Effects of Deferoxamine and Sympathectomy on Endothelin-1–Induced Contraction and Acetylcholine-Induced Relaxation Following Subarachnoid Hemorrhage in Carotid Artery

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ABSTRACT. The role of endothelium-related factors in the pathogenesis of cerebral vasospasm after subarachnoidal hemorrhage (SAH) has gained interest since the discovery of EDRF and of endothelin-1 (ET-1). The effect of SAH and both treatment of deferoxamine (DFO) and sympathectomy on endothelium-dependent vasodilation and ET-1–induced vasoconstriction of isolated rabbit carotid artery was examined using an isometric tension recording method. Thirty-five rabbits were divided into four groups: control animals, 7 days after SAH, treatment with DFO after SAH for 7 days and sympathectomy after SAH. Acetylcholine (10-8 to 10-5 M) was used to evoke concentration-dependent vasodilation of isolated arterial rings previously contracted by 10-6 M phenylephrine. In the animals killed 7 days after SAH, acetylcholine-induced relaxation was suppressed and the degree of relaxation of this group was 50% of the initial contractile tone in response to the 10-5 M acetylcholine. These relaxant responses did not return to control values in carotid arteries obtained from animals treated with DFO and subjected to sympathectomy. In isolated carotid arteries, ET-1 (10-10 to 10-8 M) produced concentration-dependent contractions. These contractile responses were significantly enhanced in animals 7 days after SAH compared with controls and did not return to control values in carotid arteries obtained from animals both treated with DFO and sympathectomized for 7 days after SAH. The present experiments suggest that impairment of endothelium-dependent vasodilation and the hyperreactivity of ET-1 of the carotid artery as well as cerebral arteries may be involved in the pathogenesis of cerebral vasospasm. Both treatment with DFO and sympathectomy during the chronic stage for vasospasm after SAH did not affect these vascular responses of the extradural part of the carotid artery to ET-1 and acetylcholine. Copyright © 1997 Elsevier Science Inc.

INTRODUCTION

Cerebral vasospasm is the leading cause of morbidity and mortality in patients with SAH from aneurysm rupture (Kassell et al., 1982). Despite extensive clinical and experimental studies, the pathogenesis of vasospasm is still controversial and poorly understood. The cause of vasospasm is presently considered to be multifactorial. Recently, it has been considered that vasospasm is related to an impairment of the vasodilating activity of cerebral arteries following aneurysm rupture, because endothelial degeneration and diminished production of vasodilatory substances in cerebral arteries have been reported following SAH (Sasaki et al., 1981a,b).

The obligatory role of the endothelium in the arterial relaxation response to acetylcholine was first demonstrated by Furchgott and Zawadzki (1980) in the isolated rabbit aorta. The substance released from the endothelium to elicit this relaxation was later termed “EDRF.” Recently, it has been suggested that the EDRF is not only released by vasoactive agents, but is also released spontaneously (Griffith et al., 1984; Martin et al., 1985). It has been reported that endothelial damage occurs frequently following SAH (Tanabe et al., 1978; Tani et al., 1978). Therefore, it is possible that the endothelial damage caused by SAH could also result in the impairment of the endothelium-dependent vasodilation.

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ET-1 is a potent vasoconstrictor peptide produced by vascular endothelial cells (Yanagisawa et al., 1988) and it may play an important role in vasospasm. In clinical studies, the levels of ET in plasma were significantly elevated in patients with SAH associated with symptomatic vasospasm on day 7 (Masaoka et al., 1989).

In addition the pathophysiology of chronic cerebral vasospasm is closely associated with the generation of oxygen free radicals and peroxidation of lipids. Sano et al. (1980) have summarized the potential role of lipid peroxidation in vasospasm after SAH. These findings suggest that antioxidants and free radical scavengers might be useful therapeutic agents for ameliorating SAH-induced vasospasm.

Adrenergic innervation of cerebral arteries is considered to play an important role in controlling cerebral blood flow, by exerting vasomotor effects on cerebral blood vessels. Chemical and histochemical studies found high concentrations of nonadrenergic and a rich adrenergic innervation from the superior cervical ganglion to the adventitia and the outer pial arteries in humans and other species (Edvinsson et al., 1976). Recently, it has been reported that the arteriographic narrowing of vasospasm was seen almost exclusively in the arteries lying within the subarachnoid space, although, on occasion, it can extend extradurally along the carotid or vertebral arteries to a minor degree (Willkins, 1991). However, Holst and Ericson (1988) suggested that the significant reduction in vascular diameter of intradural as well as extradural parts of the internal ca-
rotid artery might reflect an adaptation to altered cerebral metabolism after SAH. Cervical or perioral sympathectomy were occasionally used in the prophylaxis or treatment of vasospasm and chronic occlusive cerebrovascular disease (Yasarigli, 1984).

The present experiments were conducted:

1. To study the effects of SAH on endothelium-dependent vasodilation induced by acetylcholine and vasoconstriction induced by ET-1 in a rabbit single hemorrage model on day 7.
2. To study the effects of deferoxamine, an iron-chelating agent and sympathectomy on both endothelium-dependent vasodilatation and ET-1-induced contraction in carotid arteries exposed to SAH on day 7.

MATERIALS AND METHODS

Experiments were carried out on 35 adult albino rabbits of either gender weighings 2.5–3 kg. The animals were divided into four groups: (1) controls (n = 10); (2) SAH (n = 10); (3) treatment with DFO after SAH (n = 10); and (4) sympathectomy after SAH (n = 5).

The animals were anesthetized with an intramuscular injection of ketamine (25 mg/kg) and xylazine (5 mg/kg). Rabbits in group 1 were injected with 0.5–1 ml of saline into the cisterna magna. Rabbits in group 2 were given an equal volume of fresh autologous arterial blood obtained from a central ear artery puncture into the cisterna magna to generate a SAH. Group 3 rabbits were given deferoxamine (50 mg/kg) administered subcutaneously every 12 h for 7 days after SAH. Group 4 rabbits were subjected to right cervical sympathectomy and periarial sympathectomy of the right common carotid artery after SAH. The animals were placed in the supine position. A ventral cervical midline incision was performed and the adventitia was sheared. The wound was closed in layers. On day 7, in the SAH and control groups, with the animals under general anesthesia, an axilary angiogram of the basilar artery was obtained using Iopamiro 370 treatment. For the control and SAH groups, mean diameters of the basilar and common carotid artery were calculated. The presence of chronic vasospasm was confirmed (n = 5). The animals were operated on 7 days after SAH under ketamine and xylazine anesthesia. The common carotid artery was removed rapidly and placed in Krebs-Henseleit solution consisting of composition (mM): NaCl 118, KCl 4.7, NaHCO3 24.9, KHPO4 1.2, CaCl2 1.6, MgSO4 1.2, glucose 11.1. Blood vessels were carefully cleaned of fat and connective tissue and then cut into rings. These preparations were equilibrated for 2 h under a resting tension of 1.5 g in chambers containing 20 ml of Krebs-Henseleit solutions at 37°C, and gassed continuously with a 95% O2-5% CO2 mixture. During the equilibration period the tissue bath was changed every 30 min. After equilibration, carotid arterial rings were contracted using 10−3 M phenylephrine to ensure stabilization of the smooth muscles. After the contractions reached a plateau, acetylcholine (10−8 to 10−4 M) was added to the bath. In endothelium-intact preparations, acetylcholine produced relaxation in a concentration-dependent manner. In the endothelium-denuded preparations, however, relaxation to acetylcholine was not observed. Concentration–response curves for ET-1 were obtained by cumulatively increasing the total concentration in the bath. After the addition of each suplemental concentration we waited until a plateau response was obtained before adding the subsequent concentration.

Isometric tension was recorded on a Grass Model 79 E polygraph using Grass FT 03 force-displacement transducers. The observations were expressed in terms of maximum response (Em) and concentration of agonist producing half-maximum relaxation (EC50). The endothelin was expressed as a percentage of the response to 80 mM KCl. The maximum response to endothelin was not observed because 3 × 10−8 M endothelin was not added to the bath. Therefore, the sensitivity of the vessels (EC50 value) was not determined. The relaxation in response to acetylcholine was expressed as the percentage of decreased tension of contractile force induced by 10−6 M phenylephrine. Results are expressed as mean ± standard error of the mean (SEM).

The concentration–response data obtained in each individual experiment were plotted as the response/concentration (y) against the response (x). This produced a straight line relation in each experiment as predicted from the Scatchard equation for drug-receptor interaction:

\[
\text{Response/Concentration} = \frac{-1}{E_{c50}} \times \text{Response} + \frac{\text{Maximum response}}{E_{c50}}
\]

Light microscopic examinations of the carotid artery were performed to demonstrate the pathomorphologic changes of the arterial wall.

The drugs used were as follows: phenylephrine hydrochloride (Sigma), acetylcholine chloride (Sigma), endothelin-1 (Sigma), papaverine hydrochloride (Sigma), desferal (Ciba), and Iopamiro 370 (Briacco).

Statistical comparisons between groups were performed by using general linear models of analysis of variance (ANOVA) followed by the Scheffe test. An unpaired analysis (Student t-test) was used to compare mean diameters of vessels between different groups. P < 0.05 was considered statistically significant.

RESULTS

Angiograms obtained on day 7 (the day of subarachnoid blood injection being day 1) showed vasospasm of the basilar artery. The diameter of the basilar arteries after SAH was significantly decreased and found to be 0.78 ± 0.080 mm. The mean diameter of the basilar artery of control animals was 1.2 ± 0.006 mm. However, no change was observed in the mean diameter of the carotid arteries after SAH (Fig. 1; Table 1).

Light microscopic examination did not show any ultrastructural changes in the carotid arteries with SAH in comparison with normal vessels (Fig. 2).

There were no significant differences in contractile responses to 80 mM KCl in carotid arteries among the four groups. The cumulative addition of ET-1 (10−10 to 10−4 M) induced concentration-dependent contractions of carotid arteries of control rabbits (Fig. 3). In contrast, ET-1-elicited contractions were enhanced in cerebral arteries obtained 7 days after SAH (Fig. 3) with significantly higher calculated relaxation (Fig. 4). In contrast to KCl the ET-1–induced contraction of rabbit carotid artery rings is longlasting and difficult to wash out. The concentration–response curve for ET-induced contraction of the artery was shifted to the left, particularly at high concentrations in the vessels with SAH (Fig. 3). Contractile responses to ET-1 and

| TABLE 1. Basilar and carotid vessels diameters (mm) (mean ± SEM) |
|-------------------------|-----------------------|
| Control (n = 5) | SAH (n = 5) |
| Basilar artery | 1.2 ± 0.006 | 0.78 ± 0.080* |
| Carotid artery | 2.9 ± 0.245 | 2.68 ± 0.197 |

* P < 0.05 compared to control.
FIGURE 1. Angiograms of control basilar artery vessels and after SAH on day 7. Note the severe reduction in diameter of the basilar artery after SAH (b) compared with that of control (a), but no change in diameter of the carotid artery after SAH (b) compared with control (a).

FIGURE 2. Light microscopic images a section of a normal carotid artery (a) (×50) and with the experimental SAH (b) (×200) carotid artery.
**FIGURE 3.** Concentration–contractile response curves to ET-1 in the carotid artery rings of each group. The contractile effect is expressed as the percentage of that evoked by 80 mM KCl in each individual preparation. Asterisk indicates significant difference from control response. Number in parentheses indicate the number of preparations used. Vertical bars represent SEM. (□) Control (n=9); (○) SAH (n=10); (□) SAH+DFO (n=12); (△) SAH+SYM (n=9).

**TABLE 2.** $E_c$ values (mg) for $10^{-8}$ M endothelin-1 in carotid artery rings (mean ± SEM)

<table>
<thead>
<tr>
<th>Group</th>
<th>$E_c$ (mg) ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1366 ± 90</td>
</tr>
<tr>
<td>SAH</td>
<td>2156 ± 165*</td>
</tr>
<tr>
<td>SAH + DFO</td>
<td>2179 ± 170*</td>
</tr>
<tr>
<td>SAH + SYM</td>
<td>1988 ± 236*</td>
</tr>
</tbody>
</table>

* $P < 0.05$ compared to control.

**DISCUSSION**

Arterial vasospasm is the major cause of disability and death in patients with SAH (Kassell et al., 1985). The one-hemorrhage canine model of subarachnoid hemorrhage has often been used usually because it reliably produces a chronic cerebral vasospasm similar to that seen in humans (Sasaki et al., 1981c). In humans there is no vasospasm for the first 3 days and the incidence is maximal 7 days after SAH (Willkins, 1991).

Our results show that, on day 7 after SAH, ET-1-induced contractile responses increased when compared with controls. ET-1 is a 21-amino acid polypeptide and its vasoconstrictor activities are highly potent and longlasting (Yanagisawa et al., 1988). Endothelin is present in the brain, especially in the hypothalamus (Gioidi et al., 1991), and its release can be stimulated by various blood elements including thrombin (Kohno et al., 1990), platelets (Ohlstein et al., 1991) and oxyhemoglobin (Cocks et al., 1991). It has been reported that ET-1 induces vasoconstriction by acting directly on smooth muscle cells (Kobayashi et al., 1990). ET-1 stimulates phospholipase C to cause phosphoinositide breakdown leading to intracellular calcium mobilization and protein kinase C activation (Masaki et al., 1991) as well as activation of voltage-dependent calcium channels.

In clinical studies, the levels of endothelin in plasma and in cerebrospinal fluid were significantly increased in patients with SAH associated with symptomatic vasospasm on day 7 (Masaoka et al., 1989; Suzuki et al., 1989). In addition, ET-1–induced contractions of cerebral arteries from rats with SAH are much stronger than those from control rats (Alafaci et al., 1990). A single intrathecal injection of...
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ET-1 in dogs leads to constriction of the basilar artery which is still present after 3 days (Asano et al., 1989) and endothelin infused intracerebrally for 7 days in dogs reproduced both angiographic and histologic features of a double-hemorrhage model of SAH (Kobayashi et al., 1991). It is reported that SAH stimulates ET-1 synthesis in the endothelial cells of cerebral vessels (Suzuki et al., 1992). All these findings lead to the possible involvement of ET-1 as a causal factor in cerebral vasospasm following SAH. If ET-1 is responsible for vascular diseases, phosphoramidone and its related compounds may be useful therapeutics. It is reported that phosphoramidone potently prevents delayed cerebral vasospasm after SAH, in a two-hemorrhage model (Matsumuro et al., 1991). BQ-123 is an antagonist of the ETA receptor, which is the main or the only endothelin receptor on vascular arterial smooth muscle cells and is the main receptor responsible for the vasoconstrictor effect of endothelin-1. Intracisternal BQ-123 has been shown to completely prevent the decrease in CBF after SAH (Clozel and Watanabe, 1992). These results demonstrate that endothelin acting on the ETA receptor plays a role in the pathogenesis of cerebral vasospasm in the rat model of SAH (Clozel et al., 1992). Thus, one possible explanation concerning the increased ET-1-induced constriction of the carotid artery, as well as cerebral arteries, after SAH may be following sequence of events. In addition, Kim et al. (1989) speculated that the SAH may induce alterations in the function of the vascular endothelium: a reduced production of endothelium-derived relaxing factor and an increased sensitivity to the endothelium-derived vasoconstrictive polypeptide endothelin.

An intact endothelium is required for the vasodilatory effects of several endogenous vasoactive agents such as acetylcholine (Furchgott and Zawadzki, 1980). The endothelium acts by releasing a diffusible unstable endothelium-derived relaxing factor (Furchgott and Zawadzki, 1980), and it is likely to be nitric oxide (Palmer et al., 1987). It has recently been suggested that impairment of the vasodilatory activity of cerebral arteries following SAH may play an important role in the pathogenesis of vasospasm (Nakagomi et al., 1987a; Kim et al., 1989; Kanamaru et al., 1989). The present study has demonstrated that SAH inhibits relaxation of rabbit carotid arteries, as well as cerebral arteries, induced by acetylcholine. In addition, acetylcholine-induced relaxations were followed contractions
after SAH. Three major causes of the impairment of endothelium-dependent vasodilation following SAH can be postulated.

1. Denudation of the endothelium.
2. Inhibition of the production or release mechanism of EDRF in the endothelium.
3. Decreased or absent responsiveness of the smooth muscle cells to EDRF (Nakagomi et al., 1987a).

Moreover, contractions followed acetylcholine-induced relaxation may be due to breakdown of EDRF and/or homologous desensitization in EDRF receptors.

Various clinical and experimental studies of cerebral arteries in chronic vasospasm have demonstrated structural damage to each layer of the arterial wall, including the endothelium (Tanabe et al., 1978; Mayberg et al., 1990; Clower et al., 1988). The morphologic alterations of the endothelium include vacuolization and detachment and are observed at the early phase after SAH (Tanabe et al., 1978). It is likely that damage of the endothelial cells result in diminished release of EDRF and thus contributes to the observed loss of endothelium-dependent relaxation. However, in this study, light microscopic pictures did not show any ultrastructural changes in the carotid artery with SAH in comparison with a normal vessel.

A loss of endothelium-dependent relaxation in cerebral arteries after experimental SAH has been demonstrated in both the rabbit and the dog (Kim et al., 1989; Nakagomi et al., 1987a). Nakagomi et al. (1987a) found that endothelial cells were still present, although endothelium-dependent relaxation was absent after SAH. Thus, loss of endothelium-dependent relaxations are due to either a reduced release of EDRF, a decreased transfer of EDRF or a reduced responsiveness of the smooth muscle to the factor.

Hemoglobin inhibits endothelium-dependent relaxations in systemic (Nakagomi et al., 1987b) as well as cerebral arteries (Fujivara et al., 1986). Therefore, it is possible to explain the observed loss of endothelium-dependent relaxation by the inactivation of EDRF by hemoglobin and cerebral vasospasm may be caused by impairment by hemoglobin of the endothelium-dependent relaxation mechanism.

EDRF activates soluble guanylate cyclase in vascular smooth muscle and increases the conversion of GTP to cGMP, thus causing relaxation (Moncada et al., 1991). Kim et al. (1982) reported that the basal production of cGMP in rings with endothelium is impaired after SAH. These observations suggest that the loss of vascular reactivity in the canine basilar artery after SAH is due, at least in part, to an impaired production of cGMP in smooth muscle. Decreased or absent responsiveness of the smooth muscle cells to EDRF is another possible cause for the inhibition of the relaxation. However, it is unlikely that damage to the smooth muscle cells impairs the endothelium-dependent relaxation because there were degenerative changes in the smooth muscle cells on light microscopy. Moreover, the present study demonstrates that there is a selective loss of endothelium-dependent relaxation. Contrary to the endothelium-dependent relaxation in response to papaverine was not altered by SAH, indicating that the pathologic process did not change the basic ability of the vascular smooth muscle to relax.

In view of several studies that strongly supported free radical generation and lipid peroxidation as a key factor in vasospasm after SAH (Sano et al., 1980). It is plausible that continuously generated free radicals may eliminate albuminal EDRF in the period of chronic vasospasm (Kanamaru et al., 1989). Oxygen free radicals such as superoxide radical and iron-catalyzed hydroxyl radical generated by the superoxide system have been implicated in the pathologic process of ischemia, trauma and inflammation (Ikeda et al., 1989a). The hydroxyl radical attacks the fatty acid side chains and starts the process of lipid peroxidation (Halliwell et al., 1986). Additionally, some investigators have reported a close correlation between the occurrence of vasospasm and an increase in the content of lipid peroxides in the cerebrospinal fluid of patients with SAH (Sasaki et al., 1981b; Sakaki et al., 1988; Halliwell et al., 1986). DFO, a chelator of intracellular and extracellular iron, is cytoprotective in several models of tissue injury (Ikeda et al., 1989b) and has recently been shown to protect against oxyhemoglobin-induced endothelial and smooth muscle cytoskeletal injury (Comair et al., 1993). This cytoprotective effect of DFO suggests that it would have therapeutic potential for oxyhemoglobin-induced vasospasm. Two recent in vivo studies have demonstrated the effectiveness of DFO in preventing vasospasm (Vollmer et al., 1991; Harada and Mayberg, 1992). In this study, endothelium-dependent relaxations and ET-1-induced contractions were not restored in the SAH group by DFO.

Despite extensive research, the pathogenesis of the cerebral vasospasm remains obscure, and no specific therapeutic method for the treatment of cerebral vasospasm has been established (Willkins, 1991). Cervical or periarterial sympathectomy are occasionally used in the prophylaxis or treatment of vasospasm and chronic occlusive cerebrovascular disease (Yapangil, 1984). But Nagai et al. (1975) suggested that ablation of the superior cervical ganglion did not affect the chronic stage of vasospasm. In this study, sympathectomized after SAH reduced to contractile responses to ET-1 in the carotid arteries but not significantly compared with SAH. In addition, endothelium-dependent relaxations were not restored in the SAH group by sympathectomy.

In conclusion, the present experiments demonstrate that SAH impaired endothelium-dependent relaxation of the carotid arteries as well as the cerebral arteries. SAH also resulted in potentiation of the contractile response to ET-1. Together with potentiation of contractile response in the carotid artery, impairment of endothelium-dependent relaxation following SAH may play an important role in the pathogenesis of cerebral vasospasm.

References
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