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MECHANISMS OF VASCULAR DYSFUNCTION AFTER SUBARACHNOID HEMORRHAGE

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The main consequence of subarachnoid hemorrhage, for those who survive bleeding, is delayed, persistent vasospasm of intracranial conduit arteries which occurs between the third and seventh day after the insult and results in symptomatic brain ischemia in about 40% of cases. This vasospasm is considered to be a major cause of disability of post-SAH patients. Despite extensive experimental and clinical research, mechanisms of vasospasm are not fully understood. Dysfunction of the endothelium resulting in enhanced production of vasoconstrictors, phenotypic changes of the receptors in endothelium and smooth muscle cells, increased sensitivity of vascular smooth muscle cells to vasoconstrictors, release of spasmogens from lysed blood clot and inflammatory response of the vascular wall have been demonstrated and discussed as pathological mechanisms participating in the development of spasm. In recent years more attention is paid to the functional and structural changes in microcirculation and a concept of microvascular spasm is evolving. Our experimental studies in rat model of SAH strongly suggest that microcirculatory dysfunction and delayed vasospasm are related to the severity of acute, transient ischemia caused by critical decrease of perfusion pressure and active vasoconstriction immediately after the bleeding.

Key words: *SAH, acute ischemia, delayed vasospasm, microcirculation, dysfunction of endothelium*

INTRODUCTION

Subarachnoid hemorrhage (SAH) is, in most cases, caused by rupture of intracranial aneurysm. The acute symptoms of SAH depend on the amount of subarachnoid blood and are attributed to the increase of intracranial pressure (ICP), decrease of cerebral perfusion pressure (CPP) and resulting ischemia (1, 2). According to the recent review of population-based studies the mortality rate within the first 48 hours after SAH varies between 30-67 % (3). In about 70% of the survivors persistent vasospasm of extraparenchymal cerebral arteries develops in a course of 4 to 7 days. In 40% of cases vasospasm is asymptomatic whereas 30% suffer from the delayed ischemic deficit (DID) - the main cause of morbidity of post-SAH patients.

Aneurysmal vasospasm has been demonstrated on angiography for the first time 55 years ago (4). Since that time many experimental and clinical studies have been undertaken to disclose mechanisms responsible for this persistent vasoconstriction and to find proper treatment for its prevention and/or reversal. Despite all efforts no effective pharmacological treatment has been found. The most promising, currently used method to surmount vasospasm is endovascular angioplasty (5, 6). Since this treatment was not always beneficial in terms of the better neurological outcome, in some patients intra-arterial vasodilators such as papaverine or nimodipine were administered to improve the efficacy of angioplasty. Both vasodilators occurred, however, not beneficial for symptomatic angiographic vasospasm (7 - 9). Papaverine was reported to increase cerebral perfusion only transiently (7) and recently published data indicate that administration of papaverine results in a decrease of brain oxygenation which might explain why the drug does not improve neurological outcome (8). In the case of nimodipine, its well known hypotensive effect may unmake the benefits. An interesting observation is, however, that nimodipine improves outcome of the poor-grade patients (10) although it is not very effective in reversing delayed vasospasm (9). This suggests that a proper target for the therapy might be not the spastic artery but dysfunctional microcirculation. Similar conclusions can be drawn from the triple H (hypertension, hypervolemia, hemodilution) therapy (11) or intravenous bolus infusion of hypertonic saline (12). Both treatments are beneficial for poor-grade patients most probably due to the improvement of blood rheology which results in better perfusion of microcirculation.

This review summarizes current knowledge on the mechanisms of acute and delayed vasospasm of cerebral blood vessels based on experimental studies. It also presents the renewed concept of dysfunction of brain microcirculation as a cause of post-SAH disability.

Readers who are interested in the mechanisms of post SAH brain injury and details on the current treatment of vasospasm will find them in few excellent reviews published recently (13 - 19).

EXPERIMENTAL MODELS OF SAH

SAH is induced in experimental animals either by injection of fresh autologous blood or blood hemolysate into subarachnoid space (injection model), by endovascular perforation of the intracranial portion of the internal carotid artery (perforation model) or by puncture of the basilar artery (20 - 27). Although these models were established in the rat, mouse, dog, and rabbit, the most widely used species are rats (20, 24 - 26). The models differ in terms of severity of acute ischemia and delayed vasospasm as well as mortality (28, 29). Injection model in its original form (single injection of blood into cisterna magna) results in short-lasting decrease of cerebral blood flow to about 35-40% of control. Within next 15 min blood flow returns to control value (29) or to 80% of control (30). Morphometric analysis of the basilar artery (BA) in this model 48 hours after SAH showed 20% reduction of mean inner perimeter and 62% reduction of the internal diameter in comparison with control vessel (28, 29). This model was modified in order to obtain more pronounced vasospasm. In the modified version (intracisternal injection of blood two times with the interval of two days between injections) larger morphometric changes were observed during vasospasm (7 days after the first injection) but there was no difference in the severity of ischemia in comparison with single injection model (20, 29). The advantage of injection model is that there is low mortality rate: 0% mortality in single-injection and 9% mortality in double-injection SAH. Due to pronounced morphometric changes, this model is considered to be the most suitable to study mechanisms of delayed vasospasm (28, 29). In the next modification of injection model fresh autologous blood is injected to prechiasmatic cistern in such a way that ICP is maintained at the level of mean arterial blood pressure (29). Decrease of microflow in the acute phase and mortality rate in this model depend on the amount of injected blood. The amount which results in moderate mortality (25%) causes microflow decrease to about 30% of control value at the nadir. Next microflow increases more slowly than in single- or double-injection model being at the level below 70% of control for 60 min. The disadvantage of this model is that there is no vascular damage which according to our results is important for pathological processes both in the conduit ruptured artery as well as in the downstream microvessels. In the perforation model decrease of microflow is more severe and the mortality rate is higher than in the injection model, depending on the amount of blood extravasated during rupture of the vessel (25). According to our experience it depends also on the size and location of the perforated vessel. Typically on autopsy clot is bigger when the internal carotid artery is punctured at the intracranial bifurcation and smaller when anterior cerebral artery is punctured. Microflow decreases to 10-15% of control at nadir and then rises slowly reaching 45-50% of control (large SAH) or 60-70% of control (small SAH) at 60 min after the bleeding, depending on the thread used to puncture the vessel. These results are similar to the data published by Bedersen's group (25). Morphometric changes are less pronounced in

endovascular perforation than in double injection model but similar to the changes observed in single injection one (28). Endovascular perforation model with its high mortality rate (close to 50%) resembles human SAH. The more so that it mimics aneurysm rupture. According to our experimental data severity of ischemia in the acute phase in this model determines the extent of structural changes in the middle cerebral artery (MCA) and BA and also microcirculatory dysfunction is the late phase after SAH (31,32). The last SAH model which was mentioned above is that one in which puncture of the basilar artery in the rat results in the acute decrease of microflow to about 30% of control at nadir (26). Next microflow returns very slowly reaching 60% of control value at 60 min after BA puncture. Morphometric changes in BA reported at 4 days after SAH were negligible when compared with the changes reported in the other discussed models.

Summing up this brief overview of SAH models in rats it seems that they enable purpose-based choice. Endovascular perforation model with acute vasospasm resulting in severe acute ischemia and less pronounced delayed vasospasm is suitable to study mechanisms of acute vasospasm/ischemia whereas double injection model is more suitable to study mechanisms of delayed vasospasm.

MECHANISMS OF ACUTE VASOSPASM

Acute vasospasm is usually described as a decrease of cerebral microflow recorded upon induction of SAH (25, 26, 29, 32 - 34). It is accompanied by an abrupt increase of ICP with concomitant decrease of CPP (25, 29, 34). Decrease of CPP can not be, however, the only cause of vasospasm since it lasts typically no longer than 10 min (25, 29) whereas microflow is compromised for at least 30 - 60 min. Results of our experimental studies in perforation model of SAH in the rat indicate that decrease of microflow in the cerebral cortex on the site ipsilateral to the perforation is more severe than on the contralateral site (32, 33). If the increase of ICP and decrease of CPP would be the only cause of ischemia then the microflow should decline symmetrically. According to a current view, active contraction participates in the acute vasospastic/ischemic response. The potential spasmogens are released from activated platelets as well as from mechanically damaged erythrocytes (RBC) (*Fig. 1*). Activation of platelets has been demonstrated as early as 10 min after SAH induction in a perforation model by Sheba *et al.* (35). It was also reported in post-SAH patients (36). Activated platelets release strong vasoconstrictors such as thromboxane A₂ (TXA₂), serotonin, ATP and platelet-derived growth factor (PDGF). Increased concentration of these mediators in cerebrospinal fluid and plasma after SAH has been observed both in the experimental animals and in post-SAH patients (37 - 40). Some of these mediators are known to stimulate the release from endothelial cells of a powerful vasodilator nitric oxide (NO) thus counteracting their own direct excitatory effect on vascular smooth muscle cells under physiological conditions (41, 42). NO relaxes vascular

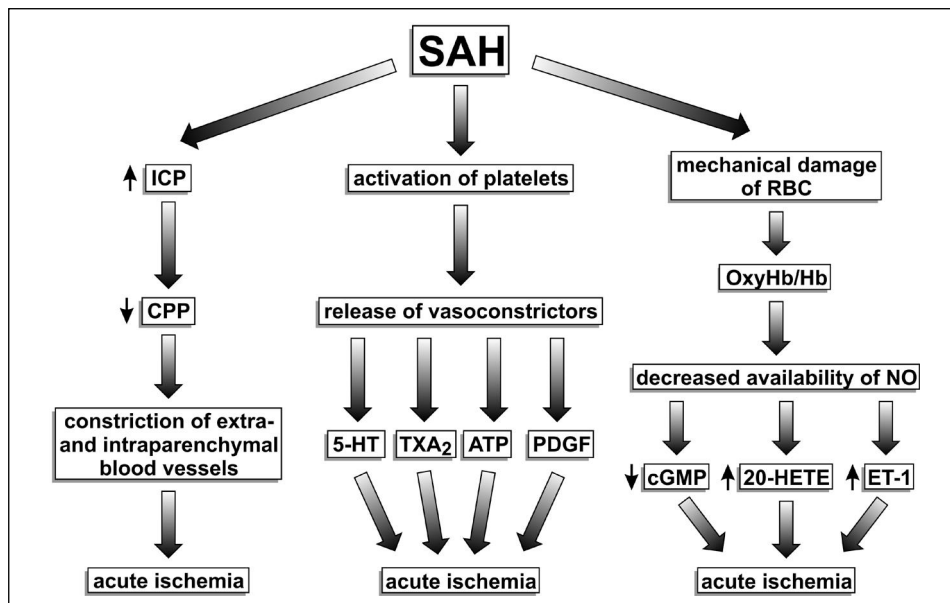


Fig. 1. Putative mechanisms of acute post-SAH vasospasm and ischemia (description in the text) Abbreviations: ICP - intracranial pressure, CPP - cerebral perfusion pressure, RBC - red blood cells, OxyHb - oxyhemoglobin, Hb - hemoglobin, NO - nitric oxide, 5-HT - serotonin, TXA₂ - thromboxane A₂, PDGF - platelet-derived growth factor, cGMP- cyclic guanosino monophosphate, 20-HETE - 20-hydroxyeicosatetraenoic acid, ET-1 - endothelin-1.

smooth muscle cells directly by stimulation of cyclic guanosino monophosphate (cGMP) production (43, 44) and indirectly by down-regulation of the production of two powerful vasoconstrictors - endothelin-1 (ET-1) and 20-hydroxyeicosatetraenoic acid (20-HETE) in vascular wall (45-47). This inhibitory mechanism is attenuated or absent after SAH due to scavenging effect of hemoglobin which binds NO decreasing its availability (48-50). Decrease of NO availability results in constriction of blood vessels exposed to fresh clot and mechanically damaged RBC which results in a decrease of cerebral blood flow. This view is consistent with the observation that acute ischemia can be reversed by NO donor S-nitrosoglutathione (51). Likewise, acute vasospasm and ischemia can be attenuated by inhibition of the synthesis of 20-HETE (47) or administration of selective endothelin receptor antagonist (45, 52). It should be mentioned that increase in the concentration of endothelin-1 in cerebrospinal fluid and/or plasma during acute ischemia may result also from the direct stimulation of its production in endothelial cells by oxyhemoglobin (53, 54). Another stimulus for ET-1 release in acute phase after SAH might be vasopressin. It is known that concentration of vasopressin in plasma increases during acute rise in ICP (55). The increase of plasma vasopressin concentration has been also demonstrated in acute phase of SAH (56). Vasopressin belongs to vasoconstrictors which under physiological

conditions attenuate their own direct activation of smooth muscle cells through the release of NO (57, 58) but it is also known that vasopressin stimulates secretion of ET-1 from cerebrovascular endothelium (59). In acute phase of SAH, when availability of NO is decreased, increase of vasopressin concentration in plasma may result in a direct vasoconstriction (60, 61) as well as in indirect one through the release of ET-1.

This discussion shows that in acute vasospasm/ischemia participate many different factors. They interact and cross-talk but the key point seems to be the impairment of nitric oxide-dependent regulation.

MECHANISMS OF DELAYED VASOSPASM

The mechanisms responsible for the delayed vasospasm are most intensively studied since the delayed vasospasm is considered to be the main cause of the disability of post-SAH patients and there is no effective prevention and treatment offered. It is generally accepted that clot formed during the process of coagulation of extravasated blood is a source of many pathogenic factors which participate in acute as well as in delayed vasospasm (13 - 19, 62). In a course of few days after SAH oxyhemoglobin is released from erythrocytes which undergo phagocytosis and lysis (62). These processes start about 16 hours after SAH. The consequences of NO trapping by oxyhemoglobin are similar to that described during acute phase of SAH but vasoconstrictory responses seem to be accentuated. This is due to the production of potent vasoconstrictors by the damaged endothelium and to the release of vasoconstrictors from the clot (*Fig. 2*). Endothelin, thromboxane A₂, serotonin levels in cerebrospinal fluid are elevated at the time of persistent vasospasm (18, 63). Moreover, upregulation of endothelin (ET_B) and serotonin (5-HT_{1B}) receptors in spastic cerebral arteries was found and increased contractile responses to ET-1 and 5-HT were reported (64, 65). Selective endothelin converting enzyme-1 inhibitor was able to attenuate SAH-induced late vasospasm which further suggest involvement of ET-1 in the mechanism of persistent vasospasm (66). Inability of NO to regulate endothelial functions seems to result in the increased production of 20-HETE (67). This view is supported by the observation that delayed vasospasm may be attenuated by the administration of selective inhibitor of the synthesis of this compound (67). In addition to NO binding by oxyhemoglobin, dysregulation of NO-dependent processes might be due to reduced expression of soluble guanylate cyclase in the spastic artery as has been demonstrated in the canine double-injection SAH model (68) or/and to increased rate of cGMP hydrolysis as reported during vasospasm in the rat (69). These observations are consistent with the impairment of NO-mediated cerebral vasodilatation after SAH (69) but seem to contradict other experimental results which demonstrated attenuation of vasospasm after administration of NO donors or after genetic manipulations targeted to increase expression of endothelial NO synthase (eNOS) (70). It should also be mentioned that

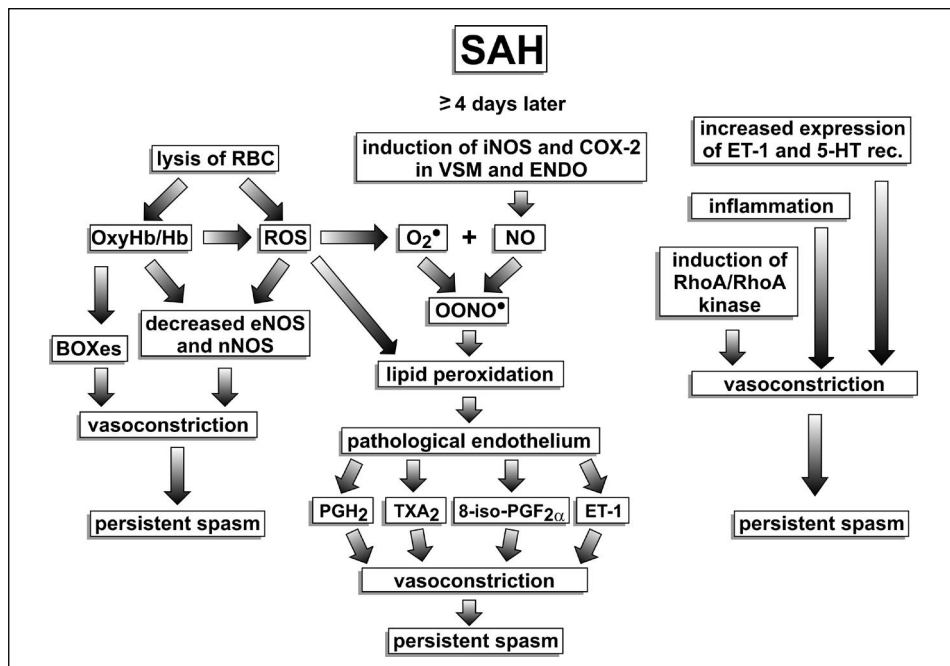


Fig. 2. Putative pathogenic factors underlying development of persistent vasospasm after SAH (description in the text)

Abbreviations: RBC - red blood cells, OxyHb - oxyhemoglobin, Hb - hemoglobin, BOXes - bilirubin oxidized products, ROS - reactive oxygen species, eNOS - endothelial nitric oxide synthase, nNOS - neuronal nitric oxide synthase, iNOS - inducible nitric oxide synthase, COX2- cyclooxygenase-2 (inducible), VSM - vascular smooth muscle, ENDO - endothelium, O₂^{•-} - superoxide anion, NO - nitric oxide, OONO[•] - peroxyntirite, PGH₂ - prostaglandin PGH₂, TXA₂ - thromboxane A₂, 8-iso-PGF_{2α} - 8-iso prostaglandin F_{2α}, ET-1, 5-HT rec. - endothelin-1 and serotonin receptors, respectively.

NO donors are beneficial in human SAH. They were reported to improve neurological status of patients with vasospasm (71). On the other hand, however, there is no discrepancy between these results (68, 69 vs. 70, 71) when one assumes that NO may act independently of cGMP (*e.g.* inhibiting synthesis of 20-HETE).

Metabolism of hemoglobin delivers many toxic mediators (62). First of all free radicals such as hydroxyl radical or superoxide anion are produced and result in oxidative stress and lipid peroxidation of cell membranes (13). Besides that, hydroxyl radical is a vasoconstrictor. After subarachnoid hemorrhage hypersensitivity to hydroxyl radicals in the rat basilar artery has been reported (72). Oxidative stress affects the function of endothelium (73) which releases vasoconstrictors such as prostaglandin PGH₂, TXA₂ and isoprostanes instead of vasodilators NO and prostacyclin (73). Superoxide anion reacts with NO which at the time of delayed vasospasm is produced in toxic amounts due to the presence of inducible NO synthase (iNOS) in endothelial, smooth muscle and adventitial cells

(74). The product of this reaction - peroxynitrite is a very powerful oxidant (75). Peroxidative damage by nitric oxide-dependent mechanism has been evidenced in chronic vasospasm after SAH (76). Recently Asaeda *et al.* (77) reported that 8-iso-prostaglandin $F_{2\alpha}$ which is known to be produced during oxidative stress is present in the cerebrospinal fluid of patients with aneurysmal SAH particularly in those with symptomatic vasospasm. In line with the participation of superoxide anion in post-SAH vasospasm it has been reported that inhibition of NADPH oxidase, which is the main source of superoxide production in the vasculature, ameliorates vasospasm after SAH in rats (78). Two other studies performed in mouse model of SAH demonstrated that in animals with overexpression of superoxide dismutase (a scavenger of superoxide anion) there is no induction of iNOS after SAH, endothelial damage is attenuated (79) and morphometric post-SAH changes ameliorated (27).

There is at least one more class of powerful spasmogens produced during metabolism of oxyhemoglobin - bilirubin oxidation products (BOXes) (19). BOXes are produced during oxidation of both bilirubin and biliverdin, were identified in cerebrospinal fluid of patients with vasospasm after SAH and the increase of their concentration correlates well with clinical occurrence of vasospasm (19). Although BOXes are vasoconstrictors by themselves their participation in vasospasm is most probably connected with the increase of the efficacy of other vasoconstrictors released during vasospasm (19).

Irrespective of the membrane mechanisms which are activated by various substances participating actively in the development of vasospasm after SAH, all of them excite smooth muscle cells either due to the increase of intracellular concentration of Ca^{2+} and/or as a result of the sensitization of contractile proteins in smooth muscle cells to Ca^{2+} , (80). Recently this mechanism is postulated as a possible cause of tonic vascular spasm both in the coronary and cerebral blood vessels (81).

One of the factors which sensitizes smooth muscle cells to Ca^{2+} is small GTPase RhoA - protein which belongs to Ras family. Addition of this peptide to smooth muscle cells in culture in which concentration of Ca^{2+} is kept constant increases their force of contraction (82, 83) whereas inhibition of RhoA activity gives opposite result (84). Moreover, it has been demonstrated that the activity of specific RhoA- dependent kinase is increased in the basilar artery during vasospasm after experimental SAH (85, 86) and that administration of RhoA kinase inhibitor results in amelioration of vasospasm (85). Recently published results of a randomized trial of the effect of an inhibitor of RhoA kinase on cerebral vasospasm and delayed cerebral ischemic symptoms after aneurysmal SAH clearly demonstrate attenuation of vasospasm and improvement of neurological deficits (87).

Concept of the sensitization of smooth muscle cells to calcium following SAH may constitute a link between acute ischemia and delayed vasospasm. Most of the spasmogens released during acute vasospasm activate RhoA/RhoA-kinase system and it is possible that its early inhibition could prevent development of late vasospasm.

It is difficult not to mention that extravasated blood is responsible for initiation of the cascade of reactions leading to inflammation (88-90). Some elements of this cascade were already discussed. The review of the inflammatory processes associated with SAH is beyond the scope of this paper.

IMPACT OF SAH ON CEREBRAL MICROCIRCULATION

About 40% of patients with SAH do not demonstrate neurological symptoms despite the presence of angiographic vasospasm (3). On the other hand, there is a certain percentage of patients who despite absence of angiographic vasospasm do have neurological symptoms (3). These observations suggest that microcirculation, which is the most important part of the vascular bed from the point of view of the supplied tissue, may be impaired independent on the state of conduit artery.

Study of the microcirculation after SAH started about 30 years ago (91, 92) to answer the question whether arterioles which regulate blood flow at the microcirculatory level are in spasm similarly to the extraparenchymal conduit vessels. Herz *et al.* (91) did experiments on guinea pig pial microvessels which were subjected to traumatic (puncture and bleeding) or nontraumatic (topical application of blood) subarachnoid hemorrhage. These experiments showed that acute SAH results in constriction of arterioles which was more pronounced when the vessel was injured. Microflow behaviour was consistent with the data obtained on arterioles. Similar results concerning morphology of arterioles were reported by Wiernsperger *et al.* (92). An interesting observation was published by Ohkuma and Suzuki (93) on morphometric analysis of intra- and extraparenchymal portions of small canine cerebral vessels after SAH. Seven days following SAH (intracisternal single injection model) intraparenchymal portion of perforating arteries were constricted whereas extraparenchymal didn't demonstrate any changes in comparison with control vessels. These data show that intraparenchymal vessels are more prone to constriction in response to extravasated blood than the extraparenchymal one and that spasm may occur in microcirculation in dissociation with the state of extraparenchymal vessels.

Microvascular spasm after SAH was also suggested to occur in patients. Analyzing angiographic images of SAH patients using digital subtraction method Ohkuma *et al.* (94) noted that in patients with no demonstrable large vessels spasm but with decreased regional cerebral blood flow there was a prolonged cerebral circulation time which suggests microvascular constriction.

Introduction of orthogonal polarization spectral imaging (OPS) enables also direct study of human cerebral microcirculation during aneurysm surgery (95, 96). Using this technique Uhl *et al.* (95) found that in patients with SAH capillary density significantly decreased and small arteries and arterioles of the cortical surface exhibited vasospasm that cannot be detected by angiography or transcranial Doppler sonography. Similarly, during acute surgery in SAH patients

Pennings *et al.* (96) observed multifocal (bead-string like) constriction of cerebrocortical arterioles in the presence of subarachnoid blood (early surgery). Both papers (95, 96) report also about functional microvascular changes such as lack of dilation in response to mild hypercapnia (95) and increased contractile response to hyperventilation (96).

Functional studies of microcirculation after experimental SAH demonstrated impairment of autoregulation (97 - 99). During acute SAH there was loss of autoregulation (97, 98) which is typical for ischemia whereas during persistent vasospasm shift of the autoregulatory curve to the right was observed (99). The latter indicates that during chronic vasospasm the capacity of microvessels to dilate in case of hypotension is decreased. Impaired vasodilatory response of cerebral microcirculation to hypercapnia was also reported (31 - 33, 97).

SAH has been also demonstrated to affect endothelium-dependent regulation of microcirculation (31 - 32, 100). Park *et al.* (100) observed that cortical microvessels had a reduced response to endothelium-dependent dilator ADP and an enhanced response to ET-1 in the acute phase of SAH in the rat (single injection model).

According to our study (31-32) response of microcirculation to endothelium-dependent vasodilator (acetylcholine) and to direct (acting on VSM) dilator (papaverine) is impaired upon reperfusion after SAH (perforation model in the rat). Thus not only endothelium-dependent regulation but smooth muscle responses are compromised. Vasodilation in response to hypercapnia was likewise abolished. Three days after SAH, however, in animals which didn't have severe ischemia upon SAH induction, slight recovery of the reactivity was observed whereas in animals with severe ischenmia upon SAH induction the was no recovery (in some of them paradoxical reaction i.e. steal effect could be observed). The interesting result is that at the time of vasospasm (3 -4 days after the bleeding) in the animals with severe ischemia upon SAH induction cerebral microflow increased in response to intravenous administration of thromboxane receptor antagonist which did not affect microflow in matching sham rats. This suggests that acute ischemia during SAH elicits progressive functional changes at the level of the microcirculation. We have also observed progressive structural changes in the conduit arteries (MCA, BA). Both, functional impairment of microcirculation and structural changes in conduit arteries correlated with the severity of SAH.

CONCLUSION

The main conclusion from the presented data is that vasospasm and/or DID develop as a result of progressive structural and functional changes in extra - as well as intraparenchymal vessels and microcirculation itself. This is a maturation process which culminates with angiographic vasospasm and/or severe microvascular dysfunction. It might be too late to start the therapy for reversal of persistent vasospasm since certain processes might be irreversible at that time.

Instead of reverse we should try to prevent but may be direct a different target. Taking into account not large arteries but the smallest blood vessels, starting to protect microcirculation at the early phase after SAH.

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