Effect of High-dose Methylprednisolone on Vasospasm After Subarachnoid Hemorrhage

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Abstract

The effects of high-dose methylprednisolone (MP) on vasospasm following subarachnoid hemorrhage (SAH) were investigated. In a double hemorrhage canine model, administration of high-dose MP (10 mg/kg, every 12 hours) reduced angiographic narrowing of the basilar artery and prevented morphological changes in the arterial wall. In addition, increased platelet aggregation observed from days 4 to 7 in untreated SAH dogs was inhibited by the MP treatment. An in vitro experiment showed that MP inhibited platelet aggregation dose-dependently. High-dose MP had a nonspecific vasodilatory effect on the smooth muscle of the basilar artery. In another SAH model with dysautoregulation of cerebral blood flow (CBF), intravenous MP administration markedly attenuated the decrease in both blood pressure and CBF caused by exsanguination. These results indicate that MP has beneficial effects in normalizing CBF dysautoregulation following SAH. High-dose MP has several advantages for preventing and improving the multiple pathological status in cerebral vasospasm following SAH.

Key words: methylprednisolone, vasospasm, platelet aggregation, dysautoregulation

Introduction

Cerebral vasospasm leading to ischemia is a major cause of morbidity and mortality following aneurysmal subarachnoid hemorrhage (SAH). The pathogenesis of vasospasm is unclear and no effective treatment for preventing or ameliorating vasospasm is available, although several methods are reported to have some success. Chyatte et al. reported that ibuprofen or high-dose methylprednisolone (MP) prevented the arterial narrowing and smooth muscle degeneration induced by experimental SAH. A multicenter, controlled, double-blind clinical study also showed that intravenous high-dose hydrocortisone ameliorated the delayed ischemic neurological deficits following SAH, although the mechanism of steroid action was not fully studied.

In addition to the arterial narrowing resulting from smooth muscle contraction and/or the intimal thickening of cerebral arteries, intravascular pathogenic states such as microthrombus and increased blood viscosity are considered important in the development of ischemic deficits in vasospasm following SAH. Impaired autoregulation of cerebral blood flow (CBF) may also aggravate the neurological symptoms. This study investigated the effects of MP on the multiple pathological status after cerebral vasospasm.

Materials and Methods

I. Effects of MP on vasospasm and morphological changes

Double hemorrhage model was produced in 16 adult mongrel dogs weighing 9-14 kg. On day 0, the dogs were anesthetized with intravenous sodium pentobarbital (30 mg/kg), intubated, and spontaneously respirated. The head was fixed in a stereotactic frame. The right vertebral artery was cannulated with a polyethylene tube (internal diameter, 0.86 mm) for angiography, and the right cervical vein was...
cannulated with a vinyl chloride tube (1.9 mm) for MP (Pridol) administration. The PCO₂ of exhaled air was between 38 and 43 mmHg. Angiography was performed using 5 ml of meglumine diatrizoate. The cisterna magna was then aseptically punctured with a No. 22 needle, and 0.2 ml/kg of cerebrospinal fluid was removed. Autologous blood (0.4 ml/kg) previously withdrawn from the femoral artery was injected into the cisterna magna over 2 minutes. After withdrawing the needle, the dog was kept in a head-down position for 20 minutes. In eight dogs, MP was intravenously administered in 10 mg/kg doses at 12-hour intervals for the next 7 days. On day 2, 48 hours after the first experimental SAH, all the 16 dogs were reanesthetized, and a second SAH was induced as described above. A further angiogram was taken on days 2 and 7 as before. The luminal diameter of the basilar artery at the same location in the mid basilar artery was measured on the angiograms.

For the morphological study, eight dogs receiving MP treatment and 15 untreated were used. The dogs were sacrificed by KCl intravenous injection (300 mg/kg) on day 7. Heparinized physiological saline was infused into the carotid and vertebral arteries followed by 1000 ml of fixative solution (2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M cacodylate buffer, pH 7.4) at 100 cmH₂O. The basilar artery was removed through a transclival approach and kept in the same fixative solution at 4°C overnight. The tissue was postfixed in 2% buffered 0804 for 2 hours, dehydrated by a graded ethanol series, and embedded in epoxy resin. Ultra thin sections were obtained using an ultra microtome (Ultrotome; LKB Produkter AB, Bromma, Sweden), stained with uranyl acetate and lead citrate, and observed under an electron microscope (100U; JEOL Ltd., Akishima).

II. Effects of MP on platelet aggregation

The changes in platelet aggregation induced by SAH were examined in the same double hemorrhage dogs used for the angiographic study. On days 0, 2, 4, and 7, blood withdrawn from the cervical vein was diluted with sodium citrate solution to a final concentration of 0.38%. Platelet-rich plasma (PRP) was obtained by centrifugation at 800 rpm for 10 minutes. Then, platelet-poor plasma was produced by centrifugation at 3000 rpm. The platelet aggregation induced by adenosine-5'-diphosphoric acid disodium salt (ADP) was measured by the change in light transmission in the aggregometer (PAT-4M; Niko Bioscience, Inc., Tokyo).

Platelet aggregation after intravenous administra-

tion of MP (10 mg/kg) was also evaluated in five normal dogs at 5, 10, 15, 30, 45, 60, 90, 120, 150, and 180 minutes after injection.

Finally, the direct inhibitory effect of MP on human platelet aggregation was studied in vitro. The inhibition of platelet aggregation was evaluated by adding 0.02 ml ADP at the minimum concentration necessary for secondary platelet aggregation to PRP (0.18 ml) containing MP (0.02 ml; final concentrations: 2.0, 1.0, 0.5, 0.25, and 0.1 mg/ml).

III. Effects of MP on cerebral artery contractile response in vitro

Adult mongrel dogs weighing 9–14 kg were anesthetized with sodium pentobarbital (30 mg/kg) and sacrificed by exsanguination from the femoral artery. The brain including the basilar artery was removed and placed in modified Krebs-Ringer bicarbonate solution (NaCl 120 mM, KCl 4.5 mM, MgSO₄ 1.0 mM, NaHCO₃ 27.0 mM, KH₂PO₄ 1.0 mM, CaCl₂ 2.5 mM, and dextrose 10.0 mM). The basilar artery was dissected under the microscope to prepare annular segments of 3 mm long. The specimens were suspended between L-shaped stainless steel rods in a 10 ml siliconized organ bath aerated with 95% O₂/5% CO₂. The pH of the solution was 7.4–7.5. The contractile force was isometrically recorded using a force displacement transducer (WT-611T; Nihon Kohden, Tokyo).

To investigate whether the contractile response of the basilar arteries to KCl, serotonin (5-HT), and prostaglandin F₂ₐ (PGF₂α) was changed by pretreatment with MP, the specimens were treated with 2 × 10⁻⁵ M MP for 3 minutes before application of the agonists. Preliminary experiments showed the ED₅₀ value of KCl, 5-HT, and PGF₂α to be approximately 30 mM, 1 × 10⁻⁵ M, and 3 × 10⁻⁷ M, respectively. These concentrations were employed in this experiment. Preliminary studies also showed the MP plasma concentration at 3, 15, 30, 60, and 120 minutes after intravenous administration of 10 mg/kg MP to be approximately 10⁻⁴–10⁻⁵ M. Therefore, the MP concentration of 2 × 10⁻⁵ M, close to the physiological plasma concentration, was used. The effects of post-treatment with MP was also investigated by inducing the contractile response with each agonist, then adding MP (2 × 10⁻⁵, 10⁻⁴, and 10⁻³ M) cumulatively.

IV. Effects of MP on blood pressure (BP), sagittal sinus pressure (SSP), and CBF

The effect of a bolus intravenous injection of MP (10 mg/kg) was evaluated in six untreated double hemorrhage dogs on day 7. The cortical CBF of the
frontal lobe was measured by the thermo-gradient method (TGA-2; Biomedical Science, Kanazawa) under controlled respiration. The SSP at the anterior portion of the sagittal sinus and the BP in the femoral artery were continuously monitored through a cannulation tube (AP-601G; Nihon Kohden). The blood gases were monitored, and PCO₂ was maintained between 33 and 35 mmHg.

V. Effects of MP on CBF dysautoregulation

The double hemorrhage model induced by injecting blood into the cisterna magna does not consistently produce CBF dysautoregulation, in contrast to the reliable, marked angiographic vasospasm of the basilar artery. Therefore, another SAH model was produced in eight dogs by microscopic puncture of the internal carotid artery under general anesthesia through a subtemporal approach after ligation of the bilateral external carotid and vertebral arteries. In this SAH model, the CBF autoregulation was evaluated by the changes in CBF when the systemic BP was altered by either exsanguination or blood transfusion. Exsanguination (10 ml/kg) from the femoral artery was performed for 1 minute, and blood transfusion (10 ml/kg) was carried out for the same period. After confirming CBF dysautoregulation, MP (10 mg/kg) was intravenously administered to evaluate the effect on the dysautoregulation.

Statistical analysis used the paired Student’s t-test. P values less than 0.05 were considered significant.

Fig. 1 Angiographic vasospasm in untreated (solid line, n = 8) and MP-treated (broken line, n = 8) double hemorrhage dogs. Vertebral angiography was performed on days 0, 2, and 7. The basilar artery diameter is indicated by the percentage of the diameter on the day 0 angiogram. Values are means ± SD. Significant differences between untreated and treated groups are observed (*p < 0.05, **p < 0.01).

Results

I. Effects of MP on vasospasm and morphological changes

Significant basilar artery vasospasm was observed in the untreated SAH dogs. The mean reduction in vessel caliber was 33.1% on day 2 and 58.6% on day 7. In contrast, the dogs receiving MP had significantly reduced vasospasm on both days 2 (18.8%) and 7 (29.6%) (Figs. 1 and 2). The characteristic electron microscopic features of the basilar arteries following SAH were: 1) corrugation of the elastic lamina; 2) endothelial degeneration (formation of vacuoles and luminal folds), swelling, and desquamation (possibly representing necrosis); and 3) vacuolar degeneration of the smooth muscle cells and enlargement of interstitial space in the media (Fig. 3). The severity of the endothelial lesions assessed by the presence or absence of vacuoles, swelling, and desquamation was less in the MP-treated dogs than in the untreated dogs (Table 1). Degeneration of the smooth muscle was also less in the treated group.
II. Effects of MP on platelet aggregation

In untreated SAH dogs, platelet aggregation was accelerated on day 2 and further increased on days 4 and 7. In MP-treated SAH dogs, a transient acceleration of platelet aggregation on days 2 and 4 was followed by the normalization on day 7 (Fig. 4). The experiment examining the sequential changes in platelet aggregation caused by the intravenous MP administration in normal dogs demonstrated a biphasic inhibition of platelet aggregation: The first inhibition was observed at 5–15 minutes and the second at 60–180 minutes (Fig. 5). In the in vitro experiment, MP inhibition of platelet aggregation was dose-dependent (Fig. 6).

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<td>Untreated dogs</td>
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III. Effects of MP on cerebral artery contractile response in vitro

Pretreatment of canine basilar arteries with MP inhibited the contractile response to KCl by 21.8 ± 3.9%. The contractile responses to 5-HT and PGF$_2\alpha$ were also inhibited by 32.4 ± 5.2% and 38.6 ± 5.4%, respectively (Fig. 7). MP post-treatment after contraction induced by KCl, 5-HT, or PGF$_2\alpha$ caused

Fig. 3 A: Electron micrograph of the basilar artery of untreated dog. There is marked corrugation of internal elastic lamina (el) with vacuoles, swelling, and desquamation of endothelium (e). B: Electron micrograph of the basilar artery of MP-treated dog. The endothelium and smooth muscle (m) appear normal. Bar = 1 μm.

Fig. 4 ADP-induced platelet aggregation in untreated (A) and MP-treated (B) double hemorrhage dogs. Platelet aggregation is evaluated by the minimum concentration of ADP necessary for developing secondary aggregation of PRP.
IV. Effects of MP on BP, SSP, and CBF

Bolus administration of MP immediately decreased the mean BP by 17.5 ± 1.7 mmHg, which gradually returned to the previous value within 30 minutes. Immediately after MP administration, the SSP transiently increased by 16.8 ± 6.1 mmH2O for 30 seconds. The changes in CBF at 40 seconds and 30 minutes after MP administration were −3.2 ± 0.9% and −1.5 ± 1.9%. Intravenous MP administration did not therefore induce a significant decrease in CBF despite marked BP decrease (Fig. 9).

V. Effects of MP on CBF dysautoregulation

Exsanguination from the femoral artery decreased the BP to 54.5 ± 1.8% and the CBF to 75.9 ± 5.3% of the original level. Subsequent blood transfusion increased both BP and CBF to 115.0 ± 3.1% and 126.0 ± 5.0%, respectively. After confirming CBF dysautoregulation, intravenous MP administration transiently decreased BP to 80.8 ± 3.2% and CBF to 91.8 ± 3.2%. About 30 minutes after the MP administration, the BP and CBF returned to the preadministration values. Repeated exsanguination decreased BP to 63.3 ± 3.5% and CBF to 85.7 ± 4.2%. Blood transfusion increased BP to 106.2 ± 3.5% and CBF to 113.0 ± 2.2%. Thus, pretreatment with MP significantly inhibited the changes in BP and CBF induced by exsanguination and blood transfusion (Fig. 10).

Discussion

High-dose glucocorticoid hormone (steroid) therapy for vasospasm has been proposed because of the vasodilatory effect, anti-inflammatory effect, and effect on CBF. The clinical use has recently been recognized for preventing neurological deterioration caused by vasospasm following SAH. A high-dose glucocorticoid concentration has not been defined. In this study, 10 mg/kg of MP was considered a "high dose," because 10⁻⁴–10⁻³ M

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MP, close to the physiological plasma concentration after intravenous administration of MP (10 mg/kg), demonstrated a vasodilatory effect.

Fox and Yasargil reported that acute basilar artery vasospasm induced by arterial puncture or topical application of barium chloride or PGF$_{2\alpha}$ in dogs and monkeys could be relieved by topical application of MP and cortisol. Hashi et al. found that a single intravenous administration of high-dose hydrocortisone (100 mg/kg) relieved vasospasm in the canine basilar artery. These results were, however, obtained in earlier, reversible vasospasm. In contrast, a double hemorrhage canine model constantly produces chronic cerebral vasospasm with similar pathological and pharmacological features to those observed in human chronic vasospasm. In 1983, Chyatte et al. reported that the pathological arterial changes observed in the double hemorrhage model were degeneration or necrosis of the intima and smooth muscle cells. Prophylactic treatment with high-dose MP (30 mg/kg, every 12 hours) and ibuprofen alleviated the chronic cerebroarterial vasospasm without structural disarrangements of vessel walls, especially the smooth muscle layers, and preserved the contractile responses to vasoactive agonists.

Our double hemorrhage model showed that histological damage is more severe in the intima than media, and MP tended to prevent damage to both the intima and media. Recently, endothelial damage in the major cerebral arteries following SAH has
been considered part of the pathogenesis of cerebral vasospasm.\(^1\) Although the mechanism of steroid action on endothelial damage remains unclear, Bjork et al.\(^2\) reported that MP treatment reduced the endothelial inflammatory response and vascular permeability by antagonizing histamine, leukotriene C\(_4\), or platelet-activating factor. In addition, MP inhibition of continuous arterial constriction might prevent mechanical injury to endothelial cells and maintain the vessel wall integrity.

In our double hemorrhage model, acceleration of platelet aggregation was observed from days 4 to 7. Thrombus formation due to increased blood coagulation, platelet aggregation, and blood viscosity is considered important in the development of symptomatic vasospasm.\(^{1,8,9,20,22,26}\) Antiplatelet drugs such as trapidi,\(^{20}\) OKY-1581,\(^{49}\) and dextran sulfate\(^{16}\) effectively prevent ischemic symptoms in vasospasm. Although the steroid inhibition of platelet aggregation has been investigated in vitro,\(^{14,17}\) the present study demonstrated that intravenous administration of high-dose MP attenuated the accelerated platelet aggregation in the double hemorrhage canine model. We also found a biphasic inhibition of platelet aggregation by intravenous MP administration in normal dogs. The first inhibition observed at 5–15 minutes after administration can probably be considered a direct effect of MP, and the second at 60–180 minutes might be due to a phospholipase-inhibitory protein, produced through several biochemical reactions, causing suppression of arachidonic acid and thromboxane A\(_2\) release.\(^{3,12,14,24}\) Improved circulation due to suppression of arterioconstriction and endothelial disorder by intravenous MP administration may also prevent platelet aggregation.

Toda et al.\(^{21}\) reported that the middle cerebral arteries 7 days after induced SAH in dogs showed significantly depressed responses to KCl, 5-HT, norepinephrine, and histamine. They suggested this nonspecific depression to be due to reduced influx, mobilization, and availability of Ca\(^{2+}\) or to histologically impaired arterial smooth muscle cells. Chyatte et al.\(^5\) showed that annular arterial specimens from dogs receiving ibuprofen or MP had better contractile responses than from untreated dogs and concluded that anti-inflammatory drugs prevented smooth muscle cell degeneration. We showed that high-dose MP have a nonspecific vasodilatory effect on smooth muscle cells, which presumably contributes to prevention of vasospasm.

Hashi et al.\(^{10}\) have recently reported that intravenous injection of hydrocortisone caused vasodilation in canine spastic cerebral artery following SAH, which tended to increase CBF. They suggested that spastic arterial dilatation associated with CBF dysautoregulation results in increased CBF and maintains cerebral perfusion pressure. In our experiment using the double hemorrhage canine model, however, intravenous administration of high-dose MP did not increase CBF, probably due to the marked fall in systemic BP.

The other SAH model produced by puncture of the internal carotid artery after ligation of the bilateral external carotid and vertebral arteries demonstrated that pretreatment with high-dose MP attenuated the BP and CBF changes induced by blood withdrawal or transfusion. These results cannot be due to the vasodilatory effects of MP only. Wilson and Fisher\(^{25}\) reported that high-dose steroid tended to normalize cardiac output, total peripheral resistance, and stroke volume. Such effects on cardiovascular functions may help attenuate the changes in BP and CBF. Pathological conditions such as cerebral vasospasm are likely to associate with CBF dysautoregulation, and reduced BP directly decreases CBF and causes symptomatic deterioration.\(^{1,10}\) Therefore, high-dose glucocorticoid presumably prevents symptomatic deterioration due to vasospasm by attenuating the decrease in perfusion pressure and maintaining the CBF.

Although the etiology of cerebral vasospasm is still poorly understood, our experimental canine study demonstrated that high-dose MP prevents vasospasm and reduces histological damage to the vessel wall. Nonspecific vasodilation on smooth muscles, anti-platelet aggregation, and hemodynamic effects for sustaining CBF autoregulation were ascertained. Therefore, appropriate use of high-dose MP has therapeutic value in cerebral vasospasm following SAH.

References

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