Anti-inflammatory effect of tepoxalin

Blood and synovial tissue studied in patients with knee arthrosis

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Our aim was to determine the amounts of eicosanoids in blood and synovial tissue of patients with knee arthrosis and to examine the effects of 2 doses of tepoxalin (50 mg twice, 200 mg twice), administered p.o. for 3.5 days.

Concentrations of leukotriene B₄ (LTB₄), LTC₄, and thromboxane B₂ (TXB₂) were measured in blood before and after oral administration of tepoxalin and release of prostaglandin E₂ (PGE₂), 6-keto-PGF₁α, and LTC₄ was measured in incubation media of synovial tissue, taken at surgery from patients treated with tepoxalin. Radioimmunoassay (RIA) was used to determine the levels of the eicosanoids.

LT and TXB₂ release was reduced by tepoxalin in both doses used. Under these conditions, PGE₂, 6-keto-PGF₁α, and LTC₄ release from synovial tissue was detectable only after stimulation with calcium ionophore A23187. Washed synovial tissue, in which tepoxalin concentrations should be reduced, released higher amounts of all eicosanoids measured than directly incubated synovial tissue did. Pain after tepoxalin administration was significantly reduced. Relevant drug concentrations were detected in plasma and synovial fluid. Tepoxalin was well tolerated and had no marked adverse effects.

At 400 mg, tepoxalin is a dual inhibitor of cyclooxygenase (CO) and 5-lipoxygenase (5-LO) in blood and synovial tissue.

2 major pathways of arachidonic acid (AA) metabolism are known. These are the cyclooxygenase (CO) and 5-lipoxygenase (5-LO) pathways which convert AA to prostaglandins (PG) and leukotrienes (LT) (Goldyne 1984, Stenson and Parker 1984, Kulkami and Parale 1985). Both PG and LT are potent inflammatory mediators (Bray 1983, Stenson and Parker 1984, Higgs and Moncada 1985, Kulkami and Parale 1985, Feuerstein and Hallenbeck 1987, Ford-Hutchinson 1990, Lewis et al. 1990). Elevated levels of LTB₄ (Klickstein et al. 1980, Davidson et al. 1982), TXB₂, PGE₂ and 6-keto-PGF₁α (the stable metabolite of PGI₂) have been detected in synovial fluid of patients with OA and RA (Trang et al. 1977, Brodie et al. 1980, Egg et al. 1980, Egg 1984).

Commonly used non-steroidal anti-inflammatory drugs (NSAIDs) are CO inhibitors. Since BW 755c, the first prototype (Blackwell and Flower 1978), the quest for 5-LO inhibitors, LT antagonists or biosynthesis inhibitors and dual inhibitors has been intense but, until recently, frustrating (Lewis et al. 1990, Ford-Hutchinson 1990). Tepoxalin [5-(4-chlorophenyl)-N-hydroxy-1-(4-methoxyphenyl)-N-methyl-1H-pyrazole-3-propanamide], a novel compound, is an orally active anti-inflammatory agent, which inhibits both CO and 5-LO activities (Depre et al. 1996). Tepoxalin also inhibits neutrophil migration, suppresses NF kappa B activation and inhibits T-cell proliferation (Zhou et al. 1994, Kazmi et al. 1995, Ritchie et al. 1995, Depre et al. 1996, Zhou et al. 1996).

We determined the clinical activity and biochemical profile of tepoxalin in blood and synovial tissue of patients with knee arthrosis.

Patients and methods

This study was approved by the local ethics committee. The patients gave informed consent and the study complied with the principles for the standard of good clinical practice for trials on medicinal products in human beings in the European Community (Pharmacol Toxicol 1990; 67: 361-72).

Patients

14 patients (10 women), mean age 67 (53-79) years, operated on with total knee replacement because of arthrosis, were examined in a clinical phase II study
Table 1. Study procedures

<table>
<thead>
<tr>
<th>Procedures</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
</tr>
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<tbody>
<tr>
<td>Drug administration</td>
<td>07.00</td>
<td>07.00</td>
<td>07.00</td>
<td>2 hrs before surgery</td>
</tr>
<tr>
<td></td>
<td>19.00</td>
<td>19.00</td>
<td>19.00</td>
<td></td>
</tr>
<tr>
<td>Plasma (LTB₄/LTC₄)</td>
<td>7.00</td>
<td>2 hrs after drug</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>19.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum (TXB₂)</td>
<td>7.00</td>
<td>2 hrs after drug</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>19.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Synovial tissue (PG/LT)</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Synovial fluid (tepoxalin)</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma (tepoxalin)</td>
<td>2 hrs after drug</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical assessment</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>

(double-blind, randomized, parallel, 2-dose levels).
The oral dosage of tepoxalin was 200 mg twice daily (n 7) or 50 mg twice daily (n 7). Tepoxalin was administered for 3 days before surgery and on day 4, 2 hours before surgery (Table 1). Participating patients had no gastrointestinal disorder or hypersensitivity to aspirin.

In order to avoid effects of previously administered drugs on the results of this study, only patients who had received their last intraarticular corticosteroid injection at least 3 months before and who had stopped all corticosteroid medication at least 4 weeks before were included. All therapy with NSAIDs had to be terminated at least 8 days before tepoxalin treatment was commenced.

Clinical parameters were evaluated. Pain was assessed daily before surgery, using visual analogue scales (VAS) asking about pain during the previous night, the same day and intermittent pain induced by a specific activity, like sitting down or climbing stairs. The maximum flexion angle of both knees was determined on every day of the study.

Blood and synovial tissue samples

Blood taken from the cubital vein was used for the measurement of TXB₂ and heparinized blood for determination of LTB₄ and LTC₄. Synovial tissue and fluid were obtained during total knee replacement, avoiding blood contamination. 2 mL of whole blood was incubated for 30 min at 37 °C, as described by Patrono et al. (1980). 2 mL of heparinized blood was incubated for 15 min at 37 °C, thereafter the divalent ionophore A23187 was added, resulting in a final concentration of 50 micromol/L. Ionophore A23187 activates Ca²⁺-dependent phospholipase and increases free AA (Borgeat and Samuelsson 1979). Incubations were then continued for an additional 30 min, as described by Carey and Forder (1986). After incubation, all samples were centrifuged at 1500 × g for 10 min at 4 °C and the serum/plasma kept frozen at −20 °C until eicosanoid RIAs were performed.

The synovial tissue was cut into fragments of about 3 × 3 mm, divided into portions of 150 mg wet weight (ww), and placed in plastic tubes. 2 portions were washed once with ice-cold Tyrode’s solution to eliminate blood traces. Another 2 portions were washed 5 times over a period of 20 min with ice-cold Tyrode’s solution. Thereafter, the synovial tissue was incubated in oxygenated prewarmed (37 °C) Tyrode’s solution for 5 min and then for an additional 20 min in Tyrode’s solution, containing 5 microg/mL ionophore A23187. After incubation, the supernatant was removed and the samples were either immediately analyzed for PG and LT or stored at −20 °C. Storage under these conditions has been shown not to affect significantly the amounts of eicosanoids detected over a period of up to 2 months.

Analysis of PG, LT and TXB₂

Levels of PG and LT in the supernatants of tissue incubates, TXB₂ in serum and of LTB₄ and cysteinyl-LT in heparinized plasma samples were determined radioimmunologically, as described previously (Peskar et al. 1979, Patrono et al. 1980, Aehringhaus et al. 1982). LTB₄ and cysteinyl-LT in plasma were determined by RIA, after Sep-Pac-purification (Dreyling et al. 1986). The validity of the radioimmunoassay (RIA) measurements was verified by high-pressure liquid chromatography (HPLC) and bioassay. HPLC analysis followed by RIA measurement of eluted fractions showed that immunoreactive LTB₄ co-chromatographed exclusively with authentic LTB₄, whereas immunoreactive LTC₄ consisted of a mixture of cysteinyl-leukotrienes C₄, D₄ and E₄ in approximately equal amounts. Release of cysteinyl-LT was calculated, using standard curves for LTC₄ and was expressed in terms of LTC₄. The details of these measurement techniques and their accuracy have been described elsewhere (Dreyling et al. 1986, Simnet et al. 1988).

Measurement of tepoxalin

The concentrations of tepoxalin and its carboxylic acid metabolite in plasma and synovial fluid were determined according to Seidemann et al. (1994) and Depre et al. (1993).

Statistics

Incubations were performed in duplicate and the means and SD values for eicosanoid release were calculated. Statistical analysis was performed using the Student’s t-test. P-values less than 0.05 were consid-
Figure 1. LTB₄ and LTC₄ levels in heparinized ionophore A 23187-stimulated blood of patients with OA, before and after oral administration of tepoxalin. (▼) 400 mg tepoxalin LTB₄, (●) 100 mg tepoxalin LTB₄, (●) 400 mg tepoxalin LTC₄, (●) 100 mg tepoxalin LTC₄. D1: 0h – blood sample before first administration of tepoxalin, D1: 2h – blood sample 2 hours after first drug intake, D4: 0h – blood sample before administration of tepoxalin on day 4, D4: Surgery – blood sample 2 hours after drug intake during surgery (n 7 respectively, for 400 mg tepoxalin LTB₄ and LTC₄ p<0.05).

Figure 2. TXB₂ levels in serum after clotting of whole blood of patients with knee arthrosis, before and after oral administration of tepoxalin. For the abbreviations, see Figure 1 (n 7 respectively, for 400 mg and 100 mg p<0.01).

Table 2. Eicosanoid release from synovial tissue (n 7 in each group). Mean (SD)

<table>
<thead>
<tr>
<th>Synovial tissue</th>
<th>Tepoxalin PGE₂ (pg/mg ww)</th>
<th>6-keto-PGF₁α (pg/mg ww)</th>
<th>LTC₄ (pg/mg ww)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact 400 mg</td>
<td>0.3 (0.3)</td>
<td>0.2 (0.2)</td>
<td>252 (51)</td>
</tr>
<tr>
<td>Washed 400 mg</td>
<td>1.6 (1.4)</td>
<td>2.0 (1.1)</td>
<td>407 (100)</td>
</tr>
<tr>
<td>Intact 100 mg</td>
<td>1.1 (0.8)</td>
<td>0.8 (0.5)</td>
<td>226 (68)</td>
</tr>
<tr>
<td>Washed 100 mg</td>
<td>5.7 (2.8)</td>
<td>6.8 (2.8)</td>
<td>298 (61)</td>
</tr>
</tbody>
</table>

Results

The oral administration of 100 mg tepoxalin reduced and of 400 mg tepoxalin significantly inhibited LTB₄ and LTC₄ release from ionophore A 23187-stimulated heparinized whole blood (Figure 1).

TXB₂ release into serum from whole blood during clotting was very significantly inhibited by both concentrations of tepoxalin (Figure 2).

Release of PGE₂, 6-keto-PGF₁α and LTC₄ from synovial tissue in the absence of ionophore A 23187 was below the detection limits of the RIAs used after administration of either dose of tepoxalin. After stimulation with ionophore A23187, LTC₄ release was easily detectable, while PGE₂ and 6-keto-PGF₁α release was still close to the detection limits of the RIAs used. After washing the synovial tissues 5 times, the release of all eicosanoids measured increased, as compared to the release from the tissues incubated immediately after one brief washing (Table 2).

Drug concentrations in plasma and synovial fluid (day 4, 2hrs after drug administration) are shown in Table 3.

Pain was significantly reduced with both concentrations from study start to surgery (Table 4).

No significant increase in maximum knee flexion was detectable.
Tepoxalin caused no clinical side-effect that required treatment or any recurring abnormal laboratory adverse effects. The most serious findings were mild diarrhea and transiently elevated liver enzymes after surgery, each in 1 case.

Discussion

As in other studies and biological systems, high interindividual variations of inflammatory mediator release were found. This resulted in a wide range of eicosanoid release levels and accounts for the very disparate baseline values. Nevertheless, release of TXB2 in clotting whole blood was significantly decreased as early as 2 hours after the first intake of tepoxalin, at either dose. There was no significant difference between the high and low doses of tepoxalin. Previous results have demonstrated CO inhibition at doses of 5–25 mg tepoxalin (Depre et al. 1993). The almost complete CO inhibition at the doses tested here was, therefore, to be expected. The higher values of LTB4 in comparison to LTC4 are in accordance with previous findings (Verpooten et al. 1993). Release of LTB4 and LTC4 in whole blood incubates was significantly decreased after oral intake of 400 mg tepoxalin, while 100 mg tepoxalin had no uniform effect and, therefore, caused no significant decrease in LT release.

Thus, our results prove that low concentrations of tepoxalin can inhibit CO, but 100 mg tepoxalin seems to be insufficient for 5-LO inhibition in blood.

Other reports have shown that the synovial tissue of patients with OA releases significant amounts of PGE2, 6-keto-PGF1α, and LTC4 after stimulation with ionophore A23187 (Wittenberg et al. 1993). Our previous findings demonstrated inhibition of PG release by diclofenac and indomethacin but no inhibition of LT release. The inhibition of both CO and 5-LO by tepoxalin was demonstrated in this study.

Eicosanoid release from extensively washed synovial tissue was significantly higher than release from briefly washed synovial tissue. This is probably due to wash out of the drug, which is a reversible enzyme inhibitor, resulting in less inhibition of CO and 5-LO.

Gastric and intestinal ulcerations are significant side-effects of CO inhibitors. It has been stated that a possible mechanism for such effects is a substrate shift by CO inhibitors. The additional effect of 5-LO inhibition might be important, as it has been suggested that the enhancement of LTs observed with NSAIDs may be due to diversion of AA to the 5-LO pathway (Szabo and Goldberg 1979, Walker et al. 1980). The formation of these LTs might be responsible for events associated with NSAID-induced gastrointestinal inflammation. The data of Kirchner et al. (1997) suggest that 5-LO inhibition may play a role in the prevention of NSAID-induced gastric inflammation. Such inflammation is characterized by the migration of polymorphonuclear leukocytes. Immunohistochemical analysis has indicated that tepoxalin inhibits this neutrophil migration (Zhou et al. 1996). LTs have been proposed as culprits in mucosal damage (Morales et al. 1992). A dual inhibitor of CO and 5-LO like tepoxalin might prevent or reduce this mechanism (Wallace et al. 1991, Wallace et al. 1993). Another point is the existence of constitutive CO (COX-1) and inducible CO (COX-2). COX-1 is present in cells under physiological conditions, whereas COX-2 is induced in pathological conditions, like inflammation (Mitchell et al. 1993). COX-1 is probably involved in production of PG in cellular "housekeeping" functions, such as coordinating the actions of circulating hormones and regulating vascular homeostasis (Meade et al. 1993). COX-2 may produce PG involved in inflammation and/or mitogenesis. Selective COX-2 inhibitors may protect cellular functions and reduce proinflammatory symptoms. To what extent tepoxalin might specifically inhibit one CO isozyme is not yet clear.

Clinically, tepoxalin caused no severe adverse reactions. It was well tolerated and pain scores were reduced. 8 of 14 patients reported better alleviation of pain with tepoxalin than with different NSAIDs they had taken before the study. Therefore, tepoxalin might be a safe and potent alternative to known

### Table 3. Drug concentrations (ng/mL). Mean (SD)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>n</th>
<th>Conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tepoxalin plasma</td>
<td>100 mg</td>
<td>7</td>
<td>0.26 (0.19)</td>
</tr>
<tr>
<td>Tepoxalin plasma</td>
<td>400 mg</td>
<td>7</td>
<td>0.42 (0.20)</td>
</tr>
<tr>
<td>Tepoxalin syn. fluid</td>
<td>100 mg</td>
<td>7</td>
<td>0.21 (0.14)</td>
</tr>
<tr>
<td>Tepoxalin syn. fluid</td>
<td>400 mg</td>
<td>7</td>
<td>0.39 (0.23)</td>
</tr>
<tr>
<td>Metabolite plasma</td>
<td>100 mg</td>
<td>7</td>
<td>1.03 (0.31)</td>
</tr>
<tr>
<td>Metabolite plasma</td>
<td>400 mg</td>
<td>7</td>
<td>4.05 (2.48)</td>
</tr>
<tr>
<td>Metabolite syn. fluid</td>
<td>100 mg</td>
<td>7</td>
<td>0.46 (0.14)</td>
</tr>
<tr>
<td>Metabolite syn. fluid</td>
<td>400 mg</td>
<td>7</td>
<td>1.98 (0.98)</td>
</tr>
</tbody>
</table>

### Table 4. Pain evaluation. Mean (SD)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>n</th>
<th>Day 0</th>
<th>Day 3</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previous night</td>
<td>100 mg</td>
<td>7</td>
<td>29 (29)</td>
<td>15 (11)</td>
<td>0.05</td>
</tr>
<tr>
<td>Previous night</td>
<td>400 mg</td>
<td>7</td>
<td>30 (34)</td>
<td>14 (20)</td>
<td>0.05</td>
</tr>
<tr>
<td>On same day</td>
<td>100 mg</td>
<td>7</td>
<td>45 (34)</td>
<td>22 (23)</td>
<td>0.05</td>
</tr>
<tr>
<td>On same day</td>
<td>400 mg</td>
<td>7</td>
<td>55 (31)</td>
<td>17 (22)</td>
<td>0.05</td>
</tr>
<tr>
<td>Intermittent</td>
<td>100 mg</td>
<td>7</td>
<td>54 (34)</td>
<td>31 (21)</td>
<td>0.05</td>
</tr>
<tr>
<td>Intermittent</td>
<td>400 mg</td>
<td>7</td>
<td>61 (34)</td>
<td>31 (37)</td>
<td>0.05</td>
</tr>
</tbody>
</table>
NSAIDs. This is even more likely because of the dual inhibition of CO and 5-LO of arachidonic acid metabolism. Our results indicate sufficient accumulation of tepoxalin in synovial tissue. This might explain the good clinical results, with reduction of pain. In our opinion, tepoxalin should be studied further and it may find a place in the therapy of inflammatory joint diseases.

Acknowledgement
The study was supported by the R.W. Johnson Research Pharmaceutical Institute, Bassersdorf, Switzerland.

References


Trang L E, Granström E, Lövgren O. Levels of prostaglandins F_{\alpha\beta\gamma} and E_{\alpha\beta\theta} and thromboxane B_{\alpha\beta} in joint fluid in rheumatoid arthritis. Scand J Rheumatol 1977; 6: 151-4.


