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Comparison of the systemic inhibition of thromboxane synthesis, anti-inflammatory activity and gastro-intestinal toxicity of non-steroidal anti-inflammatory drugs in the rat

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Introduction

Cyclooxygenase inhibition is now widely accepted as one of the major properties of non-steroidal anti-inflammatory drugs (NSAIDs). This group of compounds is diverse in chemical structure yet its members share both therapeutic and toxic effects which points to a common underlying biochemical mechanism. The side-effects most frequently associated with NSAID therapy are occult bleeding and ulcerogenicity. The available evidence suggests that inhibition of prostaglandin synthesis by NSAIDs impairs the mucosal cytoprotective mechanism and renders the epithelial cell layer more vulnerable to damage by aggressive luminal factors.

Current research is directed towards developing potent anti-inflammatory drugs with significantly improved gastro-intestinal tolerability. Following this concept, we have compared in rats several NSAIDs for anti-inflammatory activity in adjuvant arthritis, gastro-intestinal toxicity as measured by fecal blood loss, and thromboxane B_2 (TXB_2) formation by platelets as an indication of systemic cyclooxygenase activity.

Methods

TXB_2 levels were measured *ex vivo* in rat serum according to the method of Patrono *et al.* [1]. Briefly, blood was collected by orbital puncture from individual rats (n = 3–6) before, and at 1, 4 and 24 hours after a single oral drug administration. The blood samples were allowed to clot at 37 °C for 30 min., and then the serum was removed and frozen. TXB_2 levels were assayed later using a commercial radioimmunoassay kit (Amersham, no. TRK. 780) in thawed serum samples diluted 1 : 40 with assay buffer. At this high dilution serum proteins do not interfere with the assay so no extraction step is required.

The compounds studied included the reference NSAIDs aspirin, indomethacin, naproxen and nimesulide, and a new developmental compound CGP 28237 (5-methylsulfonylamino-6-phenoxy-1-indanone) which is structurally related to nimesulide.

Results and discussion

The results are summarized in Table 1.

For aspirin, the effective anti-inflammatory dose range lies close to the threshold dose for increased fecal blood loss, resulting in a therapeutic index of only 4. In the TXB_2 assay, oral aspirin doses of 100, 30 and even 10 mg/kg inhibited TXB_2 generation by more than 90% at 1 and 4 hours, and only at the lowest dose did the TXB_2 level begin to recover towards the pre-treatment level at 24 hours. Much lower doses of aspirin than those required for anti-inflammatory effects and ulcerogenicity already completely inhibited TXB_2 formation for more than 4 hours, reflecting the well known irreversible inhibition of platelet cyclooxygenase by this drug.

Table 1
Comparison of anti-inflammatory activity, gastro-intestinal toxicity, and systemic inhibition of thromboxane B_2 synthesis by NSAIDs in the rat in vivo.

Drug	Adjuv. Arthritis[1] ED_{40} (mg/kg p.o.)	GI Blood Loss[2] threshold dose (mg/kg p.o.)	Therapeutic Index GIB×3/ED_{40} Adjuv. Arth.	Thromboxane B_2[3] ED_{90} (mg/kg p.o.)
Aspirin	150	600	4	10
Indomethacin	0.5	4	8	5
Naproxen	3	20	7	3
Nimesulide	0.2	100	500	> 10
CGP 28237	0.5	Ø 400	> 800	> 100

[1] Adjuvant arthritis test: dose of drug in mg/kg which, when given orally once a day on days 11–14 after adjuvant injection, reduces paw oedema by 40% on day 15.
[2] Gastro-intestinal toxicity test: dose of drug in mg/kg which, when administered once daily for 10 days, causes a threefold increase over control in the 10-day cumulative fecal blood loss as measured by ^{51}Cr-labelled homologous erythrocytes (GIB×3).
[3] Systemic cyclooxygenase inhibition test: dose of drug in mg/kg inhibiting the formation of thromboxane B_2 in blood ex vivo by 90% after a single oral administration.

Compared to aspirin, the 2 classical NSAIDs indomethacin and naproxen exhibit an approximately 2-fold increase in therapeutic index but there is still no marked dissociation between anti-inflammatory activity and gastro-intestinal toxicity. In the TXB_2 assay both drugs inhibited TXB_2 formation by ≧90% with doses ≦5 mg/kg p.o. at 1 and 4 hours after drug administration. These doses are close to the active anti-inflammatory and ulcerogenic doses of these NSAIDs. Within 24 hours after drug administration TXB_2 inhibition had decreased to less than 50%, reflecting the plasma half-life of these drugs and the reversible nature of the cyclooxygenase inhibition.

Compared to the previously mentioned drugs, nimesulide exhibited a pronounced dissociation between anti-inflammatory potency and gastro-intestinal toxicity, resulting in a more than 50-fold increase in the therapeutic index. This potent anti-inflammatory compound only caused gastro-intestinal side-effects at high doses (≧100 mg/kg p.o.). TXB_2 formation was significantly inhibited (~80%) with 10 mg/kg but not with 1 mg/kg.

A further improvement in the dissociation between the anti-inflammatory activity and gastro-intestinal toxicity can be seen from the results for CGP 28237. This compound is a potent inhibitor of rat adjuvant arthritis (ED_{40} 0.5 mg/kg) but it caused no increase in gastro-intestinal blood loss up to the highest dose tested (400 mg/kg p.o. for 10 days). In fact, CGP 28237 was non-ulcerogenic in all the test models used so it is not possible to calculate a therapeutic index for the compound. Interestingly, in the TXB_2 assay, doses of 10 and even 100 mg/kg p.o. failed to produce a significant inhibition of serum TXB_2 within the 24 hour observation period in contrast to what had been observed with all the other NSAIDs tested. Thus there would appear to be an association between the compound's lack of inhibition in the TXB_2 assay and its outstanding gastro-intestinal tolerability, whereas the biochemical basis for the potent anti-inflammatory activity of CGP 28237 remains to be elucidated.

In conclusion, these studies have demonstrated that the TXB_2 assay is a useful adjunct to the standard screening procedures for anti-inflammatory compounds in rats. The method has provided valuable evidence for the systemic bioavailability, platelet cyclooxygenase inhibition and pharmacodynamics of several reference NSAIDs and developmental compounds. Furthermore, from the data for CGP 28237, the possibility emerges that the absence of marked inhibition in the TXB_2 assay may be an additional valuable selection criteria for potent anti-inflammatory compounds with significantly improved gastro-intestinal tolerability.

Reference

[1] C. Patrono, G. Ciabattoni, E. Pinca, F. Pugliese, G. Castrucci *et al., Low Dose Aspirin and Inhibition of Thromboxane B_2 Production in Healthy Subjects.* Thrombosis Research *17*, 317–327 (1980)