Heparin and its derivatives in the treatment of arterial thrombosis: a review

M. Dvorak[1], M. Vlasin[2], M. Dvorakova[3], P. Rauser[2], L. Lexmaulova[2], Z. Gregor[1], R. Staffa[1]

[1]2nd Department of Surgery, St. Anne's University Hospital, Faculty of Medicine, Masaryk University, Brno, Czech Republic
[2]Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic
[3]Department of Internal Medicine, Hematology and Oncology, University Hospital Brno and Faculty of Medicine, Masaryk University, Brno, Czech Republic

ABSTRACT: Arterial occlusion due to thrombosis caused by ruptured atherosclerotic plaques (Baba et al., 1975) has been recognized as a major cause of morbidity and mortality in western populations. Thrombosis may occur in various sections of arterial circulation, peripheral arteries of the limbs, coronary arteries, brain arteries, or both major and minor vessels within the abdominal cavity. The ultimate consequence is varying degrees of organ failure, mostly of ischemic origin. Arterial thrombosis represents a continuous problem, debilitating patients and decreasing their quality of life. Moreover, along with chronic heart failure, it can significantly decrease patient life expectancy. Arterial thrombosis results in ischemia, with serious systemic consequences, such as metabolic breakdown. The major goal of treatment remains fast and efficient recanalization – surgical, interventional or thrombolytic. To be able to prevent acute reocclusion with severe consequences (rhabdomyolysis, compartment syndrome, excessive tissue necrosis leading to limb amputation, etc.), several adjunctive treatment regimens have been advocated. Among others, thrombin inhibitors and platelet inhibitors have been widely used for both prophylaxis and adjunctive treatment. Direct thrombin inhibitors and antithrombin stimulators have been recognized as typical antithrombotic drugs. Direct (antithrombin-independent) thrombin inhibitors can be divided into two main categories: monovalent, active site inhibitors (argatroban, efegatran, inovastan, melagatran) and bivalent (hirudin, hirugen, hirulog, bivalirudin), while antithrombin stimulators represent standard (unfractionated) heparin (UFH) and its depolymerizing products – low molecular weight heparins (LMWH's). Recently, a clear change in the main use of heparin, as well as low-molecular weight heparins has been advocated representing a shift from treatment and prophylaxis of deep vein thrombosis to prophylaxis of thromboembolic disease following vascular, cardiovascular or orthopedic surgery, treatment of unstable angina and prevention of acute myocardial infarction. The main effect of heparins lies in their anticoagulant activity. Heparins are involved in different pathways of the coagulation cascade with anticoagulant, antithrombotic, profibrinolytic, anti-aggregative, as well as anti-inflammatory effects. Moreover, there is a little doubt about their anti-proliferative and anti-ischemic activity (Penka and Bulikova, 2006). Unlike standard heparin, low-molecular weight heparins do not affect the patient's general coagulation profile. Obviously, the difference in molecular weight results in different pharmacokinetic and pharmacodynamic properties of the agents.

Key words: coagulation; arterial thrombosis; standard heparin; low-molecular weight heparins

List of abbreviations

ABI = axillary/...index, **t-PA** = tissue-type plasminogen activator, **ADP** = adenosin diphosphate, **AG** = angiography, **AMI** = acute myocardial infarction, **APSAC** = acylated plasminogen streptokinase activator complex, **APTT** = activated partial thromboplastin time, **AS** = acute stroke, **AT** = antithrombin, **ATD** = acute thromboembolic disease, **CNS** = central nervous system, **DNA** = deoxyribonucleic acid, **DSA** = digital subtractional angiography, **DVT** = deep vein thrombosis, **FDP's** = fibrin – fibrinogen degradation products, **HCII** = heparin cofactor II, **HIT** =

heparin-induced thrombocytopenia, **IHF** = ischemic heart failure, **LDL** = low density lipoproteins, **LLID** = lower limb ischemic disease, **LMWH** = low-molecular weight heparin, **LMWH's** = low-molecular weight heparins, **MRA** = magnetic resonance angiography, **PAT** = percutaneous aspiration thrombectomy, **PDGF** = platelet-derived growth factor, **PTA** = percutaneous transluminal angioplasty, **PTCA** = percutaneous transluminal coronary angioplasty, **SCu-PA** = single chain urokinase-type plasminogen activator, **TFPI** = tissue factor pathway inhibitor, **UFH** = unfractionated heparin, **USG** = ultrasonography, **VSMC's** = vascular smooth muscle cells

Contents

1. Atherosclerosis as the main cause of thrombosis

Occlusion of peripheral vessels is in more than 90% of cases caused by atherosclerosis leading to thrombosis. Chronic vasculitis, small aneurysms, acute embolic disease, or external compression of arteries is observed less frequently. Atherosclerosis can affect arteries of the lower limbs, carotid arteries, brain arteries and, last but not least, coronary arteries (Ferrieres et al., 2006). On the other hand, upper limb arteries are only affected in extremely rare cases.

The development of atherosclerosis is rather long and without clinical signs. Atherosclerotic plaques can subsequently obstruct the blood stream within the vessel by narrowing its diameter. According to the vessels affected, clinical consequences may include: ischemic heart failure (IHF), ischemic stroke (IS), or peripheral thromboembolic disease, most frequently lower limb ischemic disease (LLID), carotid and/or renal artery occlusion.

Atherosclerosis can be defined as a degenerative process, typically with inflammatory infiltration of the vessel wall, accumulation of triglycerides and proliferation of fibrotic tissue. The early stage of atherosclerosis is more or less characterized by penetration of atherogenic lipoproteins and inflammatory cells through the endothelial layer and their accumulation in the sub-endothelial space. Later on, fibro-proliferative and degenerative processes take place, as a reaction to high levels of lipoproteins and inflammatory mediators. The development of atherosclerotic plaques currently depends on the effect of atherogenic lipoproteins in combination with endothelial damage and local inflammatory process.

Based on pathophysiology, three main stages of atherosclerosis are described: (1) early lesions with fatty stripes, (2) fibrotic and atherosclerotic plaques, (3) stage of complication development (Hansson et al., 2002). To be able to control atherosclerosis, one must pay due attention to so called "risk factors". Risk factors are recognized as reducible and non-reducible. To reducible risk factors belong insufficient physical activity, smoking (Lekakis et al., 1997), arterial hypertension (Rizzoni et al., 1998), hyperglycemia, high concentrations of non-enzymatic protein glycation products (Meeking et al., 1999), hyperhomocysteinemia, central-type obesity and, most of all, high concentrations of atherogenous low-density lipoprotein (LDL) particles (Creager et al., 1990). As non-reducible risk factors are classified: genetic factors (family history of ischemic heart failure – IHF, or other manifestation of atherosclerosis in a close relative), gender (males are at a higher risk) and age (higher risk in males over 45 and females over 55).

The therapeutic plan for atherosclerosis should take into account several factors and requires close cooperation with the patient. It begins with change in a lifestyle (smoking habits, physical activity, diet – low fat, reducing cholesterol and sodium chloride, high fibre intake). However, these adjustments are rarely by themselves sufficient. At the point of clinical manifestation of the disease, it is often necessary to commence medical treatment (Kikano and Brown, 2007). To reduce lipoproteins, hypolipidemic drugs affecting both cholesterol and triacylglycerol are widely recommended. Drugs which reduce hypertension and drugs which control diabetes mellitus can be used at the same time.

2. Arterial thrombosis of lower extremities

2.1. Definition and pathogenesis of arterial thrombosis

In organisms, there is normally a balance between coagulation and fibrinolysis. This very unstable balance is continuously maintained by the action of various enzymes, activators and inhibitors, as well as a rather complicated interaction involving cross-links and feedback systems between these substances. Damage to the endothelial layer of the vessel wall of peripheral arteries is widely considered as promoting an upsetting of this balance in favour of thrombosis activation.

Acute arterial thrombosis can be defined as sudden impairment in the perfusion of legs or acute exacerbation of pre-existing chronic ischemia of the legs, typically manifested by severe pain, paresthesia, as well as motor deficiency of various degrees, depending on extent and localization of arterial occlusion.

As mentioned before, the most frequent (85% to 95%) cause of arterial occlusion is atherosclerosis and its direct consequences, mainly acute thrombosis (Puchmayer and Roztocil, 2000). Typically, affected patients have recently undergone a peripheral vascular bypass. In clinical practice there is a distinction between prosthetic and venous bypasses; in a prosthetic bypass there is a higher probability of platelet-rich thrombus than in venous grafts. In the rest of cases, there is some other cause of occlusion, and occasionally the origin remains unknown (Puchmayer and Roztocil, 2000). Typically, an occlusion is localized to a superficial artery of the thigh and to crural arteries which are predominantly affected. Early on, the occlusion of a peripheral vascular bypass is limited to a period of up to one month following surgery, as it is caused mainly by a technical error or incorrect indication of the procedure, while an occlusion of between one and 24 months is considered as the most frequent and is caused mostly by neointimal hyperplasia. Late occlusions, occuring later than 24 months following surgery are caused most likely by progression of atherosclerosis in the site of either proximal or distal anastomosis (Whittemore et al., 1981).

Differential diagnosis of arterial embolization is sometimes tricky. As a useful tool we can apply information provided in Table 1. Quick onset and severe manifestation is typical for acute occlusion, while thrombosis is known for its slow start, discrete clinical symptomatology and more than one site of stenosis (Schuman et al., 2007). The ratio between acute occlusion (embolization) and thrombotic occlusion of peripheral arteries has been reported to be approximately 4 : 1 (Diehm et al., 2004).

Arterial thrombosis is a common cause of ischemia in lower limbs. The triggering mechanism of that ischemia is most likely a lack of energetic substrates in an environment of inadequate oxygen supply and subsequent shift to anaerobic metabolism. The speed and extent of the ensuing cellular damage strongly depends upon the discrepancy between oxygen requirements and its actual availability. Since different tissues have different metabolic activities (differing oxygen and energy consumption),

Table 1. The differences in clinical manifestation of acute embolization and thrombosis

	Thrombosis	Embolization
Onset	slow/subsequent (quick)	quick/immediate
Pre-existing symptoms	often	rarely
Duration	long	short
Atrial fibrilation, heart disease	irregularly	frequently
Other leg involvement	often	rarely
Ischaemia	whole leg	often segments
Therapy	Inciting cause w. thrombectomy	embolectomy
Risk of amputation	high	low
Long-term treatment	anti-aggregative (anticoagulant)	anticoagulant

the time period for developing cellular damage is different throughout the body.

The most ischemia-resistant part of limb is skins along with subcutaneous tissue. On the other hand, peripheral nerves are the most sensitive and ischemia-prone structures. That is why functional neurologic deficiency fades away very slowly even after successful revascularization and reperfusion. The exact timeframe for developing damaging ischemia and subsequent necrosis of the limb is hard to establish, as it depends on many factors. The thrombus accretion may proceed into side branches and eventually occlude collateral blood flow, increasing overall ischemic damage of the tissue. Delayed treatment for whatever reason may result in life-threatening situations, mainly due to metabolic breakdown during rhabdomyolysis and compartment syndrome development. These disorders should be regarded as emergencies and must be dealt with properly without any delay, even in the case of slower sub-acute development. Cases of peripheral thrombosis also benefit from early recognition and onset of therapy.

2.2. Etiology of an acute arterial thrombosis, Virchow's triad

The result of the thrombotic process is lies in the development of a highly organized mass of blood cells, such as platelets, red blood cells, white blood cells and other elements caught in a mesh of cross-linked fibrin. The predisposition factors leading to thrombus development are summarized in the so-called Virchow triad. The basics of Virchow's triad are the following: Endothelial damage – in arterial circulation there are three main causes of endothelial damage; the first is due to physiological hemodynamic stress during systolic inflation of the vessel and immediate diastolic deflation – the elasticity of the vessel puts a high demand on endothelial cells that kind wear down after a period of time. An increase in systemic blood pressure increases the hemodynamic stress on endothelial cells; the second cause of endothelial damage is atherosclerosis and the third one is direct trauma. Changes in blood flow – the most frequent changes observed are stasis and turbulence within the vessel (switch from a laminar flow to a turbulent one). Normally (laminar flow), there is a little or no contact between the endothelial membrane and circulating blood cells. However, a change in the charge of elements may play a role in the establishment of a continuous pathological insult. Positive charging of the endothelial surface starts to attract platelets and the so called opsonization of the endothelium commences. A decrease in blood flow may have two main causes: heart failure and increase in blood viscosity. Activation of coagulation is actually the least frequent cause of thrombosis. It starts due to a prothrombotic or thrombophilic condition of the patient (see chapter 2.2.1.). Moreover, systemic coagulation is activated following excessive burn injuries, heart failure, disseminated metastatic disease and long term estrogen medication, either pre-menopausal or in oral contraceptives (Vacha, 1999).

2.2.1. Thrombophilia

The enzymatic system of coagulation is generally under the control of a wide range of regulatory

mechanisms. Disturbing the rather unstable fluido-coagulation balance in one direction enhances the risk of systemic bleeding, while in the other direction, thrombosis occurs. The main reason for this may lie in the thrombophilic condition of the patient. The term "thrombophilia" suggests congenital or acquired coagulation disorders, associated with a higher risk of thrombosis development. Patients suffering from thrombophilia, congenital or acquired, are prone to first experiencing a thromboembolic attack; however, there is no direct evidence supporting a higher risk in such patients for the recurrence of thromboembolic disease. More thrombophilia cases are linked to deep vein thrombosis rather than to arterial thrombosis (Poul, 2006). However, according to the literature (Kamphuisen et al., 2000), factor V Leiden, as well as hyperhomocysteinemia affect both arterial and venous thrombosis in the same manner. Moreover, some acquired conditions, like antiphospholipidic syndrome or heparin-induced thrombocytopenia are associated with both arterial and venous thrombosis (Certik, 2003).

In thrombosis prevention, certain provoking factors and specific risk factors should be researched and dealt with specifically. This means a thorough review of the family history followed by a throrough physical examination of each at risk patient. We choose our patients based on criteria established recently (Poul, 2006). On the other hand, the likelihood of detecting thrombophilia increases in patients suffering from idiopathic thromboembolic attacks before 45 years of age, patients suffering from recurrent thromboembolia, those with thrombosis in an uncommon location, patients with arterial thrombosis before the age of 35 (Tanis et al., 2003), those with a family history of thromboembolic disease and women suffering from recurrent complications during pregnancy (Yamada et al., 2001; Pauer et al., 2003).

Knowledge of the thromboembolic condition of the patient enables modification of the dose and period of thromboprophylaxis, as well as helping in avoiding complications in women taking hormonal contraceptives, pre-menopausal estrogen supplementation and pregnant women or those planning a pregnancy (Jorgensen et al., 2002).

2.3. Clinical observations

Acute thromboembolic disease of the lower limbs can result in critical ischemia of a whole leg. Symptoms of such ischemia can be expressed by "6P": pain, paleness, lack of pulse, paresthesia, paralysis and prostration (Puchmayer and Roztocil, 2000). Secondary thrombosis mostly does not develop in such dramatic conditions as the severity of clinical symptoms depends on the level and capacity of collateral circulation.

Pain – starts suddenly, often in the area of acute occlusion of the vessel. It can progress as a more diffuse experience, throughout peripheral muscles. Sudden severe pain affects approximately 80% of patients, and its onset usually denotes the time of occlusion. In 10% of cases the pain is not so profound and in the remaining 10% there is no pain at all. This is probably due to paralysis and loss of perception.

Paleness – appears almost immediately, but after a period of time it is replaced by spotty cyanosis, caused by de-oxygenized blood focal accumulation. Changes in skin colour start usually 20 cm below the site of occlusion (collateral circulation can feed the upper portions in most cases). Basically, we recognize two types of ischemia: (1) pale and (2) blue ischemia.

Pale ischemia often reveals only little or no damage in acral vessels, including capillaries and in the venular system, which enables at least a limited perfusion of the affected area.

Blue ischemia is characterized by blue and pale spots throughout the affected area. The prognosis for this condition is poor, as tissue necrosis often follows.

Lack of pulse is another clear-cut symptom for the clinician to look for.

Paresthesia is mostly expressed as local skin hyperesthesia, followed by complete anaesthesia after some period of time. This change may be slow, irregular and may develop only subsequently, as some sensory neurons are less sensitive to oxygen deficiency than others.

Paralysis – what we observe is not usually regular paralysis with a neurological origin. It is rather muscular rigidity due to a low supply of energy (temporary or permanent ADP deficiency) and progressive local metabolic acidosis. Typically, both superficial and deep sensitivity is affected in various degrees. The onset of paralysis may denote gangrene. Whenever paralysis lasts for 12 hours or longer, the prognosis for saving the limb is rather poor. Stiff oedema throughout the gastrocnemius muscle, along with severe pain is commonly associated with muscular necrosis, as well as possibly other irreversible damage. While skin and

Table 2. Demarcation line

Location of arterial occlusion	Demarcation line
Sub-renal aorta	mid-abdominal region
Aortic bifurcation and common iliac arteries	groin
External iliac arteries	proximal femoral region
Femoral arteries	upper third of femoral region
Superficial femoral arteries	upper shank, just below the knee
Popliteal arteries	lower third of the shank

subcutaneous tissues are generally more resistant to progressive ischemia, nerves and muscles can bcome necrotic after four to six hours.

Prostration mostly does not appear as a result of shock. Physical exhaustion observed in patients suffering from thromboembolic disease is caused by vagal reflexes, initiating nausea, weakness and seizures, and sometimes even acute collapse.

The demarcation of ischemic changes marks out the location of an acute occlusion. As there is always some sort of collateral circulation feeding the tissue immediately below the actual occlusion, the demarcation line appears as outlined in Table 2.

Establishment of collateral circulation restores local temperature and results in the disappearance of paresthesia. In cases of severe damage, 100% restitution may never happen and some degree of deficiency usually persists. After the capacity of collateral perfusion reaches its maximum, symptoms are mostly the same as for chronic ischemia. Once early muscular rigidity, followed by spotty oedema and/or ulceration with crepitation appears, the prognosis is generally poor.

2.4. Diagnostic protocol for patients with suspected acute peripheral thrombosis

Primary data obtained from a patient should be based on individual history and major symptoms. Physical examination and primary vascular examination is still widely regarded as an important first step for establishing proper and early diagnosis. Inspection of colour, followed by close monitoring of any skin efflorescence, as well as drop in local temperature may help in the exact localization of vascular occlusions. Peripheral pulse palpation is another method of targeting the source of the problem. Auscultation may aid in finding murmurs, especially in the upper femoral region.

As another step, methods of diagnostic imaging should be utilized, not only for exact localization, but also for assistance in deciding upon treatment options. Basic ultrasonography, using a two-dimensional picture along with Doppler Effect measurement is currently used in clinical practice. Angiography is another method that can be used. Digital subtractional contrast angiography (DSA) is widely regarded as the "gold standard" for thorough examination of vascular damage. It enables exact visualization of the inner surface of the vessel and can outline both major and minor irregularities of the vessel wall. Magnetic resonance angiography (MRA) and CT-guided contrast angiography are other methods of choice for vascular imaging of lower extremities. Both methods are non-invasive and use contrast media for good visualization of arterial circulation with extremely high resolution. With the development of new workstations for CT scanning and magnetic resonance imaging, along with duplex sonography, these methods have become more and more useful as first-choice options in vascular diagnostic imaging (Schumann et al., 2007).

Sometimes, the justification for specific advanced diagnostic methods becomes an issue. An exact indication should come after detailed history was obtained and thorough physical examination performed. In most cases, no specific rules or guidelines exist and the steps to be followed are case-specific and depend, more or less, on the possibilities of the actual diagnostic imaging department. Moreover, many methods of imaging are complimentary and their utilization often helps in establishing a proper diagnosis. In any case, the critical consideration must always be to avoid any delay, especially in cases when there is a risk of limb amputation or exitus. In patients suffering from excessive damage or gangrene (or both) there is no need for in-depth examination and life-saving

(or limb-saving) procedures should follow. In such cases, immediate MRA or angiography should be performed without any delay. Generally, the most accurate information comes from MRA or digital subtractional angiography and all patients, especially those scheduled for surgical revascularization should undergo one of these treatments.

2.5. Treatment options for acute peripheral thrombosis

Treatment of thrombosis ought to be fast, aggressive and well targeted to be efficient. The goal is to re-establish adequate circulation and perfusion as fast as possible. This enables the saving of a limb in young patients and likely can save a life in older ones (Jivegard et al., 1988). Sufficient revascularization is the ultimate goal, depending on many factors. First of all, it requires good cooperation between the primary care provider and many specialists of secondary care, at least the radiologist and vascular surgeon or interventional specialist. The primary care physician must recognize the pathological condition and refer the patient to the specialist as soon as possible (Blacher et al., 2006). Specialists should focus on establishing a proper diagnosis and deciding on an appropriate treatment (Rice and Lumsden, 2006). A crucial point seems to be a good interpretation of the angiography, followed by thorough assessment of the degree of ischemic damage in the affected area, not forgetting the general physical condition of the patient, including all other disorders he/she suffers, with special emphasis on cardiovascular and cerebral conditions (McNamara and Gardner, 1991). All contraindications for thrombolysis should be seriously regarded, namely a cerebral stroke occurring less than six months ago, severe arterial hypertension, acute gastroduodenal ulcers, hemorrhagic diathesis, neoplasia, trauma less than 14 days ago, major surgery less than four weeks ago and arterial puncture in the groin less than seven days ago. Another condition which requires attention is thrombus formation within the left heart; this can cause embolization at any time, and at any place in systemic circulation.

We have three basic methods for revascularization: (1) surgical or invasive revascularization, (2) interventional endovascular (endoluminal) angioplasty, and (3) systemic intravenous thrombolysis. Also, percutaneous transluminal angioplasty (PTA) has been successfully applied along with local thrombolysis.

As mentioned, percutaneous transluminal angioplasty (PTA) or percutaneous aspirational thrombectomy (PAT) in combination with local or systemic thrombolysis has been advocated as the most successful method for arterial recanalization. Sometimes, interventional methods are abandoned and a surgical approach is used instead (Rocek, 2005). A very important step in the protocol is the assessment of the exact location of the thrombosis, as this affects the decision making for the basic therapeutic plan. For example, it is better to treat Aorto-femoral segments surgically (Weaver et al., 1996), while subinguinal areas are more feasible for treatment by angioplasty, thrombolysis, or by a combination of both (Weaver et al., 1996).

In order to choose the proper method, a basic scoring according to the Society of Vascular Surgery/International Society of Cardiovascular Surgery – SVS/ISCVS has been developed (Ahn et al., 1997; Rutherford et al.,1997):

Grade 1 – patients not in direct jeopardy. Patients not experiencing continuous pain. Extremity is pale, substantially colder, senso-motor functions remain. Ankle Brachial compressive index (ABI) ≥ 0.3. Mostly in patients with thrombosis of already atherosclerotic vessels.

Grade 2 – patients in jeopardy. Extremity is becoming cyanotic, senso-motor functions are impaired. Patients without persistent pain are in Group 2a, patients experiencing persistent pain are classified into Grade 2b. ABI index is < 0.3, or Doppler signal is completely missing.

Grade 3 – patients with irreversible changes. Patients experiencing severe pain, with muscular rigidity and subsequent anaesthesia of affected extremity. Doppler signal, both arterial and venous is missing. Biochemical analysis reveals muscular necrosis.

Patients from Group 1 do not need to be treated urgently, while Grade 2a patients require immediate action and those from Grade 2b group should undergo acute revascularization without any delay. Grade 3 patients should not undergo thrombolysis.

2.5.1. Surgical revascularization

After referral it is up to the vascular surgeon to make the decision regarding what kind of treatment

is to be utilized. This decision should be based on the localization of the problem, severity of tissue damage and some potential risk factors, such as other disorders ruling out general anesthesia or more invasive methods for the patient. Generally, there are few surgical procedures available:

Fogarty thromboembolectomy – this method had been regarded as a "gold standard" until recently. The key point is introducing a specially equipped balloon catheter through a arterectomy beyond the site of occlusion, then inflation of balloon by liquid (Ringer or saline solution); the obstructive thrombus is subsequently removed by gentle traction. Even though this method is highly efficient, we have to point out several disadvantages – unlike thrombolysis, this procedure requires local or general anaesthesia and we can remove only a fresh clot, not those adhering to the vessel wall. There is no direct control during the procedure; the control angiography is performed afterwards. This may lead to late recognition of vascular damage, pseudo-aneurysm formation or compartment syndrome development. With regard to late complications, neointimal hyperplasia and vascular smooth muscle cells proliferation are also possible. In these instances, clinically relevant narrowing of the vessel develops within six months following intervention (Karetova et al., 2007). After Fogarty thromboembolectomy, the formerly atherosclerotic plaque remains intact and must be removed by angioplasty or bypassed by vascular graft. Surgical bypass – bypass feasibility depends on anatomical location of the vascular defect (aorto-iliac, femoral, popliteal, crural), type of vascular graft (biological, artificial) and on the quality of the blood stream above and below the affected area. Vascular grafts have been observed to be more efficacious when an anastomosis is in the area where the side-branch of the main vessel originates. The proper choice of vascular graft is also a very important issue. Artificial grafts have been successfully used in the aorto-iliac area, revealing a five year patency as high as 90%. On the other hand, in the femoral area and below, better results have been observed after the use of biological (venous) grafts (68% of 5-year patency for venous graft versus 38% for artificial vessel above the knee, while 50% of 5-year patency for venous graft versus 12% for artificial vessel in the shank). This is the main reason behind the general recommendation of biological grafts for lower extremity bypasses (Boccalandro and Smalling, 2006). Another important factor is the quality of the blood stream at the distal part of the affected area. In patients with other disorders that may have an impact on distal blood flow, both the feasibility and success rate of surgical revascularization is rather limited. An adjunctive anticoagulant therapy, using platelet inhibitors (ASA, clopidogrel) or oral "blood thinners" (warfarin) is mandatory. Some other factors, such as dietary lipids, carbohydrates, smoking and high systemic blood pressure also may play a role. Revascularization in a moribund patient with clearly irreversible ischemic changes should not be attempted. These cases should be solved by immediate amputation to avoid systemic reperfusion injury by free radicals of oxygen and highly acidotic and hyperkalemic venous blood.

2.5.2. Interventional endovascular procedures

Percutaneous interventional angioplasty has become a widely used procedure during the last two decades. Now, all patients suffering from more systemic disorders (all geriatric patients, typically) may profit from this minimally invasive approach. In addition such methods are generally not time-consuming and do not require anaesthesia. Nowadays, two main procedures are recognized as standard methods for percutaneous angioplasty: percutaneous aspiration thromboembolectomy (PAT) and percutaneous transluminal angioplasty (PTA).

Percutaneous aspiration thromboembolectomy (PAT). This technique has some clear advantages over standard surgical thromboembolectomy. It is less invasive, resulting in less damage to the endothelium. It often reveals transversal lesions in the distal part of the occlusion, facilitating a direct approach to secondary narrowing of the affected vessel (working Party on Thrombolysis, 2003). The first experiences with this method were published by Starck (1985) and it has been a well accepted method ever since, especially for removing acute, fresh thrombi, using a thin-walled end-hole catheter, connected to a syringe, which helps to create suction pressure. The clot with all remnants is subsequently sucked through the catheter into the syringe. In some instances, a PTA should follow to open the secondary vascular narrowing due to thrombosis. With regard to disadvantages, some blood loss, incomplete thrombus removal and possible embolization of peripheral vessels may occur.

Percutaneous transluminal angioplasty (PTA). This method has been almost abandoned for the treatment of an acute thrombosis, because it does not offer any clear advantages. In fact, this method has been recently limited to an adjunctive procedure following local endovascular thrombolysis, in cases with persistent atherosclerotic narrowing of the vessel, or secondary treatment after PAT. Generally, better results have been observed in patients with short occlusions, without spread to side-branches, in patients with no diabetes, non-smokers and in those after surgical revascularization. Compared to surgical intervention, it results in lower mortality and morbidity with approximately the same efficacy. Furthermore, it does not require long hospitalization.

Interventional methods still have a certain rate of reocclusion regardless of the procedure applied. Re-thrombosis occurs more often in the femoro-popliteal area and below the knee. In general, interventional methods seem to be more feasible for the infra-inguinal area, while for the aorto-femoral area, more invasive methods, such as surgical revascularization have been recommended.

2.5.3. Thrombolysis

Since its very inception in the early eighties of last century thrombolysis has developed very quickly as a treatment option for various thrombotic disorders. The pharmacokinetics of thrombolytic drugs itself helps to break down the hemostatic balance of the patient, i.e., the equilibrium between thrombin and plasmin.

Thrombolysis can be generally divided into local and systemic. The significance of general or systemic thrombolysis is more or less historical, as systemic administration of thrombolytic drug requires higher doses, increasing the risk of systemic bleeding and, moreover, despite some attempts to develop a thrombin-specific substance, this treatment still cannot be targeted very well. For this reason systemic administration has been abandoned for treatment of acute arterial thrombosis. Local (intraarterial) thrombolysis can be also divided into two categories: (a) continuous (infusion) thrombolysis, where the arterial catheter is inserted close to the obstructive clot and an infusion of the thrombolytic drug is given over a few hours; (b) spray accelerating (infiltrative) thrombolysis, when the thrombolytic agent is directly injected into the obstructing clot. This spray injection is done under pressure repeatedly at certain intervals of time (minutes).

2.5.3.1. First generation thrombolytic agents

Streptokinase, obtained from C β-hemolytic streptococcus cultures, was the first fibrinolytic substance successfully applied (Certik, 2003). It forms complexes with circulating plasminogen, accelerating its modification into plasmin, which actually breaks down the cross-linked fibrin mesh (Marder and Francis, 1990). Streptokinase itself does not show any affinity to fibrin. This lack of affinity makes it unsafe, since it can bind anywhere in the circulation and cause some unexpected and possibly life-threatening bleeding. The systemic plasma concentration of plasminogen is about 2μM, while the concentration of its major direct inhibitor, α-2 antiplasmin is only about 1μM. Since streptokinase is a foreign protein, it can cause severe allergic reactions – as observed in 4.4% of patients during multicentric clinical trials (GISSI, 1986; ISIS-2, 1988). The biological half-life of streptokinase is about 30 minutes, while in complex with plasminogen it is extended up to 80 minutes.

Urokinase can be isolated from human urine or from human kidney cell lines. It possesses direct affinity to plasminogen, so it does not require any other binding to be effective. It is a human protein, so allergic reactions are rare. The biological half-life of urokinase is about 10 minutes.

2.5.3.2. Second generation thrombolytic agents

This group presents a tissue-type plasminogen activator (t-PA), a single chain urokinase-type plasminogen activator (SCu-PA) and acylated plasminogen streptokinase activator complex (APSAC) (Marder and Francis, 1990). The main reason for starting a new era of fibrinolytic drugs was the need for a selective thrombolysis with minimum systemic effects. Despite efforts to develop new, more selective drugs, extensive multicentric clinical trials have revealed all of them to be rather insufficient in terms of clot specificity. All the new drugs require a therapeutic dose high enough to trigger fibrinolysis throughout the circulation, which means a substantial risk of bleeding. Moreover, according to clinical

studies, all these new "clot-specific drugs reveal approximately the same rate of bleeding complications as does streptokinase" (TIMI Study Group, 1985; Magnani, 1989; White et al., 1989).

Tissue-type plasminogen activator (t-PA): was first isolated from human vascular endothelium in 1971 (Certik, 2003). Even though the natural t-PA is a single chain substance, it is rapidly converted into a double chain molecule by plasmin. However, both types are enzymatically active, with the same affinity to fibrin (Francis and Marder, 1990). The property that distinguishes t-PA from other thrombolytic drugs is mainly its affinity to fibrin, which makes this substance somewhat more clot-specific. Now, thanks to recombinant techniques, the new, pure recombinant drug, called rt-PA (or alteplase) has been manufactured. This drug is metabolized very quickly, with a systemic half life of about five minutes (Marder and Francis, 1990).

Acylated plasminogen streptokinase activator complex (APSAC): this is a complex which binds streptokinase directly to plasminogen, and is temporarily inactivated by additional acylation. It takes additional binding to fibrin to trigger de-acylation and activation of the whole complex. The advantage of this drug is that it administers plasminogen itself, helping especially patients with lower plasma levels of circulating plasminogen.

3. Heparin

Since the first synthesis of heparin (McLean, 1916) a long-lasting debate has ran regarding its inner structure, as well as about its anticoagulant properties (Casu, 1985, 1989). Heparin is extraordinary because of its variability. Chemically, it is a collection of fragments, each with different molecular weights and different modes of action. The most important action of heparin is its interference in the coagulation cascade. This polysaccharide consists of chains, containing one to four uronic acid remnants and D-glucosamine (Casu, 1989). The molecular weight of heparin may vary from 3000 to 30 000 Da, with a mean value of 15 000 Da (Hirsh, 1991). Out of these numbers, approximately one third is represented by a unique pentasaccharide, necessary for binding to antithrombin, accelerating thrombin and activated factor X inhibition (Lam et al., 1976). An additional anticoagulant activity of heparin goes through heparin cofactor II activation, which is less potent and generally requires higher systemic concentrations of heparin. The remainder of the heparin molecule does not possess any anticoagulant properties. The major anticoagulant activity is based on the number of oligosaccharide (pentasaccharide) remnants that directly affect the molecular weight of the effective agent. Generally, heparin acts on different levels of the coagulation cascade. Its properties can be defined as anticoagulative, antithrombotic, profibrinolytic and ani-aggregative, anti-inflammatory, anti-proliferative and anti-ischaemic (Lundin et al., 2000; Perretti et al., 2000; Salas et al., 2000; Trocme and Li, 2000; Yagnik et al., 2000).

3.1. Effect of heparin on the coagulation cascade

Heparin itself does not have any anticoagulation properties. It has been proven that for this effect to take place, the presence of a plasmatic cofactor is required. This cofactor has been discovered and was named antithrombin III, later abbreviated to just antithrombin (AT). Heparin binds to antithrombin through the unique pentasaccharide sequence present in approximately one third of the molecule. Stable covalent AT-heparin complexes (AT/H complexes) inactivate both thrombin (factor IIa) and activated factor X (Xa) at approximately the same level (anti IIa : anti Xa ~ 1 : 1). Similarly, activated factor IX (IXa) is inhibited by the AT/H complex (Rosenberg, 1987). Out of these enzymes, thrombin and factor Xa are more susceptible to inhibition, with thrombin being even more sensitive than factor Xa. Binding to lysine terminals of antithrombin not only creates a complex, but creates conformational changes in the active site, responsible for more effective inhibition of coagulation enzymes (Rosenberg, 1987).

To trigger inhibition of thrombin by the AT/H complex, not only the pentasaccharide terminal is required, but additionally the heparin molecule ought to be big enough to create a bridge between thrombin and antithrombin. On the other hand, bridging is not necessary for inhibition of factor Xa, where the pentasaccharide plays the most important role. For this reason the smaller molecule of heparin (less than 18 saccharides) is not as effective in terms of thrombin (factor IIa) inhibition and factor Xa affinity predominates. Instead, very small molecules, containing just one pentasaccharide become more or less selective factor Xa inhibitors.

As mentioned, antithrombin is a major cofactor of heparin; however it is not the only one. A high concentration of heparin potentiates thrombin

inhibition in an antithrombin-independent manner, through another cofactor, known as heparin cofactor II (HCII). This catalysis is also molecular weight-dependent, as it requires heparin to carry at least 24 saccharide units (Sie et al., 1986).

Heparin binds *in vivo* to platelets and then, depending on the conditions, can accelerate or inhibit platelet aggregation. Generally, high molecular weight heparin with low affinity to factor Xa affects the platelets more than low molecular weight heparins with high affinity to factor Xa.

Heparin increases coagulation times in humans and increases blood loss in rabbit animal models (Ockelford et al., 1982). Moreover, it increases the vessel wall permeability. The interaction of heparin with platelets and vascular endothelial cells can contribute to heparin-induced bleeding in a manner, independent of its previously described anticoagulant properties.

Generally, heparin disturbs haemostasis through inhibition of coagulation enzymes. This effect is facilitated by plasma cofactors and through inhibition of platelets. Heparin does not penetrate the blood vessel barrier in the placenta; also it does not reach the milk.

3.2. Limitations of heparin

The anticoagulation effects of heparin can be defined by its pharmacokinetics and its general biophysical and antihaemostatic properties. For instance, heparin bound to plasma protein or vascular endothelial cells may have rather complicated plasma recovery and clearance. Among the biophysical limitations of the AT/H complex belongs the inability to target factor Xa in the prothrombinase complex and to target thrombin bound to fibrinogen, fibrin or the subendothelial matrix. Some side-effects, like pro-aggregation effect on platelets may also restrict therapeutic effects.

The above-mentioned possible complications can be avoided by using low molecular weight heparins or synthetic heparinoids, while the inability to reach thrombin bound to fibrin (fibrinogen) can be prevented by using direct thrombin inhibitors.

3.3. Pharmacokinetics of heparin

Heparin binds to plasmatic proteins (Lindahl and Hook, 1978), as they compete for the binding site along with antithrombin (AT). It has been proven that these bonds to other proteins do make a difference in the anticoagulant activity of heparin. This might be an explanation for some cases of heparin resistance or heparin insufficiency in patients treated for thromboembolic disease (Hirsh et al., 1976).

Heparin clearance begins with a brief elimination period, followed by subsequent disappearance, which can be explained by a combination of the first-grade saturated and non-saturated mechanisms involved (De Swart et al., 1982). The saturated part of heparin clearance can be defined as its binding to the receptors on macrophages and endothelial cells. It is generally supposed that bound heparin undergoes structural changes followed by depolymerization to small derivatives (Mahadoo et al., 1977; Glimelius et al., 1978). Furthermore, the heparin – platelet interaction stimulates platelet-derived factor 4, which can eventually eliminate heparin from circulation (Dawes et al., 1978). On the other hand, the rather slower, non-saturated mode of clearance is limited to renal excretion. It is general understood that in therapeutic plasma concentrations, heparin elimination is achieved mostly through saturated pathways (Dawes and Pepper, 1979; De Swart et al., 1982).

The multifactorial mode of elimination is more likely responsible for some variability in dose response, as well as the inconsistent half-life of heparin observed in clinical settings, especially for an increased dose (100, 200 and 400 IU/kg resulting in a half-life of 60, 100, 150 and 180 minutes, respectively; Olsson et al., 1963). On the other hand, a subcutaneous route of administration results in lower bioavailability of heparin, especially in low dose regimens. The reason for this is simply that heparin which accumulates in subcutaneous depots easily binds to plasmatic proteins, which helps in its quick elimination causing decreased efficacy (Piper, 1947). However, at high doses (35 000 IU/kg per day, for example), heparin can reach up to 90% bioavailability, when administered subcutaneously (Walker et al., 1987). The anticoagulant effects of heparin can be modified by platelets, fibrin, vascular surfaces and plasmatic proteins. For instance, platelets limit the effect of heparin in two ways: (1) Factor Xa protection, since platelet bound factor Xa is inaccessible to heparin-antithrombin complexes (Marciniak, 1973; Walker et al., 1987); (2) by releasing heparin-inhibiting protein, platelet factor 4. Generally, thrombin bound to fibrin,

fibrin (fibrinogen) degradation products or the subendothelial matrix is protected from inactivation by heparin-antithrombin complexes (Bar-Shavit, 1989; Hogg and Jackson, 1989; Weitz et al., 1991). This means that higher doses of heparin, helping heparin cofactor II to take over, are needed to inhibit bound thrombin.

Various animal studies have confirmed the hypothesis that antithrombin-independent substances are needed to inhibit bound thrombin (Heras et al., 1989; Agnelli et al., 1990).

3.4. Monitoring of treatment by heparin

Heparin acts almost immediately following *i.v.* administration, with an estimated half-life of about one to two hours (dose-dependent). That is why continual infusion is substantially more efficient than *i.v.* bolus in heparin treatment. Currently, an *i.v.* bolus of higher dose followed by infusion has been recommended (Hull et al., 1986).

For subcutaneous administration usually two to three applications per day are given. Plasma response is reduced to a lower dose (about 5000 IU), or even medium dose (about 15 000 IU), while it is sufficient enough at a high dose (about 35 000 IU over 24 hours). Maximum plasma levels of heparin are reached in two to four hours following subcutaneous administration, with an estimated half-life of four to six hours (Pini et al., 1990).

Heparin is given in low doses for prophylaxis and in higher doses for treatment of thrombosis. Generally, prophylaxis using heparin is associated with a substantially lower risk of heparin-induced thrombocytopenia. Regularly, low doses are given as boluses of 5000 IU subcutaneously, two or three times a day. Low doses are efficient especially for prevention of deep vein thrombosis (very good in patients undergoing orthopedic or gynecologic surgery). On the other hand, the use of heparin in high-risk patients should be avoided even at low doses. High doses of heparin are widely used in the treatment of patients suffering from arterial thrombosis or thromboembolic disease (Hirsh et al., 1976; De Bono et al., 1992). The effects of heparin at high doses may be variable, as it depends on plasma protein concentration. Exact efficacy is hard to establish; however, there is a close relationship between clinical manifestation and the anticoagulant activity of heparin at a given dose (Hull et al., 1986; Turpie et al., 1989; Arnout et al., 1992). Moreover, the dose of heparin depends on the body weight of the patient.

The efficacy of heparin can be monitored by the screening of some laboratory parameters, enabling the evaluation and modification of the actual dose. A typical parameter of heparin treatment is measurement of Activated Partial Thromboplastin Time (APTT – more precise), Thrombin Time (TT), or Activated Clotting Time (ACT). Values of APTT are during effective treatment by heparin 1.5 to 2.5 fold elevated compared to baseline levels (no heparin). With regard to the fact that the effect of heparin is closely related to concentration of antithrombin, it is necessary to evaluate the plasma concentration of antithrombin, whenever heparin treatment fails to provide sufficient anticoagulation at a given therapeutic dose. In case of low levels of antithrombin, antithrombin concentrates can be given by infusion. To prevent thrombocytopenia, treatment by heparin should be as short as possible and the patient should be put on oral anticoagulants before the 10^{th}, and at the latest by the 14^{th} day of therapy. For a change in treatment (from heparin to oral anticoagulants), at least three days of overlap of both drugs should be established. Heparin should not be given intramuscularly.

3.5. Antidotes of heparin

Protamine sulphate is the one and only direct antidote of heparin. It should be used only in case of excessive bleeding following heparin treatment. The effective dose of protamine sulphate is 1 mg per 100 IU of heparin used, but the rather short half life of heparin should be considered. Platelet count should be measured after 48 hours of treatment. In case of a drop below 100×10^9/l, heparin should be replaced by oral anticoagulants.

3.6. Effect of heparin on vascular smooth muscle cell proliferation

Vascular smooth muscle cells (VSMC's) accumulate in various intimal defects of arteries, contributing to atherosclerotic plaque formation. This effect may play a role in re-stenosis following percutaneous coronary angioplasty (PCA) (Dartsch et al., 1989). For this reason inhibition of VSMC proliferation should be an important part of the treatment protocol for arterial thrombosis. Some *in vitro* stud-

ies suggest that heparin and its low-molecular weight fragments inhibit continuous vessel wall thickening by inhibition of VSMC proliferation. Heparin, as well as heparan sulphate, blocks cellular multiplication at the G0 and G1 stage (Castellot et al., 1985; Wright et al., 1989). This action is multifactorial, including an inhibition of mitogens in plasma, inhibition of excretion of platelet-derived growth factor (PDGF), interference with thrombin mitogenic activity, deliberation of cell-bound thrombospondin (free thrombospondin is unable to interact with PDGF) (Majack, 1985, 1986, 1988), inhibition of DNA synthesis in smooth muscle cells by intracellular heparin (Reilly et al., 1986; Wright et al., 1989), heparin-dependent inhibition of VSMC protein synthesis (Castellot et al., 1985; Cochran et al., 1985) and protecting of heparan sulphate from biodegradation by heparinase released from platelets (Fritz et al., 1985; Castellot et al., 1987; Wright et al., 1989). In the last case, the following events have been observed: (1) both heparin and heparan sulphate inhibit VSMC growth in a similar fashion (Castellot et al., 1981, 1987; Benitz et al., 1990), endothelial cells synthesize heparan sulphate in the same manner as smooth muscle cells; (2) smooth muscle cells synthesize heparan sulphate more during continuous growth than during growth on exponential cell-lines (Fritz et al., 1985); (3) media containing heparan sulphate (those fixed by heparinase from flavobacteria) increase the sensitivity of VSMC's to various mitogens and growth stimulators (Castellot et al., 1981). Heparinase itself can be released by internal platelet and monocyte activation (Oldberg et al., 1980; Castellot et al., 1982; Wright et al., 1989). It can be speculated that vascular damage itself can trigger the process of heparinase release by platelets and white blood cell accumulation in the area, giving a boost of mitogenic stimulation and smooth muscle cell proliferation. Heparin administration prior to or during this stage can slow down such process and protect heparan sulphate, which helps to keep VSMC's in a calm, non-proliferative status (Bar-Ner, 1987).

Thrombin is a well-recognized mitogen for various types of cells, including VSMC's (Chen and Buchanan, 1975; Bar-Shavit et al., 1990; Wilcox et al., 1992), but its direct influence on VSMC proliferation in patients following angioplasty has not been fully elucidated (Guyton et al., 1980; Hoover et al., 1980; Castellot et al., 1984). It has been proven that heparin fragments (including pentasaccharides) lacking anticoagulant properties have the same inhibitory effect on VSMC's as those with anticoagulation (e.g. thrombin-inhibiting) activity. This does not comply with the theory that thrombin itself is most potent stimulator of VSMC proliferation (Guyton et al., 1980; Hoover et al., 1980; Castellot et al., 1984; Wright et al., 1989; Pukac et al., 1991). Hoover et al. (1980) found that heparin can inhibit VSMC proliferation even in an environment of antithrombin-free plasma. The presence of sulphate is necessary for this process, since de-sulphated heparin loses its inhibitory properties for VSMC (Castellot et al., 1984). Other glycosaminoglycans, such as dermatan sulphate, hyaluronic acid and chondroitin sulphate do not inhibit VSMC proliferation with the same strength as heparan sulphate (Castellot et al., 1981; Fritz et al., 1985).

Some experimental studies suggest that α-thrombin bound to VSMC's may initiate their proliferation (Bar-Shavit et al., 1990; Wilcox et al., 1992). Whether this is the case for *in vivo* intimal thickening and how big is the role of thrombin catalytic activity, remains open for discussion. One possible explanation may be a direct interaction between the non-enzymatic domain in thrombin and its receptor on VSMC's. Some failures have occurred after the use of heparin in clinical studies, while experimental animal models generally showed very good results. This might be due to different dose regimens applied (Guyton et al., 1980; Clowes and Clowes, 1985, 1986; Dryjski et al., 1988; Wilson, et al., 1991; Edelman and Karnovsky, 1994).

3.7. Indications of heparin

Even though venous thromboembolism (VTE) remains the main indication, heparin has been widely used also in patients with arterial thrombosis. Especially in cases where coumarin derivatives cannot be given, heparin's ability to potentiate fibrinolysis and stimulate TFPI release can be of benefit (Penka and Bulikova, 2006).

Mueller (2004) describes the following indications recommended by the American College of Chest Physicians (ACCP):

Prophylaxis of deep vein thrombosis in general surgery, gynaecology and urology, in middle- and high-risk patients, in total hip replacement and hip arthroplasty, as well as in neurosurgery; Prophylaxis of VTE in acute myocardial infarction or acute stroke, and in high-risk patients with multiple disorders; Treatment of deep vein thrombosis; Early treatment of an acute myocardial infarction (AMI) using thrombolytics or in patients at risk of embolization; in AMI treatment, a combination

of heparin with acetyl salicylic acid (ASA) is recommended; Early treatment of an unstable angina pectoris; Uncomplicated percutaneous coronary angioplasty (PCA); Treatment of cardioembolic disease affecting large vessels, especially in connection with risk of VTE; Treatment of an acute thromboembolism; Peripheral vascular reconstructive surgery; Cardioversion in patients suffering from atrial fibrillation, during cardiopulmonary bypass, during intraarterial balloon contra-pulsation and hemodialysis; Treatment of cerebral sinus venosus thrombosis; Treatment of aseptic thrombotic endocarditis and embolization; Prophylaxis of patients with disseminated carcinoma and aseptic valvular proliferation; Selected cases of disseminated intravascular coagulopathy; Prophylaxis of pediatric patients following Blalock-Taussig shunt or following Fontan procedure; Prophylaxis in pregnant and post-parturition women with a history of deep vein thrombosis – replacement of coumarin derivatives, at least till 13 week of gravidity and again in the 3rd trimester: recommended especially in thrombophilic women with repeated abortions, preeclampsia, placental disorders and/or intrauterine growth deformity of the foetus; Anticoagulation of blood collected for laboratory analysis; Anticoagulation of catheters and cannulas during regular patient care.

3.8. Side-effects of heparin

Thrombocytopenia is probably the most frequent complication relating to heparin treatment (1.1 to 2.9% of patients). Some allergic reactions (fever, rage, rarely asthma or anaphylaxis), local bleeding (from injection site, or from ulcers), hemorrhagic diathesis (from mucous membranes, skin, to the cavities or retroperitoneally), intra-organ bleeding (CNS, adrenal glands, ovaries), heart arrhythmias, skin necrosis, alopecia, elevation of transaminase activity and hyperlipidemia may also occur. Long-term treatment may result in osteoporosis causing spontaneous bone fractures (Hirsh, 1999).

3.8.1. Heparin-induced thrombocytopenia – HIT

Heparin-induced thrombocytopenia (HIT) is a quite common immuno-mediated complication of treatment by both unfractionated heparin and low-molecular heparin fragments, especially in patients with high morbidity and mortality due to thrombosis (Boshkow et al., 1993).

Heparin binds to platelets, activating platelet factor IV and stimulating its release (Salzman et al., 1980). Then, heparin creates a complex with platelet factor IV, stimulating antibodies primarily responsible for thrombocytopenia (Kelton et al., 1994). The incidence of thrombocytopenia may vary; however, randomized studies have reported incidences of somewhere around 3% (Warkentin, 2004). This starts usually between day 5 and day 15 of treatment, with a median of 10 days (Hirsh et al., 1995). In patients with a history of previous treatment with heparin, thrombocytopenia may occur as early as one hour after the beginning of therapy. The incidence of either arterial or venous thrombosis in patients with HIT remains unknown. It has been suggested that thrombosis may develop in up to 20% of patients suffering from HIT affecting patients treated with low-molecular weight heparins, as well, even though the incidence is lower. Recently, according to some studies (Chong et al., 1989a), a synthetic heparinoid named danaparoid sodium gives the lowest rate of thrombocytopenia events, having very little contaminants and revealing almost no affinity to platelet factor IV antibodies (cross-reactions to HIT) (Chong et al., 1989b).

3.8.2. Osteoporosis

Osteoporosis has been a well-recognized complication in patients undergoing long-term treatment with heparin. Some clinical data show that over three months of treatment, spontaneous fractures occur in 2 to 3% of patients and one third of patients experience an asymptomatic but substantial loss of bone density (Dahlman, 1993; Barbour et al., 1994; Monreal et al., 1994).

It has been confirmed by a set of studies that heparin: (1) induces bone resorption (Shaughnessy et al., 1995), (2) decreases the volume of spongiose bony tissue (Muir et al., 1996), (3) decreases total amount of osteoblasts; osteoclasts prevail in an experimental rat model – the effect is observed more often in unfractionated heparin than in low-molecular weight heparins (Melissari et al., 1992).

4. Low molecular weight heparins – LMWH's

The development of low-molecular weight heparins was accelerated by the discovery that

administration of these substances decreases bleeding while maintaining the same antithrombotic efficacy when compared with heparin itself (Carter et al., 1982; Esquivel et al., 1982; Holmer et al., 1982; Cade et al., 1984; Andriuoli et al., 1985; Bergquist et al., 1985). Clinical studies show that low-molecular weight heparins (LMWH's) are effective especially in the prevention and treatment of venous thrombosis (Hull et al., 1991; Prandoni, 1991; Columbus Investigators, 1997; Klein et al., 1997; Hirsh, 1998).

LMWH's are obtained by either chemical or enzymatic de-polymerization of standard heparin (Ofosu and Barrowcliffe, 1990; Weitz, 1997). Standard – unfractionated heparin (UFH) consists of a mixture of polysaccharide chains, with molecular weights of between 3000 and 30 000 Da - with a mean molecular weight of around 15 000 Da (Anderson et al., 1979; Harenberg, 1990; Ofosu and Barrowcliffe, 1990; Hirsh, 1991; Weitz, 1997). Low-molecular weight heparins are also heterogenic, but with a narrower range (1000 to 10 000 Da; mean 3000 to 5000 Da, the numbers vary according to different authors) (Anderson et al., 1979; Harenberg, 1990; Ofosu and Barrowcliffe, 1990; Hirsh, 1991; Weitz, 1997).

Generally, low-molecular weight heparins have different anticoagulant profiles, bioavailabilities, pharmacokinetics and platelet interactions. Their safety standards have been proven to be better than standard heparin in animal models (Klement et al., 1999) (Table 3).

4.1. Anticoagulation properties of LMWH's

Like unfractionated heparin, low-molecular weight heparins possess unique anticoagulant properties through their activation of antithrombin (Rosenberg, 1975; Hook et al., 1976; Lindahl et al., 1979, 1984; Rosenberg et al., 1979; Casu et al., 1981; Choay et al., 1981, 1983; Bjork and Lindahl, 1982; Weitz, 1997; Turpie, 1998). The critical pentasaccharide is present in approximately one third of chains in UFH and in less than one third of the molecule in LMWH's. Activation by pentasaccharide is associated with conformational changes (Olson et al., 1981; Turpie, 1998), enabling inactivation of thrombin (factor IIa) and factor Xa by a fibrinogen molecule (Rosenberg, 1975). Both UFH and LMWH may act in a heparin – antithrombin complex as a catalyst of thrombin inactivation (Rosenberg, 1975; Rosenberg et al., 1979; Bjork and Lindahl, 1982; Olson and Shore, 1982; Turpie, 1998). For the heparin – antithrombin complex to create a bridge to thrombin, it takes a chain of at least 18 saccharides, while for factor Xa inactivation, just one pentasaccharide molecule is needed (Bjork and Lindahl, 1982; Olson and Shore, 1982; Turpie, 1998). Needless to say, dekaoctosaccharide (19 monomers) or a bigger molecule is present in all UFH preparations, but only in 25 to 50% of all LMWH's (Holmer et al., 1981; Lindahl et al., 1984; Holmer et al., 1986). For this reason LMWH's are more or less Xa-selective inhibitors, while the anticoagulant activity of UFH is approximately the same for both factors (anti IIa : anti Xa ~ 1 : 1) (Hirsh, 1991). At present, commercially available low-molecular weight heparins have an anti IIa : anti Xa ratio of between 1 : 2 and 1 : 4 – depending on the molecular weight of the active substances (Weitz, 1997; Turpie, 1998).

4.2. Pharmacokinetics of LMWH's

The binding affinity of the two sulfated polysaccharides to plasmatic proteins and endothelial cells indicate their pharmacokinetic properties (Barzu et al., 1984, 1985; Lane et al., 1986; Weitz, 1997; Turpie, 1998). Heparin-binding proteins tend to build stronger bonds to UFH than to LMWH's

Table 3. An overview of low-molecular weight heparins

Substance	Brand name	Half-life	Anti-Xa/anti-IIa	Mean molecular weight (Da)
Nadroparine	Fraxiparine	201	3.2	4500
Dalteparin	Fragmin	228	2.7	5000
Enoxaparin	Clexane	275	3.3	4800
Reviparin	Clivarin	180	3.5	4000
Tinzaparin	Innohep	200	1.8	4500
Certoparin	Sandoparin	258	4.2	7600

(Lane et al., 1986; Preissner and Muller-Berghaus, 1987; Sobel et al., 1991), which actually increases the bioavailability of LMWH's in plasma even at lower doses. Moreover, the dose-dependent action of LMWH's (no reduction by plasma-protein binding) means they act in a much more predictable fashion (Handeland et al., 1990). LMWH's don't bind to tissue-cultured endothelial cells (Barzu et al., 1984, 1985, 1987), which may contribute to their prolonged *in vivo* half-life (Boneu et al., 1988; Briant et al., 1989). The main mode of LMWH metabolism and excretion is through the kidney and urinary system (Palm and Mattsson, 1987; Boneu et al., 1988). LMWH's have a significantly lower affinity to von Wilebrandt factor (vWF) (Sobel et al., 1991) than UFH, which may decrease the probability of excessive bleeding complications at the same dose regimen (Esquivel et al., 1982; Andriuoli et al., 1985).

4.3. Efficacy and safety of LMWH's in an animal model

Standard (unfractionated) heparin has been compared with low-molecular weight heparins, ORG heparinoid and dermatan sulphate on various experimental animal models with regard to their antithrombotic and hemorrhagic responses (Esquivel et al., 1982; Ockelford et al., 1982; Andriuoli et al., 1985; Hobbelen et al., 1987; Van Ryn-McKenna et al., 1989; Currier et al., 1991). In one model, venostasis was achieved by vessel ligation and blood coagulation was induced by injection of serum, factor Xa, thrombin, or tissue factor (Ockelford et al., 1982; Van Ryn-McKenna et al., 1989). In these models, LMWH's are slightly less effective than UFH; however, they cause significantly less systemic bleeding under standardized conditions (Esquivel et al., 1982; Ockelford et al., 1982; Hobbelen et al., 1987). Whenever these sulphated polysaccharides are compared to each other, their effect on platelets (Fabris et al., 1983; Fernandez et al., 1986; Sobel et al., 1991), as well as on blood vessel permeability (Blajchman et al., 1989) should be kept in mind.

4.4. LMWH's in the prevention of arterial thrombosis – clinical studies

Low-molecular heparins are superior to UFH in many ways. Their significantly longer half-life and predictability of antithrombotic response allow a single dose treatment regimen with no laboratory monitoring required (Weitz, 1997; Turpie, 1998). A higher safety index allows even higher doses during treatment. In some prevention studies, an increased dose of LMWH was found to be more effective and safer in patients experiencing bleeding complications following UFH administration (Levine et al., 1991; FRISC Study Group, 1996; Zed et al., 1999). Similar results have been obtained from studies dealing with prevention and treatment of both arterial and venous thrombosis (Hull et al., 1991; Prandoni, 1991; Columbus Investigators, 1997; Klein et al., 1997; Hirsh, 1998). LMWH's have been accepted as a replacement therapy in cases of heparin-induced thrombocytopenia (Prifti et al., 2000). Moreover, osteoporosis occurs significantly less frequently in patients treated with LMWH's than in those undergoing UFH treatment (Monreal, 1994).

4.5. Administration and monitoring of LMWH's

The common route of administration for LMWH's is deep subcutaneous injection, usually once or twice a day. The dose regimen may vary according to the desired plasma levels, e.g., whether it is for prevention or treatment, or according to diagnosis and degree of risk for a specific patient.

Low-molecular weight heparins don't prolong basic coagulation times (APTT, Thrombin Time) at treatment doses. The fact that there is no need for close monitoring makes the whole therapy more convenient, and means they can be applied in an out-patient manner. However, in pregnant women and in children, close monitoring of the treatment is still recommended. The principle of plasma level establishment is based on their anti-Xa activity. Low-molecular weight heparin forms dimers with antithrombin and these complexes inactivate pre-defined amounts of activated factor X. So, a decrease in Xa levels negatively correlates with the LMWH plasma concentration. Therapeutic levels of LMWH's may vary from 0.3 to 0.7 IU of anti Xa/ml.

5. SUMMARY

Unfractionated heparin is a drug widely used in the treatment and prevention of arterial and venous thrombosis. Nevertheless, low-molecular weight

heparins, which utilize the same mode of action, and which possess better anticoagulant properties and wider safety margins represent an attractive alternative. To name some advantages, they have a longer half-life and there are clear-cut clearance mechanisms, making the dose response of the drug more predictable. That is not the case for unfractionated heparin, which can be easily administered at too high a concentration or, coversely, at too low a dosage. Therefore, to adjust the dose and prevent bleeding complications, a sustained laboratory monitoring of APTT has been designed and the patient should be closely monitored during therapy. Unlike in heparin treatment, a single dose regimen of LMWH significantly decreases the hospitalization period, allowing out-patient care. The low incidence of not only bleeding complications, but also heparin-induced thrombocytopenia (HIT) and osteoporosis makes these substances more useful even in high-risk patients.

On the other hand, we should keep in mind that LMWH's represent in fact a variety of fragments, with slightly different pharmacokinetics and different dose response. The differences stem mostly from the unique molecular structure of each fragment, which can pre-define its mode of action. Therefore, the physician must regard several aspects, namely the bioavailability of an active substance, its half-life, specific indication and safety margins of the drug. Even though close monitoring is not recommended during treatment with LMWH's, each patient should be carefully inspected prior to therapy and observed during and after treatment is stopped, to compare efficacy and safety.

6. CONCLUSION

Both unfractionated heparin and low-molecular weight heparin are useful in the treatment and prevention of arterial thrombosis. Low-molecular weight heparins, thanks to their unique molecular structure, possess some clear advantages over UFH. However, more clinical studies are needed to establish their clear superiority as well as other possible consequences of their administration.

7. REFERENCES

Agnelli G, Pascucci C, Cosmi B, Nenci GG (1990): The comparative effects of recombinant hirudin (CGP 39393) and standard heparin on thrombus growth in rabbits. Thrombosis and Haemostasis 63, 204–207.

Ahn SS, Rutherford RB, Johnston KW, May J, Veith FJ, Baker JD, Ernst CB, Moore WS (1997): Reporting standards for infrarenal endovascular abdominal aortic aneurysm repair. Ad Hoc Committee for Standardized Reporting Practices in Vascular Surgery of The Society for Vascular Surgery/International Society for Cardiovascular Surgery. Journal of Vascular Surgery 25,405–410.

Andersson LO, Barrowcliffe TW, Holmer E, Johnson EA, Soderstron G (1979): Molecular weight dependency of the heparin potentiated inhibition of thrombin and activated factor X. Effect of heparin neutralization in plasma. Thrombosis Research 15, 531–541.

Andriuoli G, Mastacchi R, Barbanti M, Sarret M (1985): Comparison of the antithrombotic and haemorrhagic effects of heparin and a new low molecular weight heparin in rats. Haemostasis 15, 324–330.

Arnout J, Simoons ML, de Bono D, Rapold HJ, Collen D, Verstraete M (1992): Correlation between level of heparinization and patency of the infarct-related coronary artery after treatment of acute myocardial infarction with alteplase (rt-PA). Journal of the American College of Cardiology 20, 513–519.

Baba N, Bashe WJ Jr, Keller MD, Geer JC, Anthony JR (1975): Pathology of atherosclerotic heart disease in sudden death. I. Organizing thrombosis and acute coronary vessel lesions. Circulation 52(Suppl.), III53–59.

Barbour LA, Kick SD, Steiner JF, LoVerde ME, Heddleston LN, Lear JL, Baron AE, Barton PL (1994): A prospective study of heparin-induced osteoporosis in pregnancy using bone densitometry. American Journal of Obstetrics and Gynecology 170, 862–869.

Bar-Ner M, Eldor A, Wasserman L, Matzner Y, Cohen IR, Fuks Z, Vlodavsky I (1987): Inhibition of heparanase-mediated degradation of extracellular matrix heparan sulfate by non-anticoagulant heparin species. Blood 70, 551–557.

Bar-Shavit R, Eldor A, Vlodavsky I (1989): Binding of thrombus to subendothelial extracellular matrix. Protection and expression of functional properties. Journal of Clinical Investigation 84, 1096–1104.

Bar-Shavit R, Benezra M, Eldor A, Hy-Am E, Fenton JWII, Wilner GD, Vlodavsky I (1990): Thrombin imobilized to extracellular matrix is a potent mitogen for vascular smooth muscle cells: nonenzymatic mode of action. Cell Regulation 1, 453–463.

Barzu T, Molho P, Tobelem G, Petitou M, Caen JP (1984): Binding of heparin and low molecular weight heparin fragments to human vascular endothelial cells in culture. Nouv Revue France Haematology 26, 243–247.

Barzu T, Molho P, Tobelem G, Petitou M, Caen J (1985): Binding and endocytosis of heparin by human endothelial cells in culture. Biochimica et Biophysica Acta 845, 196–203.

Barzu T, Van Rijn JL, Petitou M, Tobelem G, Caen JP (1987): Heparin degradation in the endothelial cells. Thrombosis Research 47, 601–609.

Benitz WE, Kelley RT, Anderson CM, Lorant DE, Bernfield M (1990): Endothelial heparan sulfate proteoglycan. I. Inhibitory effects on smooth muscle cell proliferation. American Journal of Respiratory Cell and Molecular Biology 2, 13–24.

Bergquist D, Nilsson B, Hedner U, Pedersen PC, Ostergaard PB (1985): The effect of heparin fragments of different molecular weights in experimental thrombosis and haemostasis. Thrombosis Research 38, 589–601.

Bjork I, Lindahl U (l982): Mechanism of the anticoagulant action of heparin. Review. Molecular and Cellular Biochemistry 48, 161–182.

Blacher J, Cacoub P, Luizy F, Mourad JJ, Levesque H, Benelbaz J, Michon P, Herrmann MA, Priollet P (2006): Peripheral arterial disease versus other localizations of vascular disease: the ATTEST study. Journal of Vascular Surgery 44, 314–318.

Blajchman MA, Young E, Ofosu FA (1989): Effects of unfractionated heparin, dermatan sulfate and low molecular weight heparin on vessel wall permeability in rabbits. Annals of the New York Academy of Sciences 556, 245–254.

Boccalandro F, Smalling RW (2006): Critical Limb Ischemia and Limb Salvage. The University of Texas Health Science, Center at Houston. www.uth.tmc.edu/anes/wound/critical_ischemia.htm.

Boneu B, Caranobe C, Cadroy Y, Dol F, Gabaig AM, Dupouy D, Sie P (1988): Pharmacokinetic studies of standard unfractioned heparin and low molecular weight heparins in the rabbit. Seminars in Thrombosis and Hemostasis 14, 18–27.

Boshkow LK, Warkentin TE, Hayward CP, Andrew M, Kelton JG (1993): Heparin induced thrombocytopenia and thrombosis: clinical and labolatory studies. British Journal of Haematology 84, 322–328.

Briant L, Caranobe C, Saivin S, Sie P, Bayrou B, Houin G, Boneu B (1989): Unfractioned heparin and CY 216: pharmacokinetics and bioavailabilities of the anti-factor Xa and IIa effects after intravenous and subcutaneous injection in rabbit. Thrombosis and Haemostasis 61, 348–353.

Cade JF, Buchanan MR, Boneu B, Ockelford P, Carter CJ, Cerskus AL, Hirsh J (1984): A comparison of the antithrombotic and haemorrhagic effects of low molecular weight heparin fractions: the influence of the method of preparation. Thrombosis Research 35, 613–625.

Carter CJ, Kelton JG, Hirsh J, Cerskus AL, Sandos AV, Gent M (1982): The relationship between the hemorrhagic and antithrombotic properties of low molecular weight heparin in rabbits. Blood 59, 1239–1245.

Castellot JJ Jr, Adonizio ML, Rosenberg RD, Karnovsky MJ (1981): Cultured endothelial cells produce a heparin like inhibitor of smooth muscle cell growth. Journal of Cell Biology 90, 372–379.

Castellot JJ Jr, Favreau LV, Karnovsky MJ, Rosenberg RD (1982): Inhibition of vascular smooth muscle cell growth by endothelial cell-derived heparin: Possible fate of platelet endoglycosidase. Journal of Biological Chemistry 257, 11256–11260.

Castellot JJ Jr, Beeler DL, Rosenberg RD, Karnovsky MJ (1984): Structural determinants of the capacity of heparin to inhibit the proliferation of vascular smooth muscle cells. Journal of Cellular Physiology 120, 315–320.

Castellot JJ Jr, Cochran DL, Karnovsky MJ (1985): Effect of heparin on vascular smooth muscle cell. I. Cell metabolism. Journal of Cellular Physiology124, 21–28.

Castellot JJ Jr, Wright TC, Karnovsky MJ (1987): Regulation of vascular smooth muscle cell growth by heparin and heparan sulfates. Review.Seminars in Thrombosis and Hemostasis 13, 489–503.

Casu B (1985): Structure and biological activity of heparin. Advances in Carbohydrate Chemistry and Biochemistry 43, 51–134.

Casu B (1989): Methods of structural analysis. In: Lane DA, Lindahl U (eds): Heparin: Chemical and Biological Properties, Clinical Applications. CRC Press Inc., Boca Raton, Florida. 25–49.

Casu B, Oreste P, Torri G, Zoppetti G, Choay J, Lormeau JC, Petitou M, Sinay P (1981): The structure of heparin oligosaccharide fragments with high anti-(factor Xa) activity containing the minimal antithrombin III-binding sequence. Biochemical Journal 197, 599–609.

Certik B (2003): Acute Limb Ischemia (in Czech). 1st ed. Grada Publishing, Prague. 147 pp. ISBN 80-247-0624-5.

Chen LB, Buchanan JM (1975): Mitogenic activity of blood components. I. Thrombin and prothrombin. Proceedings of the National Academy of Sciences of the United States of America 72, 131–135.

Choay J, Lormeau JC, Petitou M, Sinay P, Fareed J (1981): Structural studies on a biologically active hexasaccharide obtained from heparin. Annals of the New York Academy of Sciences 370, 644–649.

Choay J, Petitou M, Lormeau JC, Sinay P, Casu B, Gatti G (1983): Structure-activity relationship in heparin: a synthetic pentasaccharide with high affinity for antithrombin III and eliciting high anti-factor Xa activity.

Biochemical and Biophysical Research Communications 116, 492–499.

Chong BH, Fawaz I, Chesterman CN, Berndt MC (1989a): Heparin-induced thrombocytopenia: mechanism of interaction of the heparin-dependent antibody with platelets. British Journal of Haematology 73, 235–240.

Chong BH, Ismail F, Cade J, Gallus AS, Gordon S, Chesterman CN (1989b): Heparin-induced thrombocytopenia: studies with a new low molecular weight heparinoid, Org 10172. Blood 73, 1592–1596.

Clowes AW, Clowes MM (1985): Kinetics of cellular proliferation after arterial injury. II. Inhibition of smooth muscle growth by heparin. Laboratory Investigation; a journal of technical methods and pathology 52, 611–616.

Clowes AW, Clowes MM (1986): Kinetics of cellular proliferation after arterial injury. IV. Heparin inhibits rat smooth muscle mitogenesis and migration. Circulation Research 58, 839–845.

Cochran DL, Castellot JJ Jr, Karnovsky MJ (1985): Effect of heparin on vascular smooth muscle cells. II. Specific protein synthesis. Journal of Cellular Physiology 124, 29–36.

Columbus Investigators (1997): Low-molecular-weight heparin in the treatment of patients with venous thromboembolism. New England Journal of Medicine 337, 657–662.

Creager MA, Cooke JP, Mendelsohn ME, Gallagher SJ, Coleman SM, Loscalzo J, Dzau VJ (1990): Impaired vasodilation of forearm resistence vessels in hypercholesterolemic humans. Journal of Clinical Investigation 86, 228–234.

Currier JW, Pow TK, Haudenschild CC, Minihan AC, Faxon DP (1991): Low molecular weight heparin (enoxaparin) reduces restenosis after iliac angioplasty in the hypercholesterolemic rabbit. Journal of the American College of Cardiology 17(Suppl. B), 118B–125B.

Dahlman TC (1993): Osteoporotic fractures and the recurrence of thromboembolism during pregnancy and the puerperium in 184 women undergoing thromboprofylaxis with heparin. American Journal of Obstetrics and Gynecology 168, 1265–1270.

Dartsch PC, Bauriedel G, Schinko I, Weiss HD, Hofling B, Betz E (1989): Cell constitution and characteristics of human atherosclerotic plaques selectively removed by percutaneous atherectomy. Atherosclerosis 80, 149–157.

Dawes J, Pepper DS (1979): Catabolism of low dose heparin in man. Thrombosis Research 14, 845–860.

Dawes J, Smith RC, Pepper DS (1978): The release, distribution and clearance of human beta-thromboglobulin and platelet factor 4. Thrombosis Research 12, 851–861.

De Bono DP, Simoons ML, Tijssen J, Arnold AER, Betriu A, Burgersdijk C, Lopez Bescos LL, Mueller E, Pfisterer M, Van de Werf F, Zijlstra F, Verstraete M (1992): Effect of early intravenous heparin on coronary patency, infarct size, and bleeding complications after alteplase thrombolysis: results of a randomised double blind European Cooperative Study Group trial. British Heart Journal 67, 122–128.

De Swart CA, Nijmeyer B, Roelofs JM, Sixma JJ (1982): Kinetics of intravenously administered heparin in normal humans. Blood 60, 1251–1258.

Diehm C, Kareem S, Lawall H (2004): Epidemiology of peripheral arterial disease. Zeitschrift für Gefässkrankheiten. Journal for Vascular Diseases 33, 183–189.

Dryjski M, Mikat E, Bjornsson TD (1988): Inhibition of intimal hyperplasia after arterial injury by heparins and heparinoid. Journal of Vascular Surgery 8, 623–633.

Edelman ER, Karnovsky MJ (1994): Contrasting effects of the intermitent and continuous administration of heparin in experimental restenosis. Circulation 89, 770–776.

Esquivel CO, Bergqvist D, Bjork CG, Nilsson B (1982): Comparison between commercial heparin, low-molecular weight heparin and pentosan polysulfate on hemostasis and platelets in vivo. Thrombosis Research 28, 389–399.

Fabris F, Fussi F, Casonato A. Visentin L, Randi M, Smith MR, Girolami A. (1983): Normal and low molecular weight heparins: interaction with human platelets. European Journal of Clinical Investigation 13, 135–139.

Fernandez F, N'Guyen P, Van Ryn J, Ofosu FA, Hirsh J, Buchanan MR (1986): Hemorrhagic doses of heparin and other glycosaminoglycans induce a platelet defect. Thrombosis Research 43, 491–495.

Ferrieres J, Cambou JP, Gayet JL, Herrmann MA, Leizorovicz A (2006): Prognosis of patients with atherothrombotic disease: a prospective survey in a non-hospital setting. International Journal of Cardiology 112, 302–307.

Francis CW, Marder VJ (1990): Mechanisms of fibrinolysis. In: Williams VJ (ed.): Hematology. 4th ed. McGraw-Hill Publishing Company, New York. 1313–1321.

FRISC (Fragmin during Instability in Coronary Artery Disease) Study Group. (1996): Low-molecular-weight heparin during instability in coronary artery disease. Lancet 347(9001), 561–568.

Fritz LM, Reilly CF, Rosenberg RD (1985): An antiproliferative heparan sulfate species produced by postconfluent smooth muscle cells. Journal of Cell Biology100, 1041–1049.

GISSI (Gruppo Italiano per lo Studio della Streptochinasi nell' Infarcto Miocardico) (1986): Effectiveness of intravenous thrombolytic treatment in acute myocardial infarction. Lancet 1(8478), 397–402.

Glimelius B, Busch C, Hook M (1978): Binding of heparin on the surface of cultured human endothelial cells. Thrombosis Research 12, 773–782.

Guyton JR, Rosenberg RD, Clowes AW, Karnovsky MJ (1980): Inhibition of rat arterial smooth muscle cell proliferation by heparin. In vivo studies with anticoagulant and non-anticoagulant heparin. Circulation Research 46, 625–634.

Handeland GF, Abidgaard GF, Holm U, Arnesen KE (1990): Dose adjusted heparin treatment of deep venous thrombosis : a comparison of unfractionated and low molecular weight heparin. European Journal of Clinical Pharmacology 39, 107–112.

Hansson GK, Libby P, Schonbeck U, Yan ZQ (2002): Innate and adaptive immunity in the pathogenesis of atherosclerosis. Circulation Research 91, 281–291.

Harenberg J (1990): Pharmacology of low molecular weight heparins. Seminars in Thrombosis and Hemostasis 16(Suppl.), 12–18.

Heras M, Chesebro JH, Penny WJ, Bailey KR, Badimon L (1989): Effects of thrombin inhibition on the development of an acute platelet-thrombus deposition during angioplasty in pigs. Heparin versus recombinant hirudin, a specific thrombin inhibitor. Circulation 79, 657–665.

Hirsh J (1991): Heparin. The New England Journal of Medicine 324, 1565–1574.

Hirsh J (1998): Low molecular weight heparin for the treatment of venous thromboembolism. Review. American Heart Journal 135, S336–S342.

Hirsh J (1999): Low Molecular Weight Heparins. 3rd edition. B.C. Decker Inc. Hamilton, Canada. 106 pp.

Hirsh J, van Aken WG, Galus AS, Dollery CT, Cade JF, Yung WL (1976): Heparin kinetics in venous thrombosis and pulmonary embolism. Circulation 53, 691–695.

Hirsh J, Raschke R, Warkentin TE, Dalen JE, Deykin D, Poller L (1995) Heparin: mechanism of action, pharmacokinetics, dosing, considerations, monitoring, efficacy, and safety. Chest 108(Suppl.), 258S–275S.

Hobbelen PM, Vogel GM, Meuleman DG (1987): Time courses of the antithrombotic effects, bleeding enhancing effects and interactions with factors Xa and thrombin after administration of low molecular weight heparinoid ORG 10172 or heparin to rats. Thrombosis Research 48, 549–558.

Hogg PJ, Jackson CM (1989): Fibrin monomer protects thrombin from inactivation by heparin-antithrombin III: Implications for heparin efficacy. Proceedings of the National Academy of Sciences of the United States of America 86, 3619–3623.

Holmer E, Kurachi K, Soderstrom G (1981): The molecular-weight dependence of the rate-enhancing effect of heparin on the inhibition of thrombin, factor Xa, factor IXa, factor XIa, factor XIIa and kallikrein by antithrombin. Biochemical Journal 193, 395–400.

Holmer E, Mattsson C, Nilsson S (1982): Anticoagulant and antithrombotic effects of low molecular weight heparin fragments in rabbits. Thrombosis Research 25, 475–485.

Holmer E, Soderberg K, Bergquist D, Lindahl U (1986): Heparin and its low molecular weight derivatives: anticoagulant and antithrombotic properties. Haemostasis 16(Suppl. 2), 1–7.

Hook M, Bjork I, Hopwood J, Lindahl U (1976): Anticoagulant activity of heparin: Separation of high activity and low activity heparin species by affinity chromatography on immobilized antithrombin. FEBS Letters 66, 90–93.

Hoover MJ, Rosenberg R, Hearing W, Karnovsky MJ (1980): Inhibition of rat arterial smooth muscle cell proliferation by heparin. II. In vitro study. Circulation Research 47, 578–583.

Hull RD, Raskob GE, Hirsch J, Jay RM, Leclerc JR, Geerts WH, Rosenbloom D, Sackett DL, Anderson C, Harrison L, Gent M (1986): Continuous intravenous heparin compared with intermittent subcutaneous heparin in the initial treatment of proximal-vein thrombosis. New England Journal of Medicine 315, 1109–1114.

Hull RD, Raskob GE, Pineo GF, Green D, Trowbridge AA, Elliot CG, Colleagues (1991): A randomized double-blind trial of low molecular weight heparin in the initial treatment of proximal-vein thrombosis (Abstract). Thrombosis and Haemostasis 65(Suppl.), 872.

ISIS-2 Collaborative Group (1988): Randomized trial on intravenous streptokinase, oral aspirine, both or neither among 17, 187 cases of suspected acute myocardial infarction. Lancet 11, 349–360.

Jivegard L, Holm J, Schersten T (1988): Acute limb ischemia due to arterial embolism or thrombosis: influence of limb ischemia versus pre-existing cardiac disease on postoperative mortality rate. Journal of Cardiovascular Surgery(Torino) 29, 32–36.

Jorgensen PS, Warming T, Hansen K, Paltved CH, Berg HV, Jensen R, Kirchhoff-Jensen R, Kjaer L, kerbouche N, Leth-Espensen P, Narvestad E, Rasmussen SW, Sloth C, Torholm C, Wille-Jorgensen P (2002): Low molecular weight heparin (Innohep) as thromboprophylaxis in outpatients with a plaster cast: a venografic controlled study. Thrombosis Research 105, 477–480.

Kamphuisen PW, Lensen R, Houwing-Duistermaat JJ, Eikenboom JCJ, Harvey M, Bertina RM, Rosendaal FR (2000): Heritability of elevated factor VIII antigen levels in factor V Leiden families with thrombophilia. British Journal of Hematology 109, 519–522.

Karetova D, Stanek F, Ambrozy E, Bultas J, Jirat S, Linhart A, Muchova I, Tesar V (2007): Angiology in Practice (in Czech). 2nd ed. Maxdorf, Prague. 400 pp.

Kelton JG, Smith JW, Warkentin TE, Hayward CP, Denomme GA, Horsewood P (1994): Immunoglobulin G from patients with heparin-induced thrombocytopenia binds to a complex of heparin and platelet factor 4. Blood 83, 3232–3239.

Kikano GE, Brown MT (2007): Antiplatelet therapy for atherothrombotic disease: an update for the primary care physician. Mayo Clinic Proceedings. Mayo Clinic 82, 583–593.

Klein W, Buchwald A, Hillis SE, Monrad S, Sanz G, Turpie AG, van der Meer J, Olaisson E, Undeland S, Ludwig K (1997): Comparison of low molecular weight heparin with unfractionated heparine acutely and with placebo for 6 weeks in the management of unstable coronary artery disease: Fragmin in unstable coronary artery disease study (FRIC). Circulation 96, 61–68.

Klement P, Liao P, Bajzar L (1999): A novel approach to arterial thrombolysis. Blood 94, 2735–2743.

Lam LH, Silbert JE, Rosenberg RD (1976): The separation of active and inactive forms of heparin. Biochemical and Biophysical Research Communications 69, 570–577.

Lane DA, Pejler G, Flynn AM (1986): Neutralization of heparin-related saccharides by histidine-rich glycoprotein and platelet factor 4. Journal of Biological Chemistry 261, 3980–3986.

Lekakis J, Papamichael C, Vemmos C, Nanas J, Kontoyannis D, Stamatelopoulos S, Moulopoulos S (1997): Effect of acute cigarette smoking on endothelium-dependent brachial artery dilatation in healthy individuals. American Journal of Cardiology 79, 529–531.

Levine MN, Hirsh J, Gent M, Turpie AG Leclerc J, Powers PJ, Jay RM, Neemh J (1991): Prevention of deep vein thrombosis after elective hip surgery. A randomized trial comparing low molecular weight heparin with standard unfractionated heparin. Annals of Internal Medicine 114, 545–551.

Lindahl U, Hook M (1978): Glycosaminoglycans and their binding to biological macromolecules. Review. Annual Review of Biochemistry 47, 385–417.

Lindahl U, Backstrom G, Hook M, Thunberg L, Fransson LA, Linker A (1979): Structure of the antithrombin-binding site in heparin. Proceedings of the National Academy of Sciences of the United States of America 76, 3198–3202.

Lindahl U, Thunberg L, Backstrom G, Riesenfeld J, Nordling K, Bjork I (1984): Extension and structural variability of the antithrombin-binding sequence in heparin. Journal of Biological Chemistry 259 12368–12376.

Lundin L, Larsson H, Kreuger J, Kanda S, Lindahl U, Salmivirta M, Claesson-Welsh L (2000): Selectively desulfated heparin inhibits fibroblast growth factor-induced mitogenicity and angiogenesis. Journal of Biological Chemismy 275, 24653–24660.

Magnani B (1989): Plasminogen Activator Italian Multicenter Study (PAIMS): Comparison of intravenous recombinant single-chain human tissue-type plasminogen activator. Journal of the American College of Cardiology 13, 19–26.

Mahadoo J, Hiebert C, Jaques LB. (1977): Vascular sequestration of heparin. Thrombosis Research 12, 79–90.

Majack RA, Cook SC, Bornstein P (1985): Platelet-derived growth factor and heparin-like glycosaminoglycans regulate thrombospondin synthesis and deposition in the matrix by smooth muscle cells. Journal of Cell Biology 101, 1059–1070.

Majack RA, Cook SC, Bornstein P (1986): Control of smooth muscle cell growth by components of the extracellular matrix: Autocrine role for thrombospondin. Proceedings of the National Academy of Sciences of the United States of America 83, 9050–9054.

Majack RA, Goodman LV, Dixit VM. (1988): Cell surface thrombospondin is functionally essential for vascular muscle cell proliferation. The Journal of Cell Biology 106, 415–422.

Marciniak E (1973): Factor Xa inactivation by antithrombin III. Evidence for biological stabilization of factor Xa by factor V-phospholipid complex. British Journal of Hematology 24, 391–400.

Marder VJ, Francis CW (1990): Clinical aspects of fibrinolysis. In: Williams VJ (ed.): Hematology. 4th ed. McGraw-Hill Publishing Company, New York. 1543–1558.

McLean J (1916): The thrombotic action of cephalin. American Journal of Fysiology 41, 250–257.

McNamara TO, Gardner K (1991): Coaxial system improves thrombolysis of ischemia. Diagnostic Imaging 13, 122–133.

Meeking DR, Cummings MH, Thorne S, Donald A, Clarkson P, Crook JR, Watts GF, Shaw KM (1999): Endothelial dysfunction in Type 2 diabetic subjects with and without microalbuminuria. Diabetic Medicine: a journal of the British Diabetic Association 16, 841–847.

Melissari E, Parker CJ, Wilson NV, Monte G, Kanthou C, Pemberton KD, Nicolaides KH, Barrett JJ, Kakkar

VV (1992): Use of low molecular weight heparin in pregnancy. Thrombosis and Haemostasis 68, 652–656.

Monreal M, Lafoz E, Olive A, del Rio L, Vedia C (1994): Comparison of subcutaneous unfractionated heparin with low molecular weight heparin (Fragmin) in patients with venous thromboembolism and contraindications to coumarin. Thrombosis and Haemostasis 71, 7–11.

Mueller RL (2004): First-generation agents: aspirin, heparin and coumarins. Best Practice & Research. Clinical Haematology 17, 23–53.

Muir JM, Andrew M, Hirsh J, Weitz JI, Young E, Deschamps P, Shaughnessy SG (1996): Histomorphometric analysis of the effects of standard heparin on trabecular bone in vivo. Blood 88, 1314–1320.

Ockelford PA, Carter CJ, Mitchell L, Hirsh J (1982): Discordance between the anti-Xa activity and the antithrombotic activity of an ultra-low molecular weight heparin fraction. Thrombosis Research 28, 401–409.

Ofosu FA, Barrowcliffe TW (1990): Mechanisms of action of low molecular weight heparines and heparinoids. In: Hirsh J (ed.): Antithrombotic Therapy. Bailliere's Clinical Hematology. 3rd ed. Bailliere-Tindal Ltd., London. 505–529.

Oldberg A, Wasteson A, Busch C, Hook M (1980): Characterization of a platelet endoglycosidase degrading heparin-like polysaccharides. Biochemistry 19, 5755–5762.

Olson ST, Shore JD (1982): Demonstration of a two-step reaction mechanism for inhibition of alpha-thrombin by antithrombin III and identification of the step affected by heparin. Journal of Biological Chemistry 257, 14891–14895.

Olson ST, Srinivasan KR, Bjork I, Shore JD (1981): Binding of high affinity heparin to antithrombin III: Stopped flow kinetic studies of the binding interaction. Journal of Biological Chemistry 256, 11073–11079.

Olsson P, Lagergren H, Ek S (1963): The elimination from plasma of intravenous heparin. An experimental study on dogs and humans. Acta Medica Scandinavica 173, 619–630.

Palm M, Mattsson CH (1987): Pharmacokinetics of heparin and low molecular weight heparin fragment (Fragmin) in rabbits with impaired renal or metabolic clearance. Thrombosis and Haemostasis 58, 932–935.

Pauer HU, Burfeind P, Koestering H, Emons G, Hinney B (2003): Factor XII deficiency is strongly associated with primary recurrent abortions. Fertility and Sterility 80, 590–594.

Penka M, Bulikova A (2006): Antithrombotic therapy in the classic sense. KF – educational annex Cardiology Revue (in Czech) 4, 28–34.

Perretti M, Page CP (2000): Heparin and inflammation: a new use for an old GAG? Gut 47, 14–15.

Pini M, Pattacini C, Quintavalla R, Poli T, Megha A, Tagliaferri A, Manotti C, Dettori AG (1990) Subcutaneous vs intravenous heparin in treatment of deep venous thrombosis – a randomized clinical trial. Thrombosis and Haemostasis 64, 222–226.

Piper J (1947): The fate of heparin in rabbits after intravenous injection. Filtration and tubular secretion in the kidneys. Acta Pharmacologica et Toxicologica (Copenh) 3, 373–384.

Poul H (2006): Thrombophilia important in the pathogenesis of venous thromboembolism (in Czech). www.thrombosis.cz.

Prandoni P (1991): Fixed dose LMW heparin (CY216) as compared with adjusted dose intravenous heparin in the initial treatment of symptomatic therapy of proximal venous thrombosis (Abstract). Thrombosis and Haemostasis 65(Suppl), 872.

Preissner KT, Muller-Berghaus G (1987): Neutralization and binding of heparin by S-protein/vitronectin in the inhibition of factor Xa by antithrombin III. Journal of Biological Chemistry 262, 12247–12253.

Prifti E, Bonacchi M, Leacche M, Miraldi F (2000): Undergoing cardiopulmonary bypass using enoxaparin only during a cardiac transplantation procedure. European journal of cardio-thoracic surgery: official journal of the European Association for Cardio-thoracic. Surgery 17, 760–762.

Puchmayer V, Roztocil K (2000): Practical Angiology (in Czech). 1st ed. Triton, Prague. 191 pp. ISBN 80-7254-099-8.

Pukac LA, Hirsh GM, Lormeau JC, Petitou M, Choay J, Karnovsky MJ (1991): Antiproliferative effects of novel nonanticoagulant heparin derivatives on vascular smooth muscle cells in vitro and in vivo. American Journal of Patology 139, 1501–1509.

Reilly CF, Fritze LMS, Rosenberg RD (1986): Heparin inhibition of smooth muscle cell proliferation: a cellular site of action. Journal of Cellular Physiology 129, 11–19.

Rice TW, Lumsden AB (2006): Optimal medical management of peripheral arterial disease. Review. Vascular and Endovascular Surgery 40, 312–327.

Rizzoni D, Porteri E, Castellano M (1998): Endothelial dysfunction in hypertension is independent from the etiology and from vascular structure. Hypertension 31, 335–341.

Rocek M (2005): Thrombolysis of arterial occlusion of lower extremity and occlusion of peripheral bypass. In: Krajina A, Peregrin JH (eds.): Interventional Radiology; Minimally Invasive Therapy (in Czech). 1st ed. Aurius, Prague, 151–158. ISBN 80-86703-08-8.

Rosenberg RD (1975): Actions and interactions of antithrombin and heparin. Review. New England Journal of Medicine 292, 146–151.

Rosenberg RD (1987): The heparin-antithrombin system: A natural anticoagulant mechanism. In: Colman RW, Hirsh J, Salzman EW (eds.): Hemostasis and Thrombosis: Basic Principles and Clinical Practice. 2nd ed. J.B. Lippincott, Philadelphia. 1373–1392.

Rosenberg RD, Jordan RE, Favreau LV, Lam LH (1979): Highly active heparin species with multiple binding sites for antithrombin. Biochemical and Biophysical Research Communications 86, 1319–1324.

Rutherford RB, Baker JD, Ernst C, Johnston KW, Porter JM, Ahn S, Jones DN (1997): Recommended standards for reports dealing with lower extremity ischemia: revised version. Journal of Vascular Surgery 26, 517–538.

Salas A, Sans M, Soriano A, Reverter JC, Anderson DC, Pique JM, Panes J (2000): Heparin attenuates TNF-alpha induced inflammatory response through a CD11b dependent mechanism. Gut 47, 88–96.

Salzman EW, Rosenberg RD, Smith MH, Lindon JN, Favreau L (1980): Effect of heparin and heparin fractions on platelet aggregation. Journal of Clinical Investigation 65, 64–73.

Schumann R, Rieger J, Ludwig M (2007): Acute peripheral arterial occlusive disease. Medizinische Klinik (Munich) 102, 457–471.

Shaughnessy SG, Young E, Deschamps P, Hirsh J (1995): The effects of low molecular weight and standard heparin on calcium loss from fetal rat calvaria. Blood 86, 1368–1373.

Sie P, Ofosu F, Fernandez F, Buchanan MR, Petitou M, Boneu B (1986): Respective role of antithrombin III and heparin cofactor II in the in vitro anticoagulant effect of heparin and of various sulphated polysaccharides. British Journal of Hematology 64, 707–714.

Sobel M, McNeill PM, Carlson PL, Kermode JC, Adelman B, Conroy R, Marques D. (1991): Heparin inhibition of von Willebrand factor-dependent platelet function in vitro and in vivo. Journal of Clinical Investigation 87, 1787–1793.

Starck EE, Mc Dermott JC, Crummy AB, Turnipseed WD, Acher CW, Burgess JH (1985) Percutaneous aspiration tromboembolectomy. Radiology, 156, 61–66.

Tanis BC, Bloemenkamp DG, van den Bosch MA, Kemmeren JM, Algra A, van de Graaf Y, Rosendaal FR (2003): Prothrombotic coagulation defects and cardiovascular risc factors in young women with acute myocardial infarction. British Journal of Hematology 122, 471–478.

TIMI Study Group (1985): The Thrombolysis in Myocardial Infarction (TIMI) trial. New England Journal of Medicine 312, 932–936.

Trocme SD, Li H (2000): Effect of heparin-surface-modified intraocular lenses on postoperative inflammation after phacoemulsification: a randomized trial in a United States patient population. Heparin-Surface-Modified Lens Study Group. Ophtalmology 107, 1031–1037.

Turpie AG (1998): Pharmacology of the low-molecular-weight heparins. Review. American Heart Journal 135 (Suppl.), S329–S335.

Turpie AG, Robinson JG, Doyle DJ, Mulji AS, Mishkel GJ, Sealey BJ, Cairns JA, Skingley L, Hirsh J, Gent M (1989): Comparison of heigh-dose with low-dose subcutaneous heparin to prevent left ventricular mural thrombosis in patient with acute transmural anterior myocardial infarction. New England Journal of Medicine 320, 352–357.

Vacha J (1999): Pathophysiology IV (in Czech). 1st ed. Masaryk University, Brno. 271 pp. ISBN 80-210-2207-8.

Van Ryn-McKenna J, Ofosu FA, Hirsh J, Buchanan MR (1989): Antithrombotic and bleeding effects of glycosaminoglycans with different degrees of sulphation. British Journal of Hematology 71, 265–269.

Walker MG, Shaw JW, Thomson GJ, Cumming JG, Thomas ML (1987): Subcutaneous calcium heparin versus intravenous sodium heparin in treatment of established acute deep vein thrombosis of the legs: a multicentre prospective randomized trial. British Medical Journal 294, 1189–1192.

Warkentin TE (2004): Clinical picture of heparin-induced thrombocytopenia. In: Warkentin TE, Greinacher A (eds.): Heparin-induced Thrombocytopenia. 3rd ed. Marcel Dekker Inc., New York. 53–106.

Weaver FA, Comerota AJ, Youngblood M, Froehlich J, Hosking JD, Papanicolaou G (1996): Surgical revascularization versus thrombolysis for nonembolic lower extremity native artery occlusions: results of a prospective randomized trial. The STILE Investigators. Surgery versus Thrombolysis for Ischemia of the Lower Extremity. Journal of Vascular Surgery 24, 513–521.

Weitz JI (1997): Low-molecular-weight heparins. Review. The New England Journal of Medicine 337, 688–698.

Weitz JI, Leslie B, Hudoba M (1991): Thrombin remains bound to soluble fibrin degradation products and is partially protected from inhibition by heparin-antithrombin III (Abstract). Thrombosis and Haemostasis 65, 931.

White HD, Rivers JT, Maslowski AH, Ormiston JA, Takayama M, Hart HH, Sharpe DN, Whitlock RM,

Norris RM (1989): Effect of intravenous streptokinase as compared with that of tissue plasminogen activator on left ventricular function after first myocardial infarction. New England Journal of Medicine 320, 817–821.

Whittemore AD, Clowes AW, Couch NP, Mannick JA (1981): Secondary femoropopliteal reconstruction. Annals of Surgery 193, 35–42.

Wilcox JN, Ollerenshaw J, Zhong C, Hayzer DJ, Rodriguez J, Subramanian RR, Harker LA, Hanson SR, Kelly AB, Runge MS (1992): Localization of thrombin receptor expression in proliferating smooth muscle cells in vivo (Abstract). Circulation 86, 1150.

Wilson NV, Salisbury JR, Kakkar VV (1991): Effect of low molecular weight heparin on intimal hyperplasia. British Journal of Sumery 78, 1381–1383.

Wright TC, Castellot JJ, Diamond JR, Karnovsky MJ (1989): Regulation of cellular proliferation by heparin and heparan sulfate. In: Lane DA, Lindahl U (eds.): Heparin, Chemical and Biological Properties, Clinical Applications. CRC Press Inc., Boca Raton, Florida. 295–316.

Yagnik AT, Lahm A, Meola A, Roccasecca RM, Ercole BB, Nicosia A, Tramontano A (2000): A model for the hepatitis C virus envelope glycoprotein E2. Proteins 40, 355–366.

Yamada H, Kato EH, Kobashi G, Ebina Y, Shimada S, Morikawa M, Yamada T, Sakuragi N, Fujimoto S (2001): Reccurent pregnancy loss: etiology of thrombophilia. Review. Seminars in Thrombosis and Hemostasis 27, 121–129.

Zed PJ, Tisdale JE, Borzak S (1999): Low-molecular-weight heparins in the management of acute coronary syndromes. Archives of Internal Medicine 159, 1849–1857.

Received: 2010–10–07
Accepted after corrections: 2010–11–15

Corresponding Author:

MUDr. Martin Dvorak, Ph.D., 2nd Department of Surgery, St. Anne's University Hospital, and Faculty of Medicine, Masaryk University, Pekarska 53, 656 91 Brno, Czech Republic
Tel. +420 606 426 170, E-mail: martin.dvorak@fnusa.cz