Selective Thromboxane Inhibition: A New Approach to Antiplatelet Therapy

JAMES S. HOOGASIAN, M.A., ANITA M. NATALE, B.S.†, JOHN R. ARSENAULT, B.S.†
MARK H. JOHNSON, B.S.†, and PETER H. LEVINE, M.D.†‡

SUMMARY Antiplatelet drugs as exemplified by aspirin are used frequently to prevent stroke. Aspirin inhibits the formation of both the potent platelet aggregator, thromboxane A2 and the potent anti-aggregator, prostacyclin. Another approach to the inhibition of platelet aggregation might involve selective suppression of thromboxane formation. We report our experience in swine with UK-38,485, a drug which selectively inhibits thromboxane formation. The rationale and potential uses of UK-38,485 in the in vivo prevention of platelet aggregation and for the therapy of cerebrovascular disease are discussed.

ANTIPATELET THERAPY is a therapeutic modality employed frequently to prevent stroke. Enthusiasm for this approach is derived from the widespread acknowledgement that platelets are important contributors to many mechanisms which cause threatened stroke.1 Several large studies have demonstrated that aspirin, the most widely utilized antiplatelet agent, is efficacious in selected patients.2 3 A relatively large aspirin dose, 1300 mg per day, was employed in these patients and this dosage has lead to subsequent controversy.1, 4

Aspirin inhibits platelet aggregation by suppressing the enzyme cyclo-oxygenase, blocking the formation of cyclic endoperoxide, a key intermediary of prostaglandin metabolism.5 Unfortunately, the inhibition of cyclic endoperoxide has both beneficial and detrimental effects on platelet physiology because it blocks the formation of both the potent platelet aggregator, thromboxane A2 (TXA2) as well as the potent platelet anti-aggregator, prostacyclin (PGI2).6 7 Recently, it has been proposed that low dose aspirin, 80 to 240 mgs per day may be a better antiplatelet regimen than 1300 mgs per day because a low dose appears to inhibit platelet TXA2 production more than endothelial PGI2 production, but the optimal dose is uncertain, and the therapeutic margin narrow.6 8 A more satisfactory approach to this problem would be to develop compounds which selectively inhibit TXA2 synthesis while sparing PGI2 production. We report our experience with UK-38,485, an imidazole derivative, which selectively inhibits serum thromboxane production for an extended period of time, without inhibiting PGI2 formation.10

Materials and Methods

Three different groups of male, Yorkshire swine were given UK-38,485; group 1 consisted of 4 animals who received one dose of 0.5 mg/kg intravenously (I.V.); group 2 consisted of 4 animals who received a dose of 10 mg/kg orally (P.O.); and group 3 consisted of 4 animals who received 10 mg/kg P.O. daily for 10 days. All animals had baseline and post-drug serum samples drawn under anesthesia. The animals were sedated with 35 mg/kg Ketamine Hydrochloride I.M. and were maintained with a mixture of 1 1/2% Halothane and 30% Nitrous Oxide delivered via cone or endotracheal tube by a Bird Veterinary Respirator.

Bleeds drawn for the I.V. Study were immediately brought to the research laboratory where they were incubated for the remaining portion of an hour at 37°C. Bloods drawn for the P.O. Study were immediately placed in a portable 37°C water bath and transported to the laboratory, where they continued to incubate at 37°C for the remainder of an hour. The blood was then spun at 2,200 g for twenty minutes at 4°C. The serum was removed by pipet and respun as above. The resultant cell-free serum was then aliquoted into 1 ml plastic tubes and rapidly frozen at −70°C until assayed.

The TXA2 level was measured in serum as a function of its stable hydrolysis product, TXB2. Serial dilutions of serum were assayed utilizing the highly specific New England Nuclear Thromboxane B2 (TH) RIA kit, (Boston, MA) and the lower limit of sensitivity for the assay was 5 pg/0.1 ml.

The prostacyclin (PGI2) level was also measured in serum as a function of its stable hydrolysis product, 6-keto-PGF1α. Samples to be assayed for 6-keto-PGF1α were extracted prior to assay using Octadecylsilyl Silicon columns (Waters Associated, Milford, MA) as described by Powell.11 Extracted samples were evaporated under Nitrogen at 37°C, reconstituted with 50mM Phosphate buffer, pH 7.3, containing 0.1% gelatin and 0.01% Thimerosal, and frozen at −70°C until assayed. Samples were assayed utilizing the highly specific New England Nuclear 6-keto-PGF1α(3H) RIA kit.

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Serum TXB₂ levels were assayed at a minimum of three dilutions and in duplicate. Serum 6-keto-PGF₁α samples were extracted at least in duplicate and assayed. Assayed samples were mixed with 10 mls 3α70B scintillation cocktail (Research Product International Corporation, Mount Prospect, ILL) and counted for ten minutes each in a Beckman LS-100C Liquid Scintillation System (Irvine, CA).

Data was analyzed by use of a T test or a one or two way analysis of variance as appropriate.

Results

As shown in table 1, the effect of a single 0.5 mg/kg dose of UK-38,485 given I.V. was maximal at 1 hour but a significant inhibition ($p < .05$) of serum TXB₂ formation was still present 6 hours after drug injection as demonstrated by one-way analysis of variance. Serum PGI₂ concentrations were not significantly changed. A single 10 mg/kg dose of UK-38,485 given P.O. reduced mean serum TXB₂ to 14% of baseline levels at 6 hours, and to 28% of baseline levels at 24 hours. The 6 hour values were significantly reduced ($p < .05$) compared to baseline values but the 24 hour values were not significantly reduced, as shown by a two-way analysis of variance. Individual values are outlined in figure 1. Serum PGI₂ (mean ± S.E.) at baseline, 6 hours and 24 hours were 441 ± 96, 373 ± 84, and 56 ± 11 pg/0.1 ml. No significant changes were appreciated.

Administration of UK-38,485 at 10 mgs/kg once daily for 10 days resulted in the prolonged suppression of serum TXB₂. The serum TXB₂ concentration prior to the start of the drug was 7191 pg/0.1 ml ± 1111 (mean ± 1 S.E.). The mean serum concentrations (± 1 S.E.) of TXB₂ at days 2, 4, 6, 8, and 10 of drug treatment were 230 ± 27, 191 ± 70, 432 ± 302, 444 ± 121 and 546 ± 108 pg/0.1 ml, respectively (fig. 2). All mean serum TXB₂ values obtained while on UK-38,485 were significantly lower than baseline pre-drug TXB₂ levels ($p < .01$) as demonstrated by group T test. A trend toward a significantly higher level of TXB₂ was seen by day 10, but the suppression was still quite marked when compared to the baseline values. The mean serum concentrations of PGI₂ (± 1 S.E.) in pg/0.1 ml at baseline and days 2, 4, 6, 8 and 10 were 359 ± 29.2, 640 ± 64.2, 674 ± 156.5, 422 ± 45.7, 675 ± 73.2 and 729 ± 92.4. The PGI₂ levels on days 2, 8, and 10 were significantly elevated ($p < .05$) when compared to baseline but the levels on days 4 and 6 were not elevated.

No observable hemorrhagic or other side effects were appreciated in any of the swine given either an I.V. or P.O. single dose of UK-38,485 or with maintenance over the 10 day period.

Discussion

UK-38,485 selectively inhibits thromboxane production while sparing prostacyclin production. The drug was effective when given either I.V. or P.O. The suppressive effect when the drug was given over the long term period of 10 days was striking and appeared to reach a steady state of thromboxane inhibition which was more effective than a comparable single oral dose. Additionally, during prolonged dosing there was a trend toward enhanced PGI₂ synthesis. UK-38,485 did not cause any serious side effects in our swine. UK-38,485 has been administered to humans and a similar marked inhibition of thromboxane production over a 1 week dosing period was observed without any drug related side effects.¹²

The theoretical advantages of inhibiting TXA₂ production while sparing PGI₂ production relate to the pivotal opposing effects on platelet aggregation of the two compounds. Ex vivo testing of the platelet aggregating response to stimuli such as ADP, collagen and
platelet clot formation and suggest that this may also contribute to the antiplatelet aggregatory effects of thromboxane inhibitors.

In another experiment, a thromboxane synthetase inhibitor was also very effective in preventing death in an arachidonate-induced platelet mediated sudden death model.

These experiments lend further support to the proposal that agents such as UK-38,485 should be considered as a potential therapy to prevent stroke or other vascular disorders in which platelets play an important role. The use of a thromboxane synthetase inhibitor will eliminate the question of what aspirin or other cyclo-oxygenase inhibitor dosage is most effective in blocking TXA₂ production while sparing PGI₂ production. Additional experimental evidence of human in vivo suppression of platelet aggregation and safety of administration will be necessary but thromboxane synthetase inhibition with UK-38,485 or a related compound offers a new and exciting potential approach to further study the role of antiplatelet therapy in the prevention of ischemic cerebrovascular disease. UK-38,485 appears to be well suited for this type of trial because once daily use markedly effects TXA₂ production while sparing PGI₂, and the effect persists over an extended period.

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References


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Figure 2. The effects of 10 mg/kg of UK-38,485 given daily to 4 swine for 10 days on serum TXB₂ concentration (mean ± S.E.).

Arachidionate when subjects or serum samples have been pre-treated with thromboxane synthetase inhibitors have yielded conflicting results.13 Some observers report no inhibitory effects to any aggregating substances while others note inhibition of arachidonate induced aggregation by thromboxane synthetase inhibitors. The lack of an in vivo effect on platelet aggregation has been ascribed to the accumulation of other prostaglandin products of cyclo-oxygenase which could potentially induce platelet aggregation.16

Recently, several elegant in vivo experiments to assess platelet aggregation in the presence of thromboxane synthetase inhibitors have been published. Randall and Wilding demonstrated that a thromboxane synthetase inhibitor significantly reduced Indium-labelled platelet accumulation at a site of electrically induced rabbit carotid artery injury.17 Hall et al also showed that a different selective thromboxane synthetase inhibitor significantly blocked the in vivo accumulation of Indium-labelled platelets at a site of de-endothelialized rabbit abdominal aorta. These studies are important because they demonstrate a clear in vivo inhibitory effect by thromboxane synthetase inhibitors on platelet accumulation at a site of endothelial injury, a situation which may relate directly to one pathophysiologic mechanism of stroke and/or transient ischemic attack. Although species differences regarding the effect of thromboxane synthetase inhibitors on platelet activity may exist, these studies suggest that this class of drug does prevent platelet clot formation in vivo. We await similar demonstrations of antiplatelet effectiveness in swine and humans.

Specific thromboxane synthetase inhibitors have also been evaluated experimentally in therapeutic trials to prevent pathologic conditions which may be platelet mediated. Coronary artery thrombosis was prevented in 90% of dogs treated with a thromboxane synthetase inhibitor while cyclo-oxygenase inhibitors were only effective in 50 to 58% of the animals.19 It was also observed that in the absence of platelet thromboxane production, prostacyclin's anti-aggregatory effects were markedly enhanced. The enhanced anti-aggregatory effects of prostacyclin in the presence of thromboxane synthetase inhibitors may be an important contribution to the in vivo effectiveness of these drugs in preventing platelet deposition at sites of arterial injury. Defreyen et al have observed an increase in PGI₂ production in the presence of thromboxane synthetase inhibition and suggest that this may also contribute to the antiplatelet aggregatory effects of thromboxane inhibitors.20 In another experiment, a thromboxane synthetase inhibitor was also very effective in preventing death in an arachidonate-induced platelet mediated sudden death model.21

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Cerebral Blood Flow Measurement in Cerebrovascular Occlusive Diseases

TAKEHIKO YANAGIHARA, M.D.,* AND HEINZ W. WAHNER, M.D.†

SUMMARY In order to evaluate cerebral blood flow (CBF) patterns among individual patients with increased statistical confidence, CBF measurements were carried out using the 133Xe-inhalation method and external head detectors. F1 values representing gray matter flow from 3 to 6 head detectors were averaged to form 16 different regions for each cerebral hemisphere. Normative values were obtained from 46 healthy volunteers, and data from individual regions were analyzed for absolute blood flow rates (ml/100g/min), for concordance between right and left hemispheres and as percent of mean hemispheric flow. CBF measurements were then carried out among 37 patients with cerebrovascular occlusive diseases, and results were compared with normative values. A high incidence of abnormal flows were detected among symptomatic patients with intracranial arterial stenosis or occlusion and those with extracranial internal carotid artery occlusion. By using the above method for data analysis, it was possible to delineate hyperperfused areas among these patients. Even though the 133Xe-inhalation method has inherent limitations, this is a practical and safe method for measurement of CBF which can provide reliable information useful for management of patients with cerebrovascular occlusive diseases, particularly when the results are presented with statistical confidence.

Since the development of a method for measurement of cerebral blood flow (CBF) in 1948 by Kety and Schmidt,1 CBF measurement by intra-arterial and intravenous administration and inhalation of gamma-emitting radioisotopes have been used successfully for patients with various neurologic and psychiatric disorders. More recently three-dimensional measurements of regional CBF (rCBF) with gamma-emitting2 or positron-emitting3,4 radioisotopes have become available. The concept of measuring CBF by inhalation of 133Xe gas was developed by Mallett and Veall5 and has been modified for practical use by Obrist et al.,6 Risberg et al.,7 and Meyer et al.8 With the currently utilized method, it has been possible to evaluate CBF in patients with cerebrovascular diseases.9-12 Although these studies clearly demonstrated the feasibility of this method for quantitation of rCBF, it is still difficult to express the results from individual patients with statistical confidence. Yet, there is a clinical need of CBF measurements for identification of patients with cerebrovascular occlusive diseases who are amenable to surgical
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