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Review

Exploring ischemia-induced vascular lesions and potential pharmacological intervention strategies

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Summary. Structural changes in vessels under the influence of ischemia play an important role in the pathogenesis of many diseases, most important of which are stroke and myocardial infarction or myocardial insult. Over the years, information has been gathered, which implicate a role for ischemic vascular changes in the pathogenesis of crush-syndrome, atherosclerosis and other vascular diseases. When blood vessels are damaged they become unresponsive to a stimulus, which normally elicits vasodilatation and can lead to intraluminal thrombosis and ischemic events. The aim of this review is to explore the structural changes seen in vessels affected by ischemia reperfusion injury. With ischemia, the development of observable changes to vascular structure is multifactorial. One key factor is reperfusion ischemic injury. Moreover, the duration of the ischemic event is an important factor when determining both the prognosis and the type of morphological change that is observable in affected vessel walls. In this regard, the deleterious progression of blood flow impairment and its severity depends on the specific organ involved and the type of tissue affected. Further, there are regional differences within affected tissues and the degree of microvascular injury is well correlated with differences in the nature and severity of the ischemic event. Any method aimed at preventing and treating ischemic reperfusion injuries in vessels, based on these investigations, should likewise be able to decrease the early signs of brain, cerebrovascular and heart injury and preserve normal cellular architecture.

Key words: Ischemia, Vascular Endothelium, Myocardial ischemia, Brain Ischemia and reperfusion, Drug treatment

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Introduction

It is widely accepted that the duration of an irreversible ischemic event is 10-15 minutes for the brain. At the same time, the observable changes in the vessel wall of the large vessels are proportional to the duration of ischemia. Even brief ischemia and reperfusion can cause functional coronary vascular injury, which is characterized by increased microvascular permeability and impaired endothelium-dependent vasodilation. Reperfusion after prolonged ischemia leads to the appearance of a large number of smooth muscle cells (SMC) within the vessel intima. Despite the fact that endothelial cells (EC) are very resistant to anoxia, we should underscore the fact that these cells can display a very heterogeneous response in sensitivity to ischemic events. Another factor in ischemic events is a negative reperfusion or called "*no-reflow*" phenomenon in the microvessels. Longer periods of ischemia (60 or more minutes), result in "*no-reflow*" phenomenon and degenerative vascular changes during reperfusion. EC swell up, lose their pynocytic vesicles and form spherical cytoplasmic protrusions into the capillary lumen. Prolonged ischemia (over 3 hours) causes the rupture of microvascular walls (Nevalainen et al., 1986).

The duration of reperfusion, also may play an important role in the observable morphological changes of vessels after long-term ischemia. After specific reperfusion duration, the EC enters the vessel space in the reflow zones with numerous protrusions. Observable chromatin condensation occurs in the nucleus and a decreased number of picnotic vesicles become evident. These observations, as well as the detection of capillary rupture and hemorrhage in the *no-reflow* and microthrombosis zones are commonly made by light microscopy (LM). For example, during reconstructive surgery, it is often necessary to clamp the abdominal aorta. This temporary compression is accompanied by ischemia of the muscles in the lower extremities (Sidorenko et al., 1987). Ischemia-reperfusion of this

sort alters the redox state of the affected tissue, which has the following effect on the observable morphological changes.

Ischemia can induce coronary endothelial dysfunction as well, which is characterized by a lower response to endothelium-dependent vasodilators, such as acetylcholine (Ach) and serotonin (5HT). However, there is an unaltered response to endothelium-independent vasodilators, such as nitroglycerin (NTG) (Martorana et al., 1998). Because the endothelial compartment interacts with circulating blood and the adventitial compartment with the surrounding tissue, reperfusion of the ischemic myocardium results in structural changes in the capillary bed, which may contribute to decreased microcirculatory blood flow ("no reflow") and heart sufficiency to induce lipid peroxidation and other oxidative stresses. It also induces EC swelling and a reduction in luminal spaces. In addition, the luminal plasmalemma membrane blebs and capillary "constriction" occur under ischemic conditions regardless of the glutathione status or any change in malonaldehyde (MDA) concentrations (Molyneux et al., 2002).

Ischemia can be mediated by other factors as well. Cerebral vasospasm after subarachnoid hemorrhage (SAH) is a prolonged contraction that leads to cerebral ischemia or infarction. Morphological studies of cerebral arteries during vasospasm have shown extensive necrosis of SMC and desquamation and dystrophy of EC. The mechanism of cellular death is unknown (Zubkov et al., 2000). Despite years of research, delayed cerebral vasospasm remains a serious complication of subarachnoid hemorrhage. Recently, it has been proposed that endothelin-1 (ET-1) mediates vasospasm (Aliev and Burnstock, 1998). In this regard, a group of researchers examined this hypothesis in a series of experiments aimed at elucidating the role of ET-1 in SAH in a primate model of SAH. Serial ET-1 levels were measured in samples from the perivascular space using a microdialysis technique and in cerebrospinal fluid (CSF) and plasma during the development and resolution of delayed vasospasm (Pluta et al., 1997). In the perivasculature, no correlation was found between the perivascular levels of ET-1 and the development of vasospasm or its resolution (Pluta et al., 1997). In addition it has been speculated that ET-1 is released from astrocytes, but not EC, during hypoxia and is released from the brain after transient ischemia. There is no relationship between ET-1 and vasospasm *in vivo* or between ET-1 and oxyhemoglobin, a putative agent of vasospasm, *in vitro*. The increase in ET-1 levels in CSF after SAH from a ruptured intracranial aneurysm appears to be the result of cerebral ischemia rather than reflecting the cause of cerebral vasospasm (Pluta et al., 1997). The mechanism of ischemic influence on the vessel wall must be divided into 2 stages: the stage of proper ischemia (hypoxia), and the metabolic breaches connected with it, as well as the stage of reperfusion of the ischemic vessel by oxygenized blood, which plays a key role in post-ischemic pathology formation (Aliev et al., 1993).

The effect of ischemia on the central large arteries

Age-matched shame-operated rat did not show any particular changes in the vascular wall cell ultrastructure (Fig. 1A,B). The structural changes in the vessel wall of the large arterial vessels are proportional to the duration of ischemia (Fig. 2A-C). In the earlier stage (during the first 60 minutes) changes are characterized by the appearance of “craters”, “swellings”, and increased permeability of the vessel wall (Fig. 2A; Kawamura et al., 1974; Aliev and Mironov, 1987). However, during the earliest ischemic events, the ultrastructural changes or the changes of the endothelial surface, i.e., after 15 minutes of ischemia (Dauber et al., 1990), or even after 90 minutes of ischemia (Tsao et al., 1990) was not detectable. One of the earliest sighs the effect of ischemia is the activation and penetration of leukocytes through the endothelial layer (probably via extended interendothelial contacts) into the subendothelial space. After 2-hours of ischemia, leukocytes penetration can reach even the muscular layer (Fig. 2B; Park et al., 1985). Further, as the pathological processes advance, there is a gradual denudation of the vessel internal surface was seen (Elemer et al., 1976; Bhawan et al., 1977). At this point, the process of migration of smooth muscle cells (SMC) into the intima begins (Guyton and Karnovsky, 1979; Park et al., 1985). The same pattern of ischemic events was seen also after the sudden death of the organism. More recently, we have demonstrated (Aliev and Mironov, 1987) that after the 15 minute postmortem period the number and the amount of adherent fibrin films increases on the luminal surface of the EC. The ischemic events also affected the cytoskeleton of the EC. Morphologically, the structure of the cytoskeleton becomes more visible compared to sham groups (Aliev and Mironov, 1987, 1989). Individual craters are rarely observed on the surface of the EC. After 30 minutes, the above-mentioned changes start intensifying. In addition, the numbers of spindle-shaped EC frequently were seen in the endothelial monolayer. After 1 hour, the cytoplasm of the EC contained some myelin-like figures. The number of Weibel-Palade bodies especially in the perinuclear zone of the cell body significantly increases. In several EC, swelling accompanies the rupture of the surrounding plasmalemma. The stress reaction of the cytoskeleton is increased, especially in the cortical zone of the cytoplasmic matrix. At the end of a 2-hour postmortem period, the number of leukocytes adherent to endothelium increases and coexists with the presence of a fibrin film on the endothelial surface (Aliev and Mironov, 1987, 1989).

It has been well documented that the mitochondria appear to be a primary target in ischemia/reperfusion - induced tissue damage (Aliev and Mironov, 1989; Cirillo et al., 1994). The role of mitochondrial lesions in the

pathogenesis of non-reversible cellular ischemia is controversial. Recently, the release of an unidentified substance or modifier, which has been described as a sulfhydryl group (SH) inducible factor, and mitochondrial permeability transition pore (MPT) inductor phenyl arsine oxide (PAO) has been recently found in vitro on isolated guinea pig and rat heart mitochondria (Brandao et al., 2003). The factor was also released under conditions of oxidative stress. Further, the inability to restore mitochondrial function is correlated with the inability to reverse cell damage in various tissues (Aliev and Mironov, π1989; Cirillo et al., 1994; Brandao et al., 2003). In contrast to healthy mitochondria, cells affected by ischemia, generally have an edematous matrix. Disintegration of the cell cytoplasmic matrix also correlates with the duration of ischemia (Aliev and Mironov, 1987; Aliev et al., 1993). After 4 hours, the presence of microdefects, such as deendothelialized zones with adherent platelets on the subendothelial zone, was seen. The nuclear chromatin usually is sharply concentrated on the inner part of the nuclear membrane. Moreover, the electron density of nuclear matrix substantially decreases. Dystrophic changes to cytoskeletal structures also were observed (Aliev and Mironov, 1987; Aliev et al., 1993). The edema of the subendothelial layer roughly increases. By the end of the 6th hour, the above-mentioned changes become universal and almost all are present in nearly all

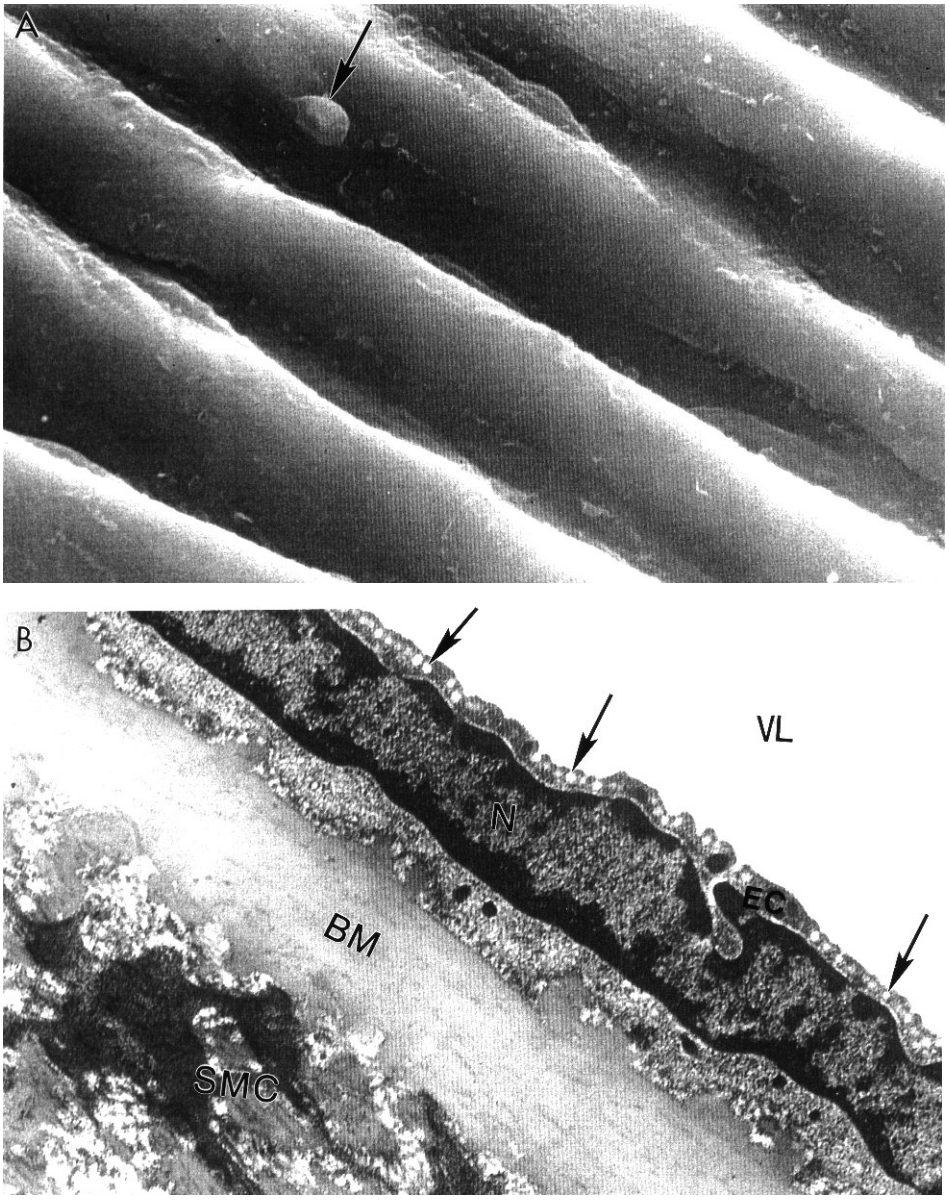

Fig. 1. The ultrastructural characteristics of the rat abdominal aortic EC from the shame operated animals. **A.** Scanning electron microscopic (SEM) characteristics of the luminal surface of the rat abdominal aorta. Endothelial monolayer did not show any visible changes in the morphology of their luminal surface. Single arrow indicates adhered erythrocytes. Original Magnification x 4,900. Sample was obtained by using perfusion fixation following the preparation of the SEM native specimens. Aortic samples were examined by using Hitachi -405A SEM with accelerating voltage 20kV. **B.** Transmission electron microscopic (TEM) characteristics of the rat abdominal aortic endothelium from the shame-operated animals. EC did not show any particular changes in their ultrastructure (arrows indicate cytoplasmic vesicles). TEM, original magnification x 10,600. Aortic tissue was obtained by using perfusion fixation and following preparation of the sample for routine TEM. Ultrathin sections were examined by using TEM Jeol 1010.
BM: basal membrane; EC: endothelial cell; N: cell nucleus, SMC: smooth muscle cell; VL: vessel lumen.

EC. The number and amount of lipid droplets, which accumulates in the cytoplasm and ischemic electron-dense material, appears to be a permanent feature of the mitochondrial matrix (Aliev and Mironov, 1987; Aliev et al., 1993). Most of the mitochondria are subjected to lysosomal transformation. By the end of a twenty-four hour post ischemic event, practically all of EC denudates and/or desquamates (Aliev and Mironov, 1987; Aliev et al., 1993). This observation demonstrates that the aortic endothelial changes after ischemia are sufficient to match changes to vessels after the sudden death of the organism (Aliev and Mironov, 1987). Hypoxia, having acted on the aortic endothelium, causes some staged viability changes to the EC. The distinguishing feature of ischemia was an active involvement of blood cells in this process. For example, after the 1 hour reperfusion in the skin arteries stasis of erythrocytes occurs, as well as the accumulation of platelets, the adhesion of monocytes and neutrophils and the damage of the EC intensifies (Marzella et al., 1988). Under the influence of ischemia in veins the leukocytes also adhered to endothelium (Bednar et al., 1984; Mullane and McGiff, 1985; Granger et al., 1989; Zeintl et al., 1989; Erlansson et al., 1991) and accompanies the infiltration of leukocytes and platelets through the endothelial monolayer. However fibrin accumulation has not been seen universally (Endrich et al., 1990). The EC and SMC have a very low basal rate of energy consumption. Thus, hypoxic disturbance is hardly probable during incomplete occlusion of the vessel when a definite (very low) level of blood flow is preserved (Headrick et al., 1990). In this regard, during the early period of anoxia, the decrease of blood pressure and accumulation of metabolic products appeared to be the main factor responsible for damage of the vessel wall cells including the EC (Aliev and Mironov, 1987; Aliev et al., 1993). It has been demonstrated that the maintenance of blood pressure during ischemia and the transport of metabolic products from the vessel essentially reduces EC ultrastructural damage in the main vessels (Aliev and Mironov, 1987).

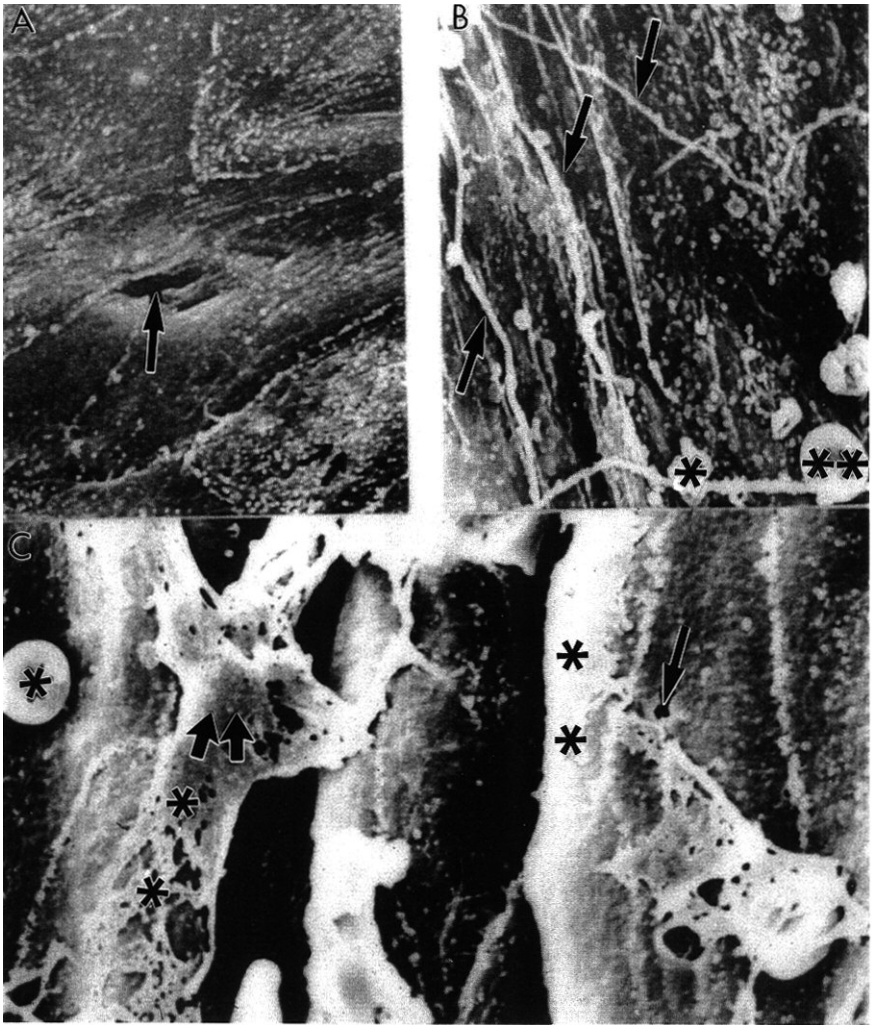

Fig. 2. Three dimensional characteristics of the changes on the rat abdominal aorta after the ischemia without reperfusion. **A.** 1 hour ischemia. Main changes show the extension of inter-endothelial contacts (indicated by single arrow). The nuclear portion of the luminal plasmalemma of the EC shows the overexpression of microblebs (indicated by short thick arrow). SEM x 3,200. **B.** 2 hours ischemia. Main changes of the endothelial layer shows an increased number of microvillies and blebs on the EC luminal surface, formation of fibrin film and adhesion of erythrocytes (double asterisk) and platelets (single asterisk) on the luminal surface of the EC. SEM, original magnification x 3,000. **C.** 2 hours ischemia. The vessel surface is characterized by the presence of fibrin films (double arrow), adhesion of erythrocytes (single asterisk) and craters (indicated by single arrow). SEM, original magnification x 4,000. All samples were obtained using perfusion fixation followed by the preparation of the SEM native specimens. All aortic samples were examined by using Hitachi -405 A SEM with the accelerating voltage of 20kV.

Therefore, ischemia is complex processes, which essentially changes the function of the vessel wall. The permeability of the endothelium increases (Finck et al., 1986) and during the first few minutes of anoxia the production of nitric oxide (NO) by the endothelium is already altered (Headrick et al., 1990). Moreover, these abnormalities are associated with increased vasoconstriction as a reaction of the vessel in response to the influence of thrombin and endothelial damage (Ku, 1982). However, more study is needed to determine the real nature of this process.

Morphological changes in microvessels affected by ischemia

The changes in microvessels in the presence of ischemia are notable and because of definite specificity of the tissue and organs. Thus, 10 and 60 minutes after coronary occlusion the number of EC with cytoplasmic edema increase and tissue edema, which leads to a decline in capillary space. The number of pynocytic vesicles in the EC cytoplasm decreases rapidly. After 120 minutes, all EC already demonstrate signs of dystrophic changes, such as the swelling of mitochondria and endoplasmic reticulum. If the period of ischemia is prolonged (more than 6 hours), the intensity of the injury is intensified and process is generalized to the capillary perivascular regions (Armiger and Gavin, 1975). Importantly, the activity of lysosomal enzymes is dramatically increases and probably plays an active role in the processes of cell degeneration.

In the microvessels of skeletal muscle after 1-3 hours of ischemia, cytoplasmic edema and subcellular compartment injury can only been found in several EC (Gidlof et al., 1988; Cirillo et al., 1992). In human biopsy material, it has been demonstrated that ischemia, which appears during the development of a "*dry*" heart, leads to edema of endothelial cytoplasm, thinning of peripheral zones, and development of cytoplasmic protrusions and blebs of luminal plasmalemma. This accompanies the formation of perivascular edema (Schaper et al., 1982). Diffuse edema of the EC has been found in kidney ischemia (Flores et al., 1972). After ischemia in the brain, the perivascular edema of glial cells and the formation of blebs and microtrusion on the luminal surface of endothelium have been reported (Chiang et al., 1968; Arsenio-Nunes et al., 1973). Scanning electron microscopy (SEM) observation of coronary arterial surfaces shows the formation of craters after the infusion of injury stimuli-containing solution (Sala et al., 1996). This abnormality accelerates when infusion solution contains purified human leukocytes (PMNL). The number of adherent PMNL to the EC is correlated with an increased number of microvilli on the EC and the presence of nonviable, desquamate and/or fusiform EC. SEM and transmission electron microscopy (TEM) of myocardial microvessels, shows the presence of perivascular and intermuscle edema, presence of activated PMNL and a decreased number of active or so-called patent microvessels (Sala et al., 1996). Taken together, these data provide evidence of a close interaction between PMNL and myocardial EC, resulting in enhanced sulfidopeptide-leukotrienes (sLT) formation via transcellular biosynthesis, which originates from a transfer of PMNL-derived leukotrienes class A4 (LTA4) to EC. These potent proinflammatory autacoids are responsible for coronary vasospasm and the observed morphological alterations (Sala et al., 1996). These morphological alterations were significantly blunted by 5-lipoxygenase inhibitor MK-886 or SKF 104353 (Sala et al., 1996).

Subcellular mechanism of the vascular lesions during ischemia/reperfusion

Microvessels

The structural changes of the EC, after ischemia and reperfusion, have been reviewed in detail (Armiger and Gavin, 1975; Marzella et al., 1988; Gidlof et al., 1988; Mehta et al., 1989; Cirillo et al., 1994). The reperfusion of ischemic tissues leads to various injuries of the microcirculatory bed, such as edema of the EC, the increase of vessel permeability, the intensification of capillary filtration and capillary thrombosis (Engler et al., 1983; Gidlof et al., 1988; Granger, 1988; Aliev et al., 1993). These changes induce acute inflammatory reaction, such as activation and following adhesion and diapedesis of leukocytes to interstitial tissue (Zeintl et al., 1989).

The short-term or transitory ischemia, but not long-term ischemia is usually followed by reactive hyperemia. The duration of ischemia, has a direct effect on blood flow, which decrease and dependent on the specific organ. For example, it takes up to 10-15 minutes for non-reversible damage in the brain (Fischer and Ames, 1972; Wade et al., 1975), 60-90 minutes for myocardium (Kloner et al., 1974, 1975), 1-2 hours for the kidney (Flores et al., 1972), 6-8 hours for skin (Willms-Kretschmer and Majno, 1969) and 6-10 hours for peripheral muscle (Aliev et al., 1993).

The main damaging effect of the no reflow phenomenon in microvessels appears to be lesions of the vascular EC. Free radicals as a product of ischemia appeared to be primarily responsible for ischemic damage (Aliev et al., 1993; Cirillo et al., 1994; Salvatico et al., 1994; Sala et al., 1996). In addition, erythrocyte stasis enhances these lesions. Ischemia of the skeletal muscles (90-180 minutes) induces decreases the number of perfused capillaries, which is associated with an increased loss in their vasomotor response. Very often, the capillary lumen is completely occupied by activated leukocytes. However, the intensity of these lesions varies and mainly depends on the resistance of the EC plasmalemma to the injury stimuli (Gidlof et al., 1988). The abnormal blood flow and cardiac metabolic condition appears to be a major factor that influences the progress of ischemic cardiac lesions. Myocardial

ischemia is promoted by either an increase in oxygen demand or a shortage of oxygen supply (Asano et al., 2003). The influence of ischemia on myocardium perfusion, *in vivo*, can be partially dependant from the occlusion of vessels by leukocytes and aggregation of platelets (Engler et al., 1986; Mehta et al., 1989; Reynolds and McDonagh, 1989; Eidt et al., 1989). The number of infiltrated leukocytes in tissue is correlated with the degree of tissue edema. For instance, the edematous regions with low capillary blood flow are predominantly characterized by an increased number of infiltrated leukocytes to interstitial tissue. Moreover, the cytoplasm of the EC in these vessels is also edematous and shows the changes of the chemical properties of their glycogalix (Armiger and Gavin, 1975). However, a study by Hauschild U. and coworkers (Baghirzade et al., 1970) considered that the decline in the capillary space induced by the increase of perivascular tissue edema appears to be the main factor influencing the capillary bed after ischemia of the myocardium. A short period (20 minutes) of myocardial ischemia leads to the dilation of the microvascular bed because of the increase in tissue adenosine level. The longer period (60 minutes) leads to injury of the vessels and to the appearance of the "*no-reflow*" phenomenon. Thus, the vascular EC appears to be a primary target for ischemia. One of the earlier markers of EC changes is the formation of edematous structures in their cytoplasmic matrix, which appear as decreases in the electron density of the cytoplasmic matrix, loss of pynocytic vesicles with spherical cytoplasmic protrusions into the vessel space. Prolonged ischemia (3 hours) leads to the rupture of the vessel wall. These microvessels further appear to be the source for the development of hemorrhages. The occlusion of capillaries by erythrocytes is often observed. However, the "*no-reflow*" phenomenon can appear without the occlusion of vessels (Nevalainen et al., 1986). According to Lamping and Gross (1985), Haundeschild and Gould (1979) and Smith (1980) 10-15 minutes of complete coronary occlusion leads to decreased heart perfusion and, therefore, induces non-reversible myocardial tissue damage.

The duration of recirculation is the key factor in the future initiation of post-ischemic tissue damage. A short period of reperfusion (5-20 minutes) does not have additional damaging effects on the EC after 40-minutes of myocardial ischemia. Surprisingly, increasing the time of reperfusion (90 minutes) results in the presence of a vessel with an increased number of protrusions, decreased numbers of pynocytic vesicles and condensation of nuclear chromatin on post-ischemic cellular compartments. However, the presence of fibrin accumulation was substantially less evident throughout the zone occupied by "*no-reflow*" phenomenon. Consequently, in the presence of long-term ischemia, the formation of a large number of non-reversible damage of EC, which leads in future to the "*no-reflow*" phenomenon (Kloner et al., 1975). Further, accelerated effects of this damage increase the risk for generalization of tissue and/or cell damage to the entire organ.

Although ultrastructural studies suggest that coronary vascular injury is a result of prolonged ischemia and subsequent reperfusion, it remains unclear whether functional changes in coronary microvascular systems develops after the brief "in vivo" ischemia or not (Dauber et al., 1990). In other organs, EC permeability appears to be a sensitive indicator of functional or reactive change on the vascular beds. The application of new techniques such as double-indicator method of assessing vascular protein permeability, a method that is both sensitive and specific for vascular injury, can be utilized to investigate the effects of ischemia/reperfusion on the coronary microvascular function. The endothelium-dependent vasodilation of isolated coronary vascular rings shows one of the earlier markers in the changes of coronary vascular bed affected by ischemia. Microvascular permeability was quantitatively assessed as a protein leak index by measuring the rate of extravascular accumulation of radiolabeled protein (indium113m transferrin) normalized for vascular surface area (technetium 99m erythrocytes). Anesthetized dogs underwent zero (control), 15, 30, or 60 minutes of left anterior descending coronary artery occlusion followed by 60 minutes of reperfusion (Dauber et al., 1990). Even 15 minutes of ischemia increased the protein leak index by 50% (3.16±0.30 ischemic vs. 2.09±0.11 control). Longer periods of ischemia increased the protein leak index in proportion to the duration of ischemia. The protein leak index increased in three fold (6.51±0.60) after 60 minutes of ischemia. At each point of ischemic duration, there was significant regional variation in the protein leak index that correlated with the severity of ischemic blood flow to that region measured with microspheres (Dauber et al., 1990).

EC injury also was evident after 15 and 30 minutes of ischemia as impaired vasodilation of isolated coronary rings in response to the endothelium-dependent vasodilators acetylcholine and the calcium ionophore A23187. Electron Microscopy (EM) and *in vitro* direct immunofluorescence revealed evidence of vascular injury after 60 minutes but not after 15 minutes of ischemia (Dauber et al., 1990). Blood reperfusion after ischemia of the myocardium is followed by the increase of permeability of microvessels for proteins and the formation of large intercellular pores that probably indicate the nature of non-reversibility of the lesions induced by ischemia (Dauber et al., 1990). The same pattern of the vessel lesions such as the formation of the rupture of capillaries and hemorrhage produced by the effect of post-ischemic "*no-reflow*" phenomenon and microthrombosis has been reported more recently by using light microscopy (Lang et al., 1974; Krug, 1979).

Short-term ischemia does not induce particular changes in a reperfusion period and had organ specific features. For example, in the striated muscles 1-3 hours of ischemia following 5 hours of recirculation was unable to induce EC damage (Gidlof et al., 1988).

However, a study by Hartsock and colleagues (1989) demonstrated that at the end of 3 hours of ischemia some of the post-ischemic area shows the formation of thrombosis in post-ischemic vessels. Normal functional activity of capillaries vessel requires at least 30 minutes of reperfusion time (Hartsock et al., 1989).

Duration of ischemia has a direct effect on the degree of the lesions. In the striated muscles after 4 hours ischemia, following 4 hours of reperfusion causes the activation and adhesion of leukocytes to the EC and accumulation of fibrin film on the endothelial surface was seen. In addition, the migration of activated leukocytes, through extended inter-endothelial junctions, also observed. Especially after 6 hours of ischemia, and following 24 hours of reperfusion, the presence of adherent leukocytes to the intermuscle area was seen throughout post-ischemic regions, where the desquamation and denudation of the EC has been observed (Aliev et al., 1993). Moreover, as we have described (Aliev et al., 1993) these changes, which are associated with the decreased number of normal (e.g., perfused or patent) capillary is caused by microthrombosis. It has been well documented that the formation of microthrombosis is caused by the decrease in anti-thrombogenic activity of the luminal plasmalemma of the EC by action of the injury stimuli (Aliev et al., 1993; Cirillo et al., 1994; Sala et al., 1996). Moreover, the luminal plasmalemma of the EC has anti-inflammatory and anti-platelet activity that decreases after ischemia, especially after long-term ischemia/reperfusion (Aliev et al., 1993; Cirillo et al., 1994). Therefore, long-term ischemia/reperfusion induces an increased risk of adhesive response of platelets and leukocytes following their migration to the perivascular space. However, these changes have organ specific features. For example, in the liver the ischemia/reperfusion injury induces the occlusion of microvessels by neutrophils (Jaeschke et al., 1990). In addition, the initial phase of ischemia (the first 60 minutes) is caused by the activation of liver parenchymal cells. The stasis of erythrocytes, the aggregation of platelets, the activation and adhesion of neutrophil and the injury in EC of the large and small vessels have been found in skin scrap after 4-12 hours of ischemia following 1 hour of reperfusion (Marzella et al., 1988). Almost the same pattern of tissue damage has been reported in a kidney model of ischemia/reperfusion (Yamamoto et al., 1984). One of the specific features of ischemia/reperfusion in the kidney appears to be the squeezing of microvessels, which injures of the parenchymal cells (Yamamoto et al., 1984). In the post-ischemic brain, the *no-reflow* phenomenon, induced by the formation of perivascular edema of glial cells, coexists with clusters of blebs and microvillies on the luminal surface of the EC (Chiang et al., 1968; Arsenio-Nunes et al., 1973; Dietrich et al., 1984). The same pattern of cellular damage of gut mucous membranes occurs after the 3 hours of ischemia and especially during reperfusion. Neutrophil accumulation is often observed (Kubes et al., 1990). Especially 10 minutes after reperfusion the extravasations of leukocytes dramatically increases (Zimmerman and Granger, 1990), which probably will initiate damage generalization to the whole organ.

The large arterial vessels

The sensitivity of the main arterial vessels to ischemia with or without reperfusion is characterized by their heterogeneous distribution (Fig. 2A-C). We have showed that in a rat model of ischemia/reperfusion, induced by the clamping of the infrarenal segment of the abdominal aorta/following blood recirculation, has a time-dependent progression of lesion development. Increasing the time of reperfusion after long-term ischemia accelerates the non-reversible damage in a lesioned endothelium (Aliev and Mironov, 1989). After prolonged periods of ischemia (two, 4, especially 6 and 24 hours), following recirculation, the number of non-reversible damaged EC were correlated with the duration of ischemia (Aliev and Mironov, 1989). Reendothelization after prolonged periods of ischemia induces mitosis of the EC, which is located close to the ischemic zone (Aliev and Mironov, 1989). Very often, the presence of an island of mitotic EC was seen through out the post-ischemic area (Aliev and Mironov, 1989). The flattening, migration and proliferation of EC, located in the islands of viable endothelium appears to be a permanent feature of the post-ischemic aortic wall (Aliev and Mironov, 1989). Prolonged ischemia (6, especially 24 hours) following long-term reperfusion induces future proliferation and migration of a large number of SMC from the media to the intimal layer (Aliev and Mironov, 1989). After a certain time (7 and 14 days after the recirculation) this aortic tissue shows the presence of an intimal thickness, which can lead to the formation of arterial stenosis (Aliev and Mironov, 1987, 1989). This abnormality is much more evident at the site of high hemodynamic stress area, where the injuries to the EC occurs more frequently (Aliev and Mironov, 1989).

Subcellular mechanisms of the vascular injury during ischemia

It has been well documented that the vascular endothelium is very resistant to anoxia (Buderus et al., 1989) and that the affect of hypoxia on the EC, after reoxygenation, is completely reversible (Johns et al., 1989). However, the sensitivity of the EC is characterized by their heterogeneous response to the injury stimuli. For example, brain EC are characterized by a higher sensitivity to ischemia compared to other organs and tissues (Dietrich et al., 1984; Aliev and Mironov, 1989). Hypoxia immediately affects EC function (Buderus et al., 1989; Johns et al., 1989). Ischemia negatively influences the expression of Willebrand't factor (factor VIII) in microvessels EC

(Sasaki et al., 1988). Hypoxia also leads to increases in the procoagulant activity of the EC (Gertler et al., 1991) and changes the barrier function, including the fluidity of plasmalemma (Guarnieri et al., 1980). It has been shown that decreases in plasmalemma fluidity leads to the decline in the release of endothelial nitric oxide synthase (eNOS)-dependent NO from the EC (Quillen et al., 1990). Other cellular organelles actively involved during ischemia/reperfusion are the EC cytoskeleton. The cytoskeleton of the EC can be damaged by the action of various injury stimuli, including ischemia alone or with reperfusion (Aliev and Mironov 1987; Hinshaw et al., 1988).

Another leading factor for ischemic injury of the vascular EC and SMC is an imbalance in the K^+/Na^+ pump (Avtsyn and Shakhlamov, 1979). This imbalance induces the release of K^+ ions from the cell and influx .better of Na^+ into the cell cytoplasm. This abnormality causes the development of cytoplasmic edema (Avtsyn and Shakhlamov, 1979).

It has been widely accepted that prolonged ischemia causes necrosis and apoptosis of cardiac myocytes and vascular cells. However, the mechanisms of ischemia-mediated cell death are still poorly understood. Ischemia is associated with both hypoxia and acidosis due to increased glycolysis and lactic acid production. It has been speculated that hypoxia does not induce the cardiac myocytes apoptosis in the absence of acidosis (Kubasiak et al., 2002). Recent study by Kubasiak and coworkers showed that hypoxia-acidosis-associated cell death is mediated by BNIP3, a member of the Bcl-2 family of apoptosis-regulating proteins (Kubasiak et al., 2002). Chronic hypoxia induces the expression and accumulation of BNIP3 mRNA and protein in cardiac myocytes, but acidosis was required to activate the death pathway (Kubasiak et al., 2002). Acidosis stabilized BNIP3 protein and increased its association with the mitochondria (Kubasiak et al., 2002). Cell death by hypoxia-acidosis can be blocked by pretreatment with antisense BNIP3 oligonucleotide (Kubasiak et al., 2002). The potential mechanism for this process includes extensive DNA fragmentation and opening of the mitochondrial permeability transition pore, but no apparent caspase activation (Kubasiak et al., 2002). Overexpression of wild-type BNIP3, but not a translocation-defective mutant, activated cardiac myocytes death occurs only when the myocytes were acidic acidosis (Kubasiak et al., 2002). This pathway may play a significantly role in muscle loss after myocardial ischemia (Kubasiak et al., 2002).

The myocardial ischemia causes gene overexpression and synthesis, and release of a large amount of vasoactive substance from the coronary vascular EC and/or from cardiac myocytes (Aliev and Burnstock, 1998). Some of these substances appear to be protective and include NO and bradykinin (Aliev and Burnstock, 1998). One hypothesis for the pronounced anti-arrhythmic effects of preconditioning involves the early generation of bradykinin and, subsequently NO. Evidence for early bradykinin release has come from clinical studies involving patients undergoing either coronary reconstructive surgery, in which four of five patients demonstrated elevated kinin levels in coronary sinus blood before or after the balloon catheterization procedure (Parratt et al., 1997). The recovery potential of any tissue following certain periods of ischemia is dependent on the ability of the microvascular system to restore blood flow. In the ischemic myocardium, a reduction in capillary cross-sectional dimensions occurs, which is likely to contribute to the "*no-reflow*" injury (Lawrenson et al., 2002).

Injury stimulus, such as ischemia, decreases the antioxidant defenses of the cell. Decreased activity leads to impairment of cellular defenses against the toxic influence of oxygen free radical species (Ferrari et al., 1986). After, ischemia especially after long-term ischemia following a reperfusion period the toxic action of reactive oxygen species leads to oxidative stress, which decreases the antioxidant activity of the EC (Ferrari et al., 1986; Aliev et al., 1993, 2003; Cirillo et al., 1994; Salvatico et al., 1994). Oxygen free-radicals play an important role in these processes (Ferrari et al., 1986; Aliev et al., 2002). In fact, it has been shown that the EC, under the influence of anoxia and reoxygenation, produces the large quantity of free oxygen radicals (Zweier et al., 1988). On the other hand, the increased permeability of microvessels caused by blood reperfusion considerably increases the weakness of microvascular systems through inhibition of xanthine oxidase and scavengers of free oxygen radicals (Granger et al., 1989; Aliev et al., 2002, 2003).

The formation of the additional amount of oxygen free-radicals by the EC, in which xanthine dehydrogenase can be converted into xanthine oxidase during hypoxia, plays a role as an additional source of free radicals (Chambers et al., 1985; Granger, 1988). The released of adenozid, for example, from the ischemic cardiomyocytes, can be the precursor for hypoxanthine that is used by the EC. The EC of microvessels have better access to a released pool of adenozid than the EC of large vessels, which lie more proximal to cardiomyocytes. Thus, the EC of microvessels generate the greater quantity of free oxygen radicals, which can lead to selective dysfunction to the EC which cover them (Quillen et al., 1990).

Oxygen free-radicals appear to be a main factor inducing ischemia/reperfusion injury of the vessel wall, which is responsible for normal vascular function (Ku, 1982; Rubanyi and Vanhoutte 1986; Lesnefsky et al., 1987). It has been demonstrated that the significant reduction in endothelium-dependent NO release appears to be an important factor inducing secondary damage of post-ischemic tissue (Tsao et al., 1990; Lefer et al., 1991; Tanaka et al., 1991). One of the potential mechanisms of post-ischemic damage of cardiac tissue appears to be the reaction of NO with oxygen free-radicals (Quillen et al., 1990; Aliev and Burnstock, 1998). On the other hand, ischemia following blood

recirculation decreases the formation of endothelium-dependent relaxing factor via the reduction in the availability of NO for vasorelaxation, because NO is utilized for the neutralization of the free oxygen species and by decreasing its synthesis de novo (Quillen et al., 1990; Aliev and Burnstock, 1998). Finally, abnormal NO activity and continued formation of oxygen free-radicals reduce the response of the SMC to NO and affects the release of prostacyclin PGI_2 or increases the release of endothelial content vasoconstrictors (Miller and Vanhoutte, 1985; Rubanyi and Vanhoutte, 1985; Aliev and Burnstock, 1998). Moreover, it has been demonstrated, clearly, that the oxygen free-radicals are able to decrease the endothelium-dependent vasorelaxation without resulting in endothelial damage. Several enzymes have been considered in this process. It has been showed that elastase, which leads to EC denudation, appeared to be involved in these processes (Gidlof et al., 1988; Inauen et al., 1990). The xanthine oxidase-derived oxidants released during reperfusion can interact with cell membrane components and probably plays a crucial role in the formation and release of substances, which activate and stimulate the adhesion of leukocytes to the microvascular endothelium (Granger et al., 1989; Grisham et al., 1990). Lipid peroxidation and activation of phospholipase A_2 products plays a central role in this process (Otamiri et al., 1988) and phospholipids can accelerate the extravasations of leukocytes after ischemia-reperfusion (Kubes et al., 1990). The ability of leukocytes to adhere to the endothelium is conditioned by a balance between adhesive forces and glycoprotein, which is released by membranes and is localized on the surface of activated leukocytes. The hemodynamic activity of the vessel wall also appears to be another important factor that initiates this process (Kubes et al., 1990; Aliev et al., 1993; Cirillo et al., 1994).

EC, which is exposed to hypoxia release chemotractant for neutrophil (Fletcher et al., 1990), which seems to plays a crucial role in the pathogenesis of ischemia/reperfusion injuries (Hernandez et al., 1987; Granger, 1988; Vedder and Harlan, 1988; Suzuki et al., 1989; Otamiri, 1989). Platelet-derived activating factor (PDAF) also plays an important role in the interaction between the circulating leukocytes and the EC of microvessels after ischemia-reperfusion and it induces increased permeability of the endothelial monolayer (Braquet et al., 1987; Lewis et al., 1988; Kubes et al., 1990). The oxidant product of ischemia enhances the synthesis all of these factors from the post-ischemic EC (Lewis et al., 1988) and, therefore, prolongs post-ischemic effects over entire organs and tissues.

Prevention of reperfusion induced vessel lesions

During the past decade, the prevention of ischemia/reperfusion has been studied extensively (Cirillo et al., 1994; Aliev and Burnstock, 1998). The application of various therapeutic approaches has opened new avenues for better understanding of the pathogenetic mechanisms of ischemia reperfusion. It has been shown that the infusion of the vessel using a solution containing dextran, or a similar supplement, before the restoration of blood recirculation was able to improve vessel permeability (Rosen et al., 1987). However, this feature is selective and tissue and organ-dependent. For example, Tanaka and coworkers (Tanaka et al., 1991) demonstrated that the removal of oxidative products (e.g. cathabolits) from the ischemic zone, by low oxidase perfusate prior to reperfusion, is not able to reduce the size of a myocardial infarction. Therefore, low oxidase perfusate essentially has no influence on the process of myocytes necrosis during the post-ischemic period. However, adding substances, which are able to increase the osmotic tension of the perfusate, induces restoration of normal vessel resistance (Gidlof et al., 1988). A similar effect has been found when dextran was added into the perfusate (Klar et al., 1990). Further, we have demonstrated that cloricromene usage (during 3 hours of ischemia and 3 hours of reperfusion) almost completely eliminated the described ultrastructural changes in the hindlimb muscle capillaries (Aliev et al., 1993). The protective action of cloricromene was not seen only in the EC of microvessels. We have found completely preserved muscle tissue after long-term ischemia following reperfusion (Aliev et al., 1993). Importantly, the ultrastructure of muscle mitochondria is completely preserved and almost overlaps with the shame-operated animals (Aliev et al., 1993). These protective effects of cloricromene restore the number of active or so-called perfused capillaries via the blocking the aggregation of platelets (Cirillo et al., 1992, 1994; Salvatico et al., 1994). In addition, cloricromene also prevents the formation of microthrombi and adhesion/or migration of leukocytes from the blood stream to the interstitial tissue (Cirillo et al., 1992, 1994; Aliev et al., 1993; Salvatico et al., 1994). The protective effect of cloricromene also has been found in a in vivo model of cardiac ischemia reperfusion (Milei et al., 1992). Milei and coworkers demonstrated that 50 minutes of myocardial ischemia followed by 20 minutes of reperfusion induces non-reversible myocardial injury and infarction (Milei et al., 1992). Interestingly, the myocardial mitochondria shows severe damage, whereas cloricromene, which has been continuously infused during the period of ischemia, decreased the signs of injury and preserved myocyte architecture (Milei et al., 1992). During ischemia, the percentage of normal mitochondria was lower in the placebo group ($p<0.0001$); and, on reperfusion, the percentage of severely damaged mitochondria was increased in the placebo group ($p <0.0001$). However, the direct addition of cloricromene to myocardial homogenates in vitro did not reduce hydroperoxide-induced chemiluminescences (Milei et al., 1992). We have showed that in large vessels, one of the crucial factors for preventing ischemia induced damage appears to be the presence of an adequate intraluminal blood pressure during the

anoxic period. This induces the contraction of vessels and accelerates the alterations of the EC and their subsequent desquamation and denudation, which accelerates further when the blood flow is restored (Aliev and Mironov, 1987; Cirillo et al., 1992; Aliev et al., 1998, 2001). In addition, the application of anticoagulants such as heparin, before induction of long-term ischemia, is able to prevent, to a large degree, subsequent EC injury.

Conclusion

Data gathered from this review indicates that the duration of ischemia and reperfusion appears to be a key factor for ischemia-induced vessel lesions. Particularly, the vascular endothelium plays a central role in the pathogenesis of these lesions and their subsequent complications. The interaction between blood cells and the vascular endothelium during ischemia/reperfusion appears to be a trigger for these lesions. Applications of pharmacological substances appear to be are able to diminish these lesions and, therefore, minimize ischemia induced vascular complications.

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