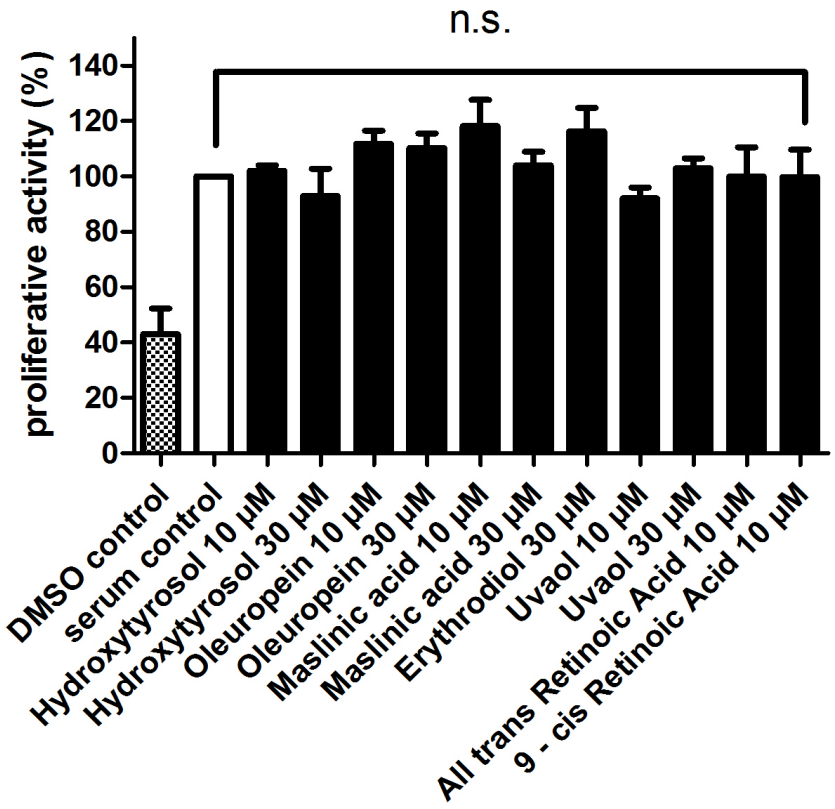

Figure 27: Proliferative activity of various olive oil compounds
Results obtained from the resazurin conversion assay of various olive oil compounds, performed as described in detail in the chapter "Materials and Methods". The compounds were all tested in a concentration of 10 µM and 30 µM. Final DMSO concentration in all samples was 0.1%. Each bar represents the mean of 3 experiments. All values were normalized to the serum control. Results are shown as mean with standard error of the mean; DMSO control: untreated, unstimulated cells; serum control: untreated cells stimulated with NBS; n.s. not significant (one-way ANOVA/Tukey)

Average proliferative activity of VSMCs treated with the olive oil compounds hydroxytyrosol, all trans retinoic acid and 9-cis retinoic acid all tested at 10 µM was nearly identical with the value obtained for the serum control (proliferative activity for

hydroxytyrosol 102 %, for all trans retinoic acid and for 9-cis retinoic acid 100 % on average). VSMCs treated with hydroxytyrosol at 30 µM and uvaol at 10 µM led to the lowest proliferative activity of the tested olive oil components with 93 % and 92 %, respectively on average. The application of maslinic acid at 10 µM (proliferative activity 118 % on average) and erythrodiol at 30 µM (proliferative activity 116 % on average) led to the highest proliferative activity of VSMCs, although only the value obtained for erythrodiol 10 µM was statistically significant, like shown in **figure 15**, chapter three. Proliferative activity of VSMCs treated with oleuropein in a 10 µM concentration was 112 % on average, whilst the application of a 30 µM concentration led to a proliferative activity of 110% on average. Treatment of cells with maslinic acid in a concentration of 30 µM resulted in an average proliferative activity of 104 %.

4.9 Various compounds declared to be antiproliferative in the literature

Although the following compounds are stated to carry out an antiproliferative effect on VSMCs in the literature, these findings could not be approved upon serum stimulation in the resazurin conversion assay, conducted in the context of this work (**figure 28**).

The application of compound T0901317, as well as parthenolide and IKK inhibitor IV did not result in any statistically significant effects (proliferative activity for T0901317 82%, for parthenolide 80% and for IKK inhibitor IV 72% on average). An application of oleocanthal resulted in the highest proliferative activity with 115% on average. Leoligin was tested in the three concentrations 0.1, 1 and 5 µM. None of these concentrations could reduce proliferative activity of VSMCs strong enough to be of statistical significance (Proliferative activity for 0.1 µM 94%, for 1 µM 76% and for 5 µM 80% on average). I3MO was tested in 5 µM and 10 µM concentration. Only the higher concentration was able to reduce proliferative activity of VSMCs significantly, like shown in **figure 10**, chapter one. Proliferative activity for VSMCs treated with the lower concentration was 80 % on average.

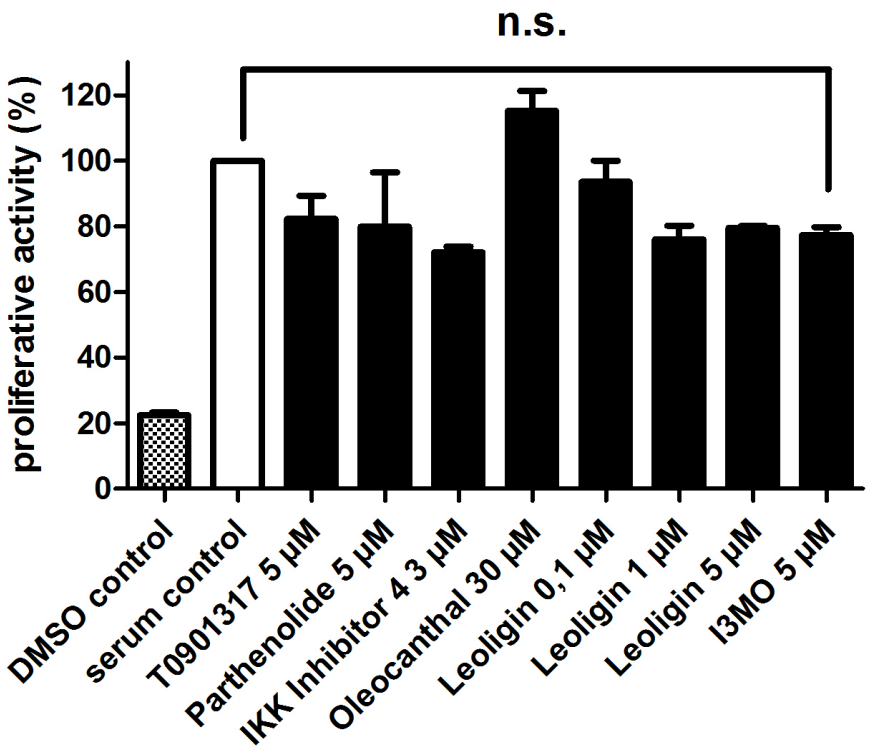

Figure 28: Proliferative activity of various compounds, declared to be antiproliferative in the literature

Results obtained from the resazurin conversion assay of various compounds, which should have an antiproliferative activity in conformity with the literature, performed as described in detail in the chapter “Materials and Methods”. Final DMSO concentration in all samples was 0.1%. Each bar represents the mean of 3 experiments. All values were normalized to the serum control. Results are shown as mean with standard error of the mea; DMSO control: untreated, unstimulated cells; serum control: untreated cells stimulated with NBS; n.s. not significant (one-way ANOVA/Tukey)

E) DISCUSSION

1. Compounds, which were found to influence VSMC proliferation

1.1 Cortisol and dexamethasone

Cortisol and dexamethasone are both stated to have antiproliferative properties on VSMCs. Hence glucocorticoids are currently clinically tested for their application as anti-restenotic agents.[75] The Dexamet™ stent, available from Abbott vascular devices is a stent, loaded with dexamethasone, that is invented to treat patients with an acute coronary syndrome.[91] Safety and efficacy of this drug eluting stent were demonstrated in the STRIDE trial. The result of this study is a binary restenosis rate of 13.3%.[92] The DESIRE study analyzes the outcomes of patients with non-ST-segment elevation acute coronary syndrome, 6 months after the implantation of a Dexamet™ stent. The primary endpoint, which is defined as death, MI and ischaemia-driven target vessel revascularisation, was reached in 11.5% of patients and angiographic restenosis rate was 33.3%, resembling that of a bare metal stent. Consequently although the risk for clinical events was rather low, the stent had no distinct anti-restenosis effect. This fact excludes a significant antiproliferative effect of the dexamethasone DES, which is justified by a too low concentration of dexamethasone or an inefficient release in this study. In addition to the use of corticosteroids in stents, they are also used as systemic immunosuppressors to prevent a restenosis after stent implantation.[91] However, it must be pointed out that the side effects of glucocorticoids include a raise in blood pressure, lipid levels and levels of various clotting factors.[75]

Corresponding with the literature that state glucocorticoids to act antiproliferative, this work revealed that cortisol and dexamethasone were both able to reduce the number of metabolic active cells with a significance level of $p<0.01$. VSMCs treated with cortisol in a concentration of 10 µM showed a proliferative activity of 54%, while the treatment of cells with dexamethasone in a 2.5 µM concentration resulted in a proliferative activity of 56% on average, compared to the serum control.

1.2 Indirubin-3´-monoxime

In vitro and *in vivo* studies with mice arteries could already prove the antiproliferative activity of I3MO on PDGF-BB stimulated VSMCs, by preventing cell cycle transition from G_0 phase to G_1 phase. The underlying mechanism of this action is the inhibition of signal transducer and activator of transcription-3 (STAT3) phosphorylation. Furthermore I3MO was demonstrated to reduce the formation of a neointima,[81] and it was shown to inhibit VSMC migration from the media into the intima.[93]

Within the context of this work utilizing serum instead of PDGF as a mitogenic stimulus, I3MO was tested at a 5 µM and a 10 µM concentration. Indeed the application of I3MO in a 10 µM concentration could significantly reduce the number of metabolic active VSMCs. Proliferative activity of serum-stimulated VSMCs was only 61% in comparison with the serum control (significance value $p<0.05$). However the application of the lower concentration did not reveal a significant effect maybe because of a too strong dilution.

1.3 *Semecarpus anacardium* extract

Semecarpus anacardium, also known as Bhilawa, is used in Ayurveda, the traditional Indian medicine. The active compounds of the plant are bhilwanol, jeediflavone, semecarpuflavone, gulluflavone, anacardoside, biflavanone and anacardic acid.[94] The plant is said to be active against liver cancer, urinary bladder cancer, oesopheagal cancer, breast cancer and chronic myeloid leukemia in different preparations. Furthermore the plant is known to be able to correct hypoglycaemia and to control an abnormal peroxidation of lipids.[95] Via an *in vitro* study, the impact of *Semecarpus anacardium* methanolic extract on African green monkey kidney Normal cell line (Vero) and Human Epidermoid Larynx Carcinoma cell line (Hep 2) was tested. It revealed a cytotoxic growth inhibition of larynx carcinoma cells, but it did not influence the healthy body cells, qualifying the plant for an antitumor agent.[94]

In accordance with this data, the testing of *Semecarpus anacardium* extract demonstrated a very strong antiproliferative effect on VSMCs. This effect was in high gear with only 14% of the cells still being able to conduct the reduction reaction of the indicator dye resazurin (significance value of $p<0.001$). An observation under

the light microscope showed that most of the cells had a morphology indicating possible death or apoptosis induction upon treatment with *Semecarpus anacardium* extract. However to definitely confirm this statement further research is necessary.
The application of cytotoxic agents represents a strategy to inhibit VSMC proliferation. A cytotoxic drug that has been used in drug eluting stents to prevent an in-stent restenosis is paclitaxel. However the utilization of cytotoxic agents shows considerable side effects, resulting in a destruction and death of the cells that may lead to a weakening of the vessel wall, making it prone for aneurysm formation. Consequently a cytostatic approach seems to be more adequate to inhibit VSMC proliferation, being able to block progression of the cell cycle without an induction of cell death.[12]

1.4 Baicalein

Baicalein was isolated from the plant *Oroxylum indicum*. This component is reported to act as a lipoxygenase inhibitor and as inhibitor of VSMC proliferation, through an inhibition of cell cycle progression. The underlying mechanism seems to be an inhibition of the G_1/S phase transition.[96]
Within the framework of this diploma work utilizing quantification of the metabolic activity of serum-stimulated VSMC, this literature finding could not be confirmed. Surprisingly the application of baicalein at a concentration of 10 µg/ml even led to a statistically significant increase in the number of metabolic active VSMCs. Proliferative activity was shown to be 158% on average, compared to sole serum stimulation with a significance value of $p<0.05$.

1.5 Erythrodiol

The consumption of olive oil is associated with a reduced mortality due to cardiovascular diseases. Olive oil components were shown to influence various processes associated with the development of atherosclerosis. They show anti-inflammatory properties and reduce endothelial activation. The mechanisms behind these actions are amongst others an inhibition of VCAM-1 and ICAM-1 expression, a reduction in monocyte adhesion and chemotaxis, an inhibition of the transcription

factor NF-κB, an antioxidant effect and a decreasing effect on LDL cholesterol levels. Feeding rats with a diet rich in olive oil results in a reduction of platelet hyperactivity and a delayed development of aortic thrombotic occlusions. Furthermore olive oil components are shown to decrease blood pressure, by leading to a vasorelaxation in rat aortas.[97]

Amongst all the olive oil components that were tested only the application of erythrodiol at 10 µM resulted in a significant effect. It led to an increase in the number of metabolic active vascular smooth muscle cells, which resulted in an increase in proliferative activity on average by 32%, compared to sole serum stimulation (significance value $p<0.05$).

2. Concluding remarks

Except from cortisol and dexamethsone, tested in a 10 µM and 2,5 µM concentration, respectively and indirubin-3´-monoxime tested at 10 µM, all the compounds, that were declared in the literature to exhibit an antiproliferative activity, did not show any corresponding effects in this work. This nonconformity with the literature`s declarations may be explained by the use of differential analysis methods and also with the use of diverse stimulating agents for VSMC proliferation.

A significant difference of this work if compared with other studies, also dealing with the identification of antiproliferative substances, is the use of diverse analysis methods. In plenty of these studies, VSMCs were stimulated to proliferate with the isolated growth factor PDGF, whilst in this work new-born bovine serum was used as stimulating agent. This reagent contains various growth factors, like PDGF, FGF, VEGF, EGF et cetera. As a result the substances, which were tested, needed to be able to inhibit multiple growth factors pathways at the same time, so that the overall result would be inhibition of cell proliferation. Consequently a direct comparison is not always possible. Nevertheless the use of new-born bovine serum represents an interesting experimental approach because it may be more adequate to imitate *in vivo* conditions because in the human organism, there are also multiple growth factors acting in parallel, that need to be inhibited by an effective treatment, and not only a single one.

A possible explanation for the contrary effects, received from the application of baicalein and erythrodiol, in comparison with the literature, may be that traces of

these substances, which may have rested in the wells after the wash step with buffer, could have reduced the indicator dye resazurin to resorufin, resulting in a high fluorescence activity.

Although the resazurin conversion assay is relatively cheap, rapid and easy to perform, however its big disadvantage is the fact that this assay is not able to differentiate between an antiproliferative effect and a cytotoxic effect because dead cells and metabolic inactive cells are both not able to conduct the reduction of the indicator dye. Thus a potential cytotoxic effect can only be visualized by observating the cells with a light microscope because cytotoxity is always accompanied by a characteristic change in VSMC morphology, which is visualized in **figure 14**. The application of substances, which show cytotoxic effects, involves the risk for severe side effects. Therefore the development of a method, which is able to differentiate between a cytotoxic and an antiproliferative effect, which may be for example based on the inhibition of growth factor signalling cascades or on an action at the level of the cell cycle, represents an interesting challenge. A promising approach to detect viable cells more reliably may be the use of CellTracker™ Green CMFDA. This reagent can pass through the membrane of cells. Inside the cells it undergoes an esterase reaction, leading to fluorescence and a reaction of the dye`s chloromethyl group with intracellular thiols generates a product, which is trapped inside the cells.[56] Consequently this method allows to detect the number of cells who have an intact cell membrane and thus are viable and healthy. Those cells with a destroyed barrier can`t trap the dye anymore and are not detected. To simultaneously measure and discriminate cell proliferation and cytotoxicity, very interesting direction for future studies might be to develop a combined assay quantifying the metabolic activity of the cells by resazurin conversion with membrane integrity quantification utilising CellTracker™ Green CMFDA.

In conclusion it must be noted that to verify all these results obtained from an application of the resazurin conversion assay, further research is necessary because results received from only one isolated assay are not enough convincing to make a definite statement if a sample is indeed able to influence the proliferation of VSMCs.

REFERENCES

1. Meens, M.JPMT., Pfenniger, A. & Kwak, B.R. Risky communication in atherosclerois and thrombosis formation. *Swiss Med Wkly* **142**, 1-10 (2012)
2. Kumar, V., Fausto, N. & Abbas, A.K. Robbins and cotran pathologic basic of disease. Saunders W.B. **Seventh Edition** (2004)
3. Doran, A.C., Meler, N. & McNamara, C.A. Role of Smooth muscle cells in the initiation and early progression of atherosclerosis. *Arterioscler Thromb Vasc Biol* **28**, 812-819 (2008)
4. Nakel, T. Diploma thesis, University of Vienna (2011)
5. Hansson, G.K. Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med* **352**, 1685-95 (2005)
6. Moore, K.J. & Tabas, I. Macrophages in the pathogenesis of atherosclerosis. *Cell* **145**, 341-355 (2011)
7. Tedgui, A. & Mallat, Z. Cytokines in atherosclerosis: pathogenic and regulatory pathways. *Physiol Rev* **86**, 515-581 (2006)
8. Glass, C.K. & Witztum, J.L. Atherosclerosis: the road ahead. *Cell* **104**, 503-516 (2001)
9. Lusis, A.J. Atherosclerosis. *Nature* **407**, 233-41 (2000)
10. Rudijanto, A. The role of vascular smooth muscle cells on the pathogenesis of atherosclerosis. *Acta Med Indones* **39(2)**, 86-92 (2007)
11. Topol, J.T. et.al. Textbook of cardiovascular medicine. Third Edition (2007)
12. Dzau, V.J., Braun-Dullaeus, R.C. & Sedding, D.G. Vascular Proliferation and Atherosclerosis: New perspectives and therapeutic strategies. *Nat Med* **8(11)** 1249-1256 (2002)
13. Jan, M.J. et al. Inflammatory and autoimmune reactions in atherosclerosis and vaccine design informatics. *Journal of Biomedicine and Biotechnology* 1-16 (2010)
14. Falk, E. Pathogenesis of Atherosclerosis. *Journal of the American College of Cardiology* **47(8)**, C7-C12 (2006)
15. Sitia, S. et al. From endothelial dysfunction to atherosclerosis. *Autoimmunity Reviews* **9**, 830-834 (2010)
16. Packard, R.S. & Libby, P. Inflammation in atherosclerosis: From vascular biology to biomarker discovery and risk prediction. *Clinical chemistry* **54(1)**, 24-38 (2008)
17. Hansson, G.K. & Libby, P. The immune response in atherosclerosis: A double edged sword. *Nature* **6**, 508-519 (2006)

18. Singh, R.B. Pathogenesis of atherosclerosis: A multifactorial process. *Exp Clin Cardiol* **7(1)**, 40-53 (2002)
19. Libby, P. Vascular biology of atherosclerosis: overview and state of the art. *Am J Cardiol* **91(suppl)**, 3A-6A (2003)
20. Tabas, I. Macrophage death and defective inflammation resolution in atherosclerosis. *Nat Rev Immunol* **10 (1)**, 36-46 (2010)
21. Fan, J. & Watanabe, T. Inflammatory reactions in the pathogenesis of atherosclerosis. *Journal of Atherosclerosis and Thrombosis* **10(2)**, 63-71 (2003)
22. Stary, H.C. A definition of initial, fatty streak, and intermediate lesions of atherosclerosis. *Circulation* **89**, 2462-2478 (1994)
23. Stary, H.C. Lipid and macrophage accumulation in arteries of children and the development of atherosclerosis. *American Journal of Clinical Nutrition* **72(5)**, 1297S-1306S (2000)
24. Stary, H.C. A definition of acvanced types of atherosclerotic lesions and a histological classification of atherosclerosis. *Circulation* **92**, 1355-1374 (1995)
25. Owens, G.K., Kumar, M.S. & Wamhoff, B.R. Molecular regulation of vascular smooth muscle cell differentiation in development and disease. *Physiol Rev* **84**, 767-801 (2004)
26. Rzucidlo, E.M. et al. Regulation of vascular smooth muscle cell differentiation. *J Vasc Surg* **45**, 25A-32A (2007)
27. Metz, R.P., Patterson, J.L. & Wilson, E. Vascular smooth muscle cells: isolation, culture, and characterisation. *Methods in Molecular Biology* **843**, 169-176 (2012)
28. Zargham, R. Preventing restenosis after angioplasty: A multistage approach. *Clinical Science* **114**, 257-264 (2008)
29. Libby, P., Ridker, P.M. & Hansson, G.K. Progress and challenges in translating the biology of atherosclerosis. *Nature* **473**, 317-325 (2011)
30. Yan, P. et al. Biological characteristics of foam cell formation in smooth muscle cells derived from bone marrow stem cells. *Int J Biol Sci* **7(7)**, 937-946 (2011)
31. Clarke, M.C.H. et al. Chronic Apoptosis of vascular smooth muscle cells accelerates atherosclerosis and promotes calcification and medial degeneration. *Circulation Research* **102**, 1529-1538 (2008)
32. Clarke, M. & Bennett, M. Defining the role of vascular smooth muscle cell apoptosis in atherosclerosis. *Cell Cycle* **5(20)**, 2329-2331 (2006)

33. Li, J.J. Inflammation in coronary artery diseases. *Chin Med J* **124(21)**, 3568-3575 (2011)
34. Clarke, M. & Bennett, M. The emerging role of vascular smooth muscle cell apoptosis in atherosclerosis and plaque stability. *Am J Nephrol* **26**, 531-535 (2006)
35. Gorenne, I. et al. Vascular smooth muscle cell senescence in atherosclerosis. *Cardiovascular Research* **72**, 9-17 (2006)
36. Stoneman, V.E.A. & Bennett, M.R. Role of apoptosis in atherosclerosis and its therapeutic implications. *Clinical science* **107**, 343-354 (2004)
37. Libby, P. et al. Inflammation in atherosclerosis: Transition from theory to practice. *Circ J* **74**, 213-220 (2010)
38. Newby, A.C. Metalloproteinases and vulnerable atherosclerotic plaques. *Trends Cardiovasc Med* **17(8)**, 253-258 (2007)
39. Hamid, H. & Coltart, J. 'Miracle stents' - a future without restenosis. *MJM* **10(2)**, 105-111 (2007)
40. Kraitzer, A. Kloog, Y. & Zilberman, M. Approaches for Prevention of Restenosis. *Journal of Biomedical Materials Research Part B: Applied Biomaterials* **85(2)**, 583-603 (2008)
41. Curcio, A., Torella, D. & Indolfi, C. Mechansims of smooth muscle cell proliferation and endothelial regeneration after vascular injury and stenting. *Circulation Journal* **75**, 1287-1296 (2011)
42. Fuster, J.J. et al. Control of cell proliferation in atherosclerosis: insights from animal models and human studies. *Cardiovascular Research* **86**, 254-264 (2010)
43. Czekanska, E.M. Assessment of cell proliferation with resazurin-based fluorescent dye. *Methods in Molecular Biology* **740**, 27-32 (2011)
44. Boyle, J.J. et.al. Human blood-derived macrophages induce apoptosis in human plaque-derived vascular smooth muscle cells by fas-ligand/fas interactions. *Arteriosclerosis, Thrombosis, and Vascular Biology* **21**, 1402-1407 (2001)
45. Schoenhagen, P. et.al. Coronary imaging: Angiography shows the stenosis, but IVUS, CT and MRI show the plaque. *Cleveland Clinic Journal of Medicine* **70(8)**, 713-719 (2003)
46. http://dc245.4shared.com/doc/t2GACyXa/preview.html
47. Krishnan, E. et.al. Hyperuricemia and the risk for subclinical coronary atherosclerosis-data from a prospective observational cohort study. *Arthritis Res Ther* **13(2),** R66 (2011)

48. Ter Avest, E., Stalenhoef, A.F.H. & De Graaf, J. What is the role of non-invasive measurements of atherosclerosis in individual cardiovascular risk prediction? *Clinical Science* **112**, 507-516 (2007)
49. Da Luz, P,L., Nishiyama, M. & Chagas, A.C.P. Drugs and lifestyle for the treatment and prevention of coronary artery disease - comparative analysis of the scientific basis. *Braz J Med Biol Res* **44(10)**, 973-991 (2011)
50. Vaughan, c.J. et.al. The evolving role of statins in the management of atherosclerosis. *Journal of the American College of Cardiology* **35(1)**, 1-10 (2000)
51. Hermann, A.G. & Moncada, S. Therapeutic potential of nitric oxide donors in the prevention and treatment of atherosclerosis. *European Heart Journal* **26**, 1945-1955 (2005)
52. Weber, C. & Noels, H. Atherosclerosis: current pathogenesis and therapeutic options. *Nature medicine* **17(11)**, 1410-1422 (2011)
53. Grote,K. Renin-angiotensin system and atherosclerosis. *Nephrol Dial Transplant* **19**,770-773 (2004)
54. Papademetriou, V. The potential role of AT_1-rececptor blockade in the prevention and reversal of atherosclerosis. *Journal of Human Hypertension* **16**, 34-41 (2002)
55. Yi,G. et.al. Effects of aspirin on atherosclerosis and the cyclooxygenase-2 expression in atherosclerotic rabbits. *Chinese medicinal Journal* **119(21)**, 1808-1814 (2006)
56. http://tools.invitrogen.com/content/sfs/manuals/mp02925.pdf
57. Kern, M.J. The interventional cardiac catheterization handbook. Mosby. Second edition (2004)
58. http://www.uibk.ac.at/pharmazie/pharmakognosie/dnti/vision/
59. Gokhale, M. & Bansal, Y.K. An avowal of importance of endangered tree Oroxylum indicum (Linn.) Vent. *Natural Product Radiance* **5(2)**, 112-114 (2005)
60. Mollik, A.H. et.al. A comparative analysis of medicinal plants used by folk medicinal healers in three districts of bangladesh and inquiry as to mode of selection of medicinal plants. *Ethnobotany Research and Applications* **8**, 195-218 (2010)
61. Hossan, S. et.al. Traditional use of medicinal plants in Bangladesh to treat urinary tract infections and sexually transmitted diseases. *Ethnobotany Research and applications* **8**, 61-74 (2010)

62. Mishra, S.I. et.al. In vitro antioxidant potential of different parts of oroxylum indicum: A comparative study. *Indian J Pharm Sci* **72(2)**, 267-269 (2010)
63. Chakraborty, A.K., Roy, H. & Bastia, S. Evaluation of antioxidant activity of the leaves of eupatorium odoratum linn. *International Journal of Pharmacy and Pharmaceutical Sciences* **2(4)**, 77-79 (2010)
64. Umukoro, S. & Ashorobi, R.B. Evaluation of the anti-inflammatory and membrane-stabilizing effects of eupatorium odoratum. *International Journal of Pharmacology* **2(5)**, 509-512 (2006)
65. Chopra, R.N. & Chopra, I.C. Chopra´s indigenous drugs of india. Third reprint (2006)
66. Aronson, J.K. & Meyler, L. Meyler´s side effects of herbal medicines (2008)
67. Tagarelli, G., Tagarelli, A. & Piro, A. Folk medicine used to heal malaria in calabria (southern Italy). *Journal of Ethnobiology and Ethnomedicine* **6(27)**, 1-16 (2010)
68. Bhaskaran, S. et al. Inhibition of atherosclerosis in low-density-lipoprotein receptor-negative mice by sesame oil. *Journal of Medicinal Food* **9(4)**, 487-490 (2006)
69. Chicca. A. et.al. Cytotoxic activity of polyacetylenes and polyenes isolated from roots of Echinacaea pallida. *British Journal of Pharmacology* **153,** 879—885 (2008)
70. Shah, S. et.al. Evaluation of echninacea for the prevention and treatment of the common cold: A meta- analysis*. Lancet Infect Dis* **7**, 473-80 (2007)
71. Zhai, Z. et.al. Enhancement of innate and adaptive immune functions by multiple echniacea species. *J Med Food* **10(3)**, 423-434 (2007)
72. Singh, D. et al. Immunomodulatory activity of semecarpus anacardium extract in mononuclear cells of normal individuals and rheumatoid arthritis patients. *Journal of Ethnopharmacology* **108**, 398-406 (2006)
73. Zanotti, I. et.al. The LXR agonist T0901317 promotes the reverse cholesterol transport from macrophages by increasing plasma efflux potential. *Journal of Lipid Research* **49**, 954-960 (2008)
74. Blaschke, F. Liver X receptor agonists suppress vascular smooth muscle cell proliferation and inhibit neointima formation in balloon-injured rat carotid arteries. *Circ Res*. **95**, e110-e123 (2004)
75. Low, L. Vascular lesion development: influence of endogenous and exogenous glucocorticoids. Phd thesis, the university of Edinburgh (2011)

76. Weng, S.X. et.al. Parthenolde inhibits proliferation of vascular smooth muscle cells through induction of G_0/G_1 phase cell cycle arrest. *Journal of Zhejiang University Science* **10(7)**, 528-535 (2009)
77. Franco, O.L. et.al. Parthenolide modulates the NF-κB-mediated inflammatory responses in experimental atherosclerosis. *Arteriosclerosis, Thrombosis, and Vascular Biology* **26**, 1864-1870 (2006)
78. Knippers, R. Molekulare Genetik. 9. Auflage (2006)
79. Reisinger, U. et.al. Leoligin, the major lignan from Edelweiss, inhibits intimal hyperplasia of venous bypass grafts. *Cardiovasc Res* **82(3)**, 542-549 (2009)
80. Duwensee, K. et.al. Leoligin, the major lignan from edelweiss, activates cholesteryl ester transfer protein. *Atherosclerosis* **219**, 109-115 (2011)
81. Schwaiberger, A. et.al. Indirubin-3´-monoxime blocks vascular smooth muscle cell proliferation by inhibition of signal transducer and activator of transcription 3 signaling and reduces neointima formation in vivo. *Arteriosclerosis, Thrombosis, and Vascular Biology* **30**, 2475-2481 (2010)
82. Tuck, K.L. & Hayball, P.J. Major phenolic compounds in olive oil: Metabolism and health effects. *The Journal of nutritional biochemistry* **13(11)**, 636-644 (2002)
83. Carluccio, M.A. et.al. Olive oil and red wine antioxidant polyphenols inhibit endothelial activation. *Arterioscler Thromb Vasc Biol* **23**, 622-629 (2003)
84. Masters, J.R. & Stacey, G.N. Changing medium and passaging cell lines. *Nature Protocols* **2(9)**, 2276-2284 (2007)
85. https://www.beckmancoulter.com/wsrportal/bibliography?docname=BR-9713B.pdf
86. http://cellbiology.med.unsw.edu.au/units/lab/cblmethod_elisa.htm
87. http://www.promega.com/~/media/Files/Resources/Protocols/Technical%20Bulletins/101/CellTiter-Blue%20Cell%20Viability%20Assay%20Protocol.pdf
88. http://www.sciencedirect.com/science/article/pii/S1046202307000096
89. MacBeath, J.R.E., Kielty,C.M. & Adrian, C. Type VIII collagen is a product of vascular smooth-muscle cells in development and disease. *The Biochemical J* **319(Pt 3)**, 993-998 (1996)
90. Okura, T. Platelet-derived growth factor induces apoptosis in vascular smooth muscle cells: roles of the Bcl-2 family. *Molecular Cell Research* **1403(3)**, 245-253 (1998)
91. Ribichini et.al. Steroid-eluting stents in patients with acute coronary syndrome: the dexamethasone eluting stent italian registry. *Heart* **93**, 598-600 (2007)

92. Daemen, J. & Serruys P.W. Drug eluting stent update 2007: Part I: A survey of current and future generation drug-eluting stents: Meaningful advances or more of the same? *Circulation* **116**, 316-328 (2007)
93. Czaloun, C. Diploma Thesis, University of Vienna (2010)
94. Patel, S.R., Suthar, A.P. & Patel, R.M. In vitro cytotoxicity activity of semecarpus anacardium extract against hep 2 cell line and vero cell line. *International Journal of Pharm Tech Research* **1(4)**, 1429-1433 (2009)
95. Balachandran, P. & Govindarajan, R. Cancer-an ayurvedic perspective. *Pharmacological Research* **51**, 19–30 (2005)
96. Peng, C.Y. et.al. Baicalein attenuates intimal hyperplasia after rat carotid balloon injury through arresting cell-cycle progression and inhibiting ERK, Akt, and NF-κB activity in vascular smooth-muscle cells. *Naunyn-Schmiedeberg`s Arch Pharmacol* **378**, 579‑588 (2008
97. Covas, M.I. Olive oil and the cardiovascular system. Pharmacological *Research* **55**, 175-186 (2007)

F) APPENDIX

1. Abbreviations

A

ACE	Angiotensin converting enzyme
Akt	Protein Kinase B
Apo-B	Apolipoprotein B
AP-1	Activator protein-1
AT_1 receptor	Angiotensin II receptor, type 1

B

Bcl-2	B-cell lymphoma-2

C

CABG	Coronary artery bypass graft
CASMC	Coronary artery smooth muscle cell
CCR2	Chemokine (C-C motif) receptor 2
CD	Cluster of differentiation
CDK	Cyclin-dependent kinase
CETP	Cholesteryl ester transfer protein
CKI	Cyclin-dependent kinase inhibitor
CMFDA	5-Chloromethylfluorescein diacetate
COX-2	Cyclooxygenase-2
CRP	C-reactive protein
CX3CL1	Chemokine ligand 1
CX3CR1	Chemokine receptor 1

D

DCM	Dichloromethane
DES	Drug eluting stent
DMEM	Dulbecco´s modified eagle medium
DMSO	Dimethyl sulfoxide
DNTI	Drugs from nature targeting Inflammation

DPPH	2,2-diphenyl-1-picrylhydrazyl

E

EC	Endothelial cells
ECM	Extracellular matrix
EDTA	Ethylendiaminetetraacetic acid
EGF	Epidermal growth factor
ET-1	Endothelin-1
E2F	E2 promoter binding factor

F

FGF	Fibroblast growth factor

H

HDL	High density lipoprotein
HMG CoA	3-Hydroxy-3-methylglutaryl-coenzyme-A

I

ICAM-1	Intracellular adhesion molecule-1
IFN-γ	Interferon-γ
IKK	IκB-kinase
IL	Interleukin
iNOS	Inducible nitric oxide synthase
IVUS	Intravascular ultrasound
I3MO	Indirubin-3´-monoxime

L

LDL	Low density liproprotein
LXR	Liver X receptor

M

MAC-1	Macrophage-1 antigen

MAPK	Mitogen-activated protein kinase
MCP-1	Monocyte chemotactic protein
M-CSF	Macrophage colony-stimulating factor
MeOH	Methanol
MI	Myocardial infarction
MLC-1	Myosin light chain 1
MMP	Matrix metalloproteinase
MPF	Mitosis-promoting factor

N

NBS	New-born bovine serum
NF-κB	Nuclear factor 'kappa-light-chain-enhancer' of activated B-cells
NK cells	Natural killer cells
NO	Nitric oxide

O

oxLDL	Oxidized low density lipoprotein

P

PAI-1	Plasminogen activator inhibitor-1
PBS	Phosphate buffered saline
PCI	Percutaneous coronary intervention
PCNA	Proliferating-cell-nuclear-antigen
PDGF	Platelet derived growth factor
PPARγ	Peroxisome proliferator-activated receptor
P53	Protein 53

R

ROS	Reactive oxygen species

S

SAβG	Senescence-associated β-galactosidase
SMC	Smooth muscle cell
SRA	Scavenger receptor A
SR-PSOX	Scavenger receptor that binds phosphatidylserin and oxidized lipoprotein
STAT-3	Signal transducer and activator of transcription-3

T

TF	Tissue factor
TGF-β	Transforming growth factor-β
TNF	Tumor necrosis factor
TVM	Traditional vietnamese medicine

V

VCAM-1	Vascular cell adhesion molecule-1
VEGF	Vascular endothelial growth factor
VLA-4	Very late antigen-4
VLDL	Very low density lipoprotein
VSMC	Vascular smooth muscle cell

2. Curriculum vitae

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