Effect of High-Dose Methylprednisolone and U74006F on Eicosanoid Synthesis After Subarachnoid Hemorrhage in Rats

Paolo Gaetani, MD; Fulvio Marzatico, PhD; Daniela Lombardi, MD; Daniela Adinolfi, MD; and Riccardo Rodriguez y Baena, MD

Free radicals and lipid peroxidation of membrane fatty acids are thought to play a role in the pathogenesis of arterial vasospasm and the physiopathologic patterns of neuronal damage after subarachnoid hemorrhage. We have evaluated the effects of treatment with either high-dose methylprednisolone every 8 hours or a single dose of U74006F on the temporal profile of ex vivo synthesis of four selected eicosanoids in brain slices after experimental induction of subarachnoid hemorrhage in rats. Prostaglandins D₂ and E₂, prostacyclin, and leukotriene C₄ levels were determined by radioimmunoassay after 1-hour incubation of the brain slices. The synthesis of prostaglandin D₂ and 6-ketoprostaglandin F₁α at 48 hours after subarachnoid hemorrhage was significantly higher when compared to sham-operated animals (p=0.01); prostaglandin E₂ release was significantly enhanced at 6 hours after subarachnoid hemorrhage (p=0.01). The release of the lipoxygenase metabolite was significantly enhanced at 1, 6, and 48 hours after subarachnoid hemorrhage induction. Both treatment regimens significantly reduced the ex vivo synthesis of prostaglandin D₂, prostaglandin E₂, and leukotriene C₄ at 1, 6, and 48 hours after subarachnoid hemorrhage, whereas the effects on 6-ketoprostaglandin F₁α synthesis differed in the two treatment groups. U74006F enhanced the synthesis of prostacyclin metabolite in the early phase after subarachnoid hemorrhage, and high-dose methylprednisolone reduced the increasing synthesis at 48 hours. A strict comparison between the two treatments was not possible because of the different modalities of administration. However, these data suggest that the antioxidant effect of single-dose treatment with U74006F influenced the early and delayed effects on enzymatic lipid peroxidation, whereas the effects of methylprednisolone administration every 8 hours were more significant in the delayed phase. (Stroke 1991;22:215–220)

Experimental and clinical studies have emphasized the role of free radicals in the pathogenesis of vasospasm after subarachnoid hemorrhage (SAH).¹⁻³ Cyclooxygenase metabolites are mostly involved in the regulatory mechanisms of arterial tone,²⁻⁸ whereas the lipoxygenase metabolites of lipid peroxides and leukotrienes are involved in the inflammatory response⁹ and in the pathogenesis of vasogenic edema.¹⁰⁻¹¹ The latter also have a vasoconstrictor effect,¹¹⁻¹³ suggesting their possible involvement in some pathophysiologic aspects of SAH. Experimental data have suggested that treatment with high-dose methylprednisolone could reduce the inflammatory response in the subarachnoid space and prevent delayed arterial narrowing.¹⁴ However, the high-dose of glucocorticoid necessary to obtain a significant cell protection in different experimental models of central nervous system pathologies suggests that the pharmacologic effect of methylprednisolone is dose-dependent and provides both a direct and indirect antioxidant effect.¹⁵⁻¹⁸ Recently, a new series of 21-aminosteroid compounds has shown a marked activity in reducing lipid peroxidation and in protecting cell membrane from free radical reactions, without the glucocorticoid and mineral corticoid effects of other steroids.¹⁹⁻²⁰ One of these, U74006F, has shown a significant preventive effect on the development of arterial vasospasm in experimental SAH models.²¹⁻²³ We compared the effects of U74006F and high-dose methylprednisolone on the ex vivo synthesis of arachidonic acid metabolites from cerebral cortex of rats after experimental SAH to
investigate 1) whether the antioxidant activity of U74006F could influence arachidonic acid synthesis and 2) whether the effect of a single administration of U74006F is similar to that exerted after repeated methylprednisolone administration. We stress that the widely accepted treatment with methylprednisolone was used not with the intention of making a strict comparison to the aminosteroid modality of action, but to collect standardized control data to correlate with the effect of single-dose administration of U74006F.

**Materials and Methods**

We conducted experiments on male Sprague-Dawley rats (Charles River strain, Calco, Como, Italy) weighing 375–425 g, using the experimental SAH procedure according to previously reported studies. General anesthesia was induced with 3% halothane (70%/30%, O2:N2O) and maintained with 0.75% halothane in the gas mixture. A burr hole was made at the interparietal/occipital suture connection using a refrigerated twist-drill, and a small catheter (model PE-10, Clay-Adams, Parsippany, N.J.) was inserted into the cisterna magna. A femoral artery was cannulated for anerobic sampling of blood to measure pH, Pco2, and Po2, and arterial blood pressure was monitored with an indirect blood pressure sensor (model 446420, Bel-Art Products, Pequannock, N.J.). Body temperature, monitored by a rectal thermometer, was adjusted and maintained near 37°C by external heating. When the rats were in a steady respiratory state with arterial Po2 and Pco2 110 and 30–40 mm Hg, respectively, 0.35 ml autologous arterial blood was collected from the femoral artery, and an aliquot of 0.30 ml was injected into the cisterna magna via the catheter within approximately 2 minutes. Before SAH induction, a sample of cerebrospinal fluid of approximately 0.01–0.03 ml was collected and immediately frozen in dry ice and maintained in a refrigerated centrifuge at −80°C until analysis.

Three aliquots of the supernatant were kept at −80°C until analysis. We determined levels of arachidonic acid metabolites using the radioimmunoassay technique for prostaglandin D2 previously described in detail. Radioimmunoassay kits for prostaglandin E2 (NEK-020) and 6-ketoprostaglandin F1α (NEK-008) were provided by New England Nuclear (NEN) Chemicals GmbH, Drecich, FRG. The radioactive labels were 3H for prostaglandin E2 and 14C for 6-ketoprostaglandin F1α. Antiserum for these metabolites had less than 2.5% of cross-reactivity with other prostaglandins. Immunoactive leukotriene C4-like activity was detected with the radioimmunoassay technique, according to Levine et al. using an antiserum (NEN Chemicals) to leukotriene C4 that has a cross-reactivity of 10.1% with leukotriene D4, 2.3% with leukotriene E4, 0.07% with hydroxyeicosatetraenoic acid, and 0.006% with leukotriene B4. Ten milliliters Atomlight High Sample Capacity Scintillation Solution (NEF-968) was added to each sample (supplied by United Technologies Packard, Packard Instruments, Downers Grove, Ill.). Radioactivity was measured using a liquid scintillation spectrometer (model 3320, Packard Instruments) as previously described.

Results are expressed in picograms per milligram of protein. The protein content of homogenate was assayed according to Lowry et al. with serum albumin as a standard. The assay sensitivity was 15 pg/mg protein. Statistical analysis was performed using analysis of variance and Tukey’s test for multiple comparisons. Statistical significance was accepted for values of p<0.05.

**Results**

Figure 1 represents the values of prostaglandin D2 ex vivo release after the experimental procedure. The release was enhanced after the hemorrhage, and at 48 hours after SAH was significantly higher when compared to values from sham-operated animals (p<0.01). High-dose methylprednisolone significantly decreased the release at 48 hours, and the values in the treated group did not differ from those in the sham-operated group. U74006F significantly reduced the late peak of prostaglandin D2 release (p<0.05), even though this effect was significantly lower when compared to animals treated with high-dose methylprednisolone.

Figure 2 shows a significant enhancement of prostaglandin E2 release at 6 hours after the SAH procedure (p<0.01). A significant reduction in prostaglandin E2 release at 6 hours after U74006F and high-dose methylprednisolone administration was observed. In animals treated with U74006F, a signif-
significant conflicting increase in prostaglandin E₂ synthesis and release was observed at 48 hours.

The release of prostacyclin was significantly enhanced at 48 hours after SAH induction. High-dose methylprednisolone significantly reduced the release at 48 hours (Figure 3). A trend toward an inhibitory effect of U74006F at 48 hours and of methylprednisolone at 6 hours was evident, although statistical significance was not achieved because of the small number of animals used in each group and the wide range of the data. More experiments are ongoing to verify the effects on eicosanoid synthesis at 3 and 4 days after administration of U74006F and high-dose methylprednisolone.

The release of the lipoxygenase metabolite was dramatically enhanced at 1, 6, and 48 hours after SAH induction, whereas high-dose methylprednisolone significantly decreased the release at 1, 6, and 48 hours. The inhibitory effect on leukotriene C₄ synthesis capacity is characteristic and showed a progressive inhibitory trend, with greater significance in the late phase (Figure 4). On the other hand, in the early posthemorrhagic period, lipoxygenase activation was reduced by U74006F, with the effect more pronounced at 1 and 6 hours (p<0.01).

Discussion

In a previous study, we showed enhanced activity of both cyclooxygenase and lipoxygenase pathways in the cerebral cortex of rats after experimental SAH. Similar findings were reported for the vascular compartment and the subarachnoid space. The ex vivo method provides information about the residual capacity of brain tissue to synthesize arachidonic acid metabolites after a pathological event, as previously discussed. The aim of the present study was to compare the efficacy of a single dose of a new aminosteroid (U74006F) with routine standard treat-
ment with high-dose methylprednisolone, rather than a pharmacologic comparison of the effects and mechanisms exerted on eicosanoid synthesis. Thus, we administered a single dose of U74006F to verify either its antioxidant properties or a delayed effect on enzymatic lipid peroxidation, i.e., on cyclooxygenase and lipoxigenase pathways. Methylprednisolone was administered every 8 hours after the experimental procedure.

The global effect of U74006F on arachidonic acid metabolism was mostly evident during the early phase (1–6 hours) after SAH. On the other hand, the effect of high-dose methylprednisolone was evident on both metabolic pathways and was more pronounced during the late phase, 48 hours after SAH. Looking at the antioxidant properties of U74006F, the significant effect of an early single injection on the late-phase synthesis of leukotriene C₄ seems more intriguing, although no mechanistic conclusion can be drawn about the specific mechanism.

In a recent review, Braughler and Hall urged that lipid peroxidation, phospholipase activation, and fatty acid release are inextricably related. Superoxide radicals are intermediate products of arachidonic acid metabolism, in both cyclooxygenase and lipoxygenase pathways. The resulting lipid peroxidation may enhance leukotriene synthesis in a chain-branched reaction. The available data suggested that methylprednisolone acts on a glucocorticoid receptor, stimulating the synthesis of lipocortin protein, which inhibits phospholipase A₂ and the release of arachidonic acid. High-dose methylprednisolone also inhibits the hydrolysis of membrane phospholipids through a receptor-independent antioxidant mechanism. Our results confirm this hypothesis previously suggested.

Our results confirm this hypothesis previously suggested.
suggested by Hall and coworkers and answer the question of whether U74006F could indirectly inhibit leukotriene release.

Glutathione peroxidase acts on lipid hydroperoxides and has been shown to inhibit lipoxigenase. Our results point out the early effect of U74006F on leukotriene C4 release at 1 and 6 hours and suggest that the aminosteroid, besides the specific antiperoxidative activity, may also influence brain synthesis of eicosanoids after SAH.

Nevertheless, the pharmacologic effects of these two drugs should also be evaluated, looking at specific pathophysiologic aspects of SAH, that is, changes in cerebral blood flow and vasospasm. High-dose methylprednisolone was recently shown to improve cerebral blood flow without a concomitant increase of intracranial pressure after experimental SAH in cats. This suggests a direct effect of this steroid on microcirculatory regulation, which would modulate the production of vasoactive compounds, with special regard to eicosanoids. Our results further support this hypothesis by demonstrating a significant effect of high-dose methylprednisolone on both lipoxigenase and cyclooxygenase pathways and on the biosynthesis of vasoactive eicosanoids.

The role played by arachidonate metabolites in the pathogenesis of arterial vasospasm after SAH has been widely discussed. Prostaglandin D2 and leukotriene C4 have well-documented vasoconstrictive activity on cerebral arteries, with high levels also detected in the eisternal cerebrospinal fluid of patients with vasospasm after the aneurysm rupture. In the same experimental model of SAH, a biphasic pattern of arterial spasm was shown: an acute phase occurring 10–15 minutes after SAH, followed by a late phase up to 48 hours after SAH, which is more important from a metabolic point of view.

U74006F was shown to reduce the postischemic hypoperfusion of brain tissue; this effect may be related to its protective effect on cerebral microvasculature and to an inhibition of lipid peroxidation. The results of the present study suggest that U74006F may exert a significant inhibitory effect on the lipoxigenase pathway and reduce the release of spasmodenic substances such as leukotriene C4, whereas its effect on the synthesis of the vasodilating agent prostacyclin did not seem so dramatic. On the other hand, high-dose methylprednisolone significantly reduces the severity of the typical degenerative arteriopathy of the delayed vasospasm. As shown in the present study, this could be partially related to a significant inhibitory effect on brain eicosanoid synthesis in the late phase.

In conclusion, our results show significant changes of the eicosanoids from cerebral cortex after experimental SAH, indirectly stressing the involvement of oxygen free radicals and lipid peroxidation in brain response to hemorrhage. Moreover, they suggest a possible double protective mechanism for U74006F. In the early phase after SAH, the inhibitory effect on iron-catalyzed lipid peroxidation influences eicosanoid synthesis; in the late phase, a reduced synthesis of vasoconstrictive eicosanoids, such as leukotriene C4 and prostaglandin D2, could be effective in reducing or preventing vasospasm.

High-dose methylprednisolone exerts a receptor-dependent effect on phospholipase A2 and inhibits both cyclooxygenase and lipoxigenase pathways. This effect is more evident in the late phase of SAH. Both treatments could act in a complementary way to provide brain protection after hemorrhage.

Acknowledgment

The authors gratefully recognize the help and advice of Agostino Sperandeo of the Upjohn Co. Italia for his technical and scientific assistance.

References


15. Braughler JM, Hall ED: Central nervous system trauma and stroke: I. Biochemical considerations for oxygen radical for-

KEY WORDS  •  lipid peroxidation  •  methylprednisolone  •  subarachnoid hemorrhage  •  rats
Effect of high-dose methylprednisolone and U74006F on eicosanoid synthesis after subarachnoid hemorrhage in rats.

P Gaetani, F Marzatico, D Lombardi, D Adinolfi and R Rodriguez y Baena

doi: 10.1161/01.STR.22.2.215

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/22/2/215

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org/subscriptions/