Effects on platelet functions and pharmacokinetics of azapropazone and ketorolac tromethamine given as single parenteral doses

DIRK PALLAPIES, BERNHARD A. PESKAR, KAY BRUNE¹ & GERD GEISSLINGER¹

Department of Pharmacology and Toxicology, Ruhr-University, Bochum, Germany and

¹Department of Experimental and Clinical Pharmacology, Erlangen, Germany

- 1 The biosynthesis of thromboxane (TX) B₂ and immunoreactive prostaglandin (PG) F_{2α} in clotting whole blood *ex vivo* as well as collagen-induced platelet aggregation were determined before and up to 72 h after intravenous injection of 600 mg azapropazone 2H₂O and intramuscular injection of 30 mg ketorolac tromethamine in six healthy subjects. The drug doses were selected on the basis of comparable analgesic activity (maximal recommended analgesic dose).
- 2 Both platelet aggregation and prostanoid biosynthesis were inhibited by rac-ketorolac to a significantly greater extent and for a longer period of time than by azapropazone.
- 3 Correlations between serum concentrations and the inhibitory effects on TXB₂ biosynthesis were observed for both drugs. Using the sigmoidal E_{max} model the mean serum concentration of azapropazone inhibiting platelet TXB₂ generation by 50% (EC₅₀) was found to be 98.1 ± 41.9 (s.d.) µg ml⁻¹, a value 1000 times higher than that for rac-ketorolac.
- 4 The moderate inhibition of platelet function by azapropazone as compared with rac-ketorolac might be an advantage with regard to its use as a post-operative analgesic.

Keywords azapropazone ketorolac thromboxane B₂ platelet aggregation pharmacokinetics

Introduction

The non-steroidal antiinflammatory drugs (NSAIDs) azapropazone and ketorolac tromethamine are being used for the treatment of post-operative pain as well as musculoskeletal and other types of pain [1–3]. Inhibition of fatty acid cyclooxygenase is a major mechanism of action of NSAIDs [4] and seems to explain therapeutic, but also some of the side effects of NSAIDs such as prolongation of bleeding-time [4]. This effect is caused by inhibition of platelet TXA₂ biosynthesis [5]. Determination of TXB₂ release from clotting whole blood has been widely used to measure disease-related changes and drug effects on TX-forming enzymes of platelets [6]. This method is highly reproducible and estimates a capacity index for these enzymes [7]. As a functional parameter we have determined the effects of parenteral administra-

tion of azapropazone and ketorolac tromethamine on collagen-induced aggregation of platelet-rich plasma (PRP). Finally, the effects of both NSAIDs on platelet aggregation and prostanoid biosynthesis were related to their serum concentrations.

Methods

Materials

Azapropazone (Tolyprin®) was a gift from Siegfried Pharma (Zofingen, Switzerland), ketorolac tromethamine (Toratex®, Syntex, Aachen, Germany) was obtained commercially, and collagen was from Hormon-Chemie (Munich, Germany).

Protocol

All experiments were performed according to a cross-over design on six healthy males (age 29–51 years) who had not taken any medication for at least 2 weeks. Written informed consent was given by all participants and the study was approved by the University of Bochum Medical Ethics Review Committee. Azapropazone (Tolyprin®, 600 mg azapropazone 2 H₂O, corresponding to 535.8 mg azapropazone) was administered i.v. over 5 min. At least 10 days later ketorolac tromethamine (Toratex®, 30 mg, corresponding to 20.36 mg rac-ketorolac) was injected i.m.

Biosynthesis of TXB₂ and PGF_{2α} in clotting whole blood

Samples of blood (2 ml) were collected just before and at 5, 30, 60, 150 min, 6, 24 and 72 h after injection of the drugs and incubated at 37° C for 60 min as described previously [6]. Plasma samples obtained before drug injection using sodium EDTA (5 mM) as anticoagulant served as controls. After incubation the samples were centrifuged at 1500 g at 4° C for 15 min. The supernatants were stored frozen at –20° C. TXB₂ and immunoreactive PGF_{2α} (only at time points 0, 5, 150 min and 72 h) in the sera were determined radioimmunologically [6, 8] within 1 week after the 72 h sample had been obtained.

In additional experiments samples of blood (2 ml) collected from five healthy subjects who did not receive any drugs were incubated at 37° C for 60 min in the presence of various concentrations of azapropazone (8, 25, 74, 222, 666, 2000 µg ml^{–1}) or rac-ketorolac (10, 30, 100, 300, 1000, 3000 ng ml^{–1}). TXB₂ biosynthesis was determined as described above.

Collagen-induced aggregation of platelet-rich plasma

Before and at 1, 24 and 72 h after azapropazone or ketorolac tromethamine injection an additional sample of blood (27 ml) was collected into polypropylene tubes containing 3 ml of 3.8% (w/v) sodium citrate solution (giving a blood:citrate ratio of 9:1). The samples were centrifuged at 150 g at room temperature for 10 min to obtain PRP as supernatant. Platelet-poor plasma was obtained by centrifugation of the pellet at 1500 g at room temperature for 10 min and was used for dilution of the PRP to a final concentration of 2.0×10^8 platelets ml^{–1} PRP. Aliquots (1 ml) of PRP were incubated in the presence of 1 mM CaCl₂ in an Eppendorf aggregometer at 37° C for 2 min. Collagen (1–3 µg) was then added to induce aggregation. The decrease of optical density was measured for 5 min and expressed as % of the decrease induced by addition of 5 µg collagen to a control sample obtained before injection of any drug.

Assay of azapropazone and rac-ketorolac

Serum concentrations of azapropazone and rac-ketorolac were measured by reversed phase high-performance liquid chromatography assays as described

previously [9–11]. The limit of quantification (the lowest concentration that could be measured with a precision of 10%) was 0.5 µg ml^{–1} and 25 ng ml^{–1} azapropazone and rac-ketorolac, respectively. The coefficients of variation over the calibration range of 0.5–200 µg ml^{–1} and 25–10 000 ng ml^{–1} for azapropazone and rac-ketorolac, respectively, were less than 8% for both assays.

Data analysis

Non-compartmental pharmacokinetic analysis was carried out using the TOPFIT® software package [12]. The terminal elimination rate constant (λ_z) was estimated by unweighted linear least-squares regression analysis of the linear segment of the log serum drug concentration-time data. The terminal elimination half-life ($t_{1/2,z}$) was calculated from $0.693/\lambda_z$. The area under the drug concentration-time curve (AUC) was calculated by the linear and log-linear trapezoidal rules with extrapolation to infinity using $C_{(last)}/\lambda_z$. Drug clearance (CL) was calculated by dividing the dose of azapropazone or rac-ketorolac by its AUC. Mean residence time (MRT) was estimated using the area under the first moment curve (AUMC) divided by the AUC. For each subject the percentage inhibition of TXB₂ generation was related directly to the serum drug concentration using a standard sigmoidal E_{max} model [13, 14]. The data were fitted with a non-linear least-squares modelling computer program [Boomer®; 15]. The EC₅₀ (concentration of drug required to cause 50% of maximum effect) and a steepness factor for the log concentration-effect relationship (n) were estimated.

The area under the effect (serum TXB₂ and PGF_{2α} concentration; % aggregation)-time curve (AUEC) was calculated by the linear trapezoidal rule. Means $\pm$ s.e. mean of data as well as 95% confidence intervals on differences between AUEC values were calculated.

Results

Biosynthesis of TXB₂ and PGF_{2α} in clotting whole blood

In the plasma samples obtained in the presence of sodium EDTA levels of TXB₂ and immunoreactive PGF_{2α} were below the detection limits of the radioimmunoassays used (with regard to the different dilution of the biological material <1.2 ng ml^{–1} and <0.7 ng ml^{–1} for TXB₂ and PGF_{2α}, respectively). Changes in TXB₂ biosynthesis and release into serum during clotting of whole blood before and after injection of azapropazone and ketorolac tromethamine, respectively, are shown in Figure 1. Rac-ketorolac inhibited TXB₂ biosynthesis to a much greater extent and for a longer time than azapropazone. While TXB₂ biosynthesis after ketorolac tromethamine injection was significantly inhibited for at least 24 h, with almost complete inhibition

Figure 1 Time-dependent inhibition of TXB₂ biosynthesis in clotting whole blood *ex vivo* after i.v. administration of 600 mg azapropazone (●) or i.m. administration of 30 mg ketorolac tromethamine (■). Results are means $\pm$ s.e. mean; 95% confidence interval of difference between AUEC values was 3100–22 300 ng ml⁻¹ h.

for 6 h, azapropazone decreased TXB₂ biosynthesis significantly only for up to 6 h with a maximal 62% inhibition at 5 min (Figure 1). The biosynthesis of immunoreactive PGF_{2 α} before injection of ketorolac tromethamine was 8.8 ± 1.6 ng ml⁻¹ serum and before injection of azapropazone it was 8.5 ± 1.6 ng ml⁻¹ serum. In the blood samples obtained 5 and 150 min after injection of ketorolac tromethamine levels of immunoreactive PGF_{2 α} were below the detection limit of the assay (more than 89% inhibition), while 5 and 150 min after administration of azapropazone biosynthesis of immunoreactive PGF_{2 α} was inhibited only by $42 \pm 4\%$ and $17 \pm 4\%$, respectively. In the blood samples obtained 72 h after either drug, biosynthesis of immunoreactive PGF_{2 α} had recovered completely (8.9 ± 1.1 ng ml⁻¹ serum and 8.6 ± 1.1 ng ml⁻¹ serum for the ketorolac and azapropazone experiments, respectively). The 95% confidence interval for the difference in AUEC value for immunoreactive PGF_{2 α} was 87–339 ng ml⁻¹ h.

Collagen-induced platelet aggregation

Platelet aggregation induced by 1 μ g ml⁻¹ collagen was significantly ($P < 0.05$) inhibited by azapropazone only 1 h after drug injection (Figure 2). By contrast, rac-ketorolac significantly ($P < 0.01$) inhibited platelet aggregation up to 24 h after drug administration and to a much greater extent than azapropazone (Figure 2). Inhibitory effects of azapropazone on platelet aggregation induced by 3 μ g ml⁻¹ collagen were very small, but significant ($P < 0.05$), while inhibition of aggregation was markedly greater under these conditions after ketorolac tromethamine injection (Figure 2).

Pharmacokinetic-pharmacodynamic analysis of azapropazone and rac-ketorolac

At 72 h after administration of ketorolac tromethamine the serum concentrations of rac-ketorolac

Figure 2 Time-dependent inhibition of collagen-induced aggregation of PRP after i.v. administration of 600 mg azapropazone (● 1 μ g collagen; ▲ 3 μ g collagen) or i.m. administration of 30 mg ketorolac tromethamine (▼ 1 μ g collagen; ■ 3 μ g collagen). Results are means $\pm$ s.e. mean; 95% confidence intervals of differences between AUEC values were 14–2500% aggregation $\times$ h (1 μ g collagen) and 117–2264% aggregation $\times$ h (3 μ g collagen).

Table 1 Mean $\pm$ s.d. pharmacokinetic parameters describing the fate of azapropazone and rac-ketorolac following i.v. administration of 600 mg azapropazone 2H₂O (corresponding to 535.8 mg azapropazone) and i.m. administration of 30 mg ketorolac tromethamine (corresponding to 20.36 mg rac-ketorolac) to six human subjects (*median and range)

	Azapropazone	Rac-ketorolac
$t_{\max}$ (h)*		0.75 (0.5–1.0)
$C_{\max}$ (μ g ml ⁻¹)	163 ± 23	3.50 ± 0.82
AUC (μ g ml ⁻¹ h)	1111 ± 251	21.3 ± 3.3
$t_{1/2, \alpha}$ (h)	13.7 ± 3.3	5.2 ± 0.5
MRT (h)	14.5 ± 3.8	5.6 ± 0.7
CL (ml min ⁻¹)	8.4 ± 1.8	16.3 ± 2.8

Table 2 Computer-fitted parameters (EC_{50} , n) describing the relationship between the total (unbound and bound) serum concentration of azapropazone and the percentage inhibition of TXB₂ generation in six healthy subjects *ex vivo*. The standard deviations for the computer-generated individual estimates (EC_{50} , n) are given in parentheses

Subject	EC_{50} (μ g ml ⁻¹)	n
1	139 (42)	1.83 (1.08)
2	71 (8)	0.94 (0.15)
3	58 (7)	0.61 (0.08)
4	73 (3)	1.23 (0.08)
5	87 (6)	0.69 (0.06)
6	161 (52)	0.95 (0.36)
Mean $\pm$ s.d.	98 ± 42	1.04 ± 0.44

were below the quantification limit whereas azapropazone could be assayed reliably. The pharmacokinetic parameters of azapropazone and rac-ketorolac are shown in Table 1. Relationships between the percentage inhibition of TXB₂ generation *in vitro* and *ex vivo* and the log concentration of serum azapropazone are shown in Figure 3. The calculated EC_{50}

Table 3 Computer-fitted parameters (EC_{50} , n) describing the relationship between the serum concentration of azapropazone or rac-ketorolac, respectively, and the percentage inhibition of TXB_2 generation after spiking the serum of five healthy subjects with various concentrations of drug. The standard deviations for the computer-generated individual estimates (EC_{50} , n) are given in parentheses

Subject	Azapropazone		Rac-ketorolac	
	EC_{50} ($\mu\text{g ml}^{-1}$)	n	EC_{50} (ng ml^{-1})	n
1	56 (10)	1.10 (0.19)	87 (9)	1.28 (0.15)
2	112 (12)	2.08 (0.37)	141 (6)	1.64 (0.11)
3	54 (3)	1.65 (0.15)	82 (8)	1.05 (0.11)
4	42 (42)	0.94 (0.24)	58 (4)	1.40 (0.12)
5	90 (5)	1.95 (0.19)	74 (14)	1.08 (0.19)
Mean $\pm$ s.d.	71 $\pm$ 29	1.54 $\pm$ 0.51	88 $\pm$ 31	1.29 $\pm$ 0.24

Figure 3 Relationships between the percentage inhibition of TXB_2 generation and log serum concentration of azapropazone ($\blacktriangle$; $\circ$) and rac-ketorolac ($\blacksquare$; $\square$) in healthy subjects (solid symbols: *ex vivo*; open symbols: *in vitro*). The solid line indicates the relationship from the computer-generated sigmoidal $E_{\max}$ model analysis based on *ex vivo* data; the dotted lines represent that of *in vitro* data.

(95.0 $\mu\text{g ml}^{-1}$) and steepness factor n (1.0) of the pooled data were in the range of the mean values calculated from the individual subjects (Tables 2 and 3) *in vitro* and *ex vivo*. Up to 24 h following i.m. administration of ketorolac tromethamine inhibition of platelet TXB_2 generation consistently exceeded 80% (Figure 3). Thus, based on these data an *in vivo* EC_{50} could not be estimated for rac-ketorolac. The computer-generated parameters (EC_{50} ; n) based on the *in vitro* data for azapropazone and rac-ketorolac are presented in Table 3.

Discussion

While plasma TXB_2 concentrations were below the detection limit of the radioimmunoassay used, TXB_2 biosynthesis in clotting blood was measurable with basal values (before drug administration) comparable with those described by Alessandrini *et al.* [7]. Consequently, TXB_2 determined by this method is released during clotting, especially from platelets stimulated by endogenous thrombin [6]. Both of the NSAIDs tested not only inhibited TXB_2 biosynthesis, but also that of immunoreactive $PGF_{2\alpha}$ to a comparable extent. Thus, inhibition of fatty acid cyclooxygenase is the mechanism of action of these drugs. Our results show that inhibition of cyclooxygenase in human platelets by rac-ketorolac is greater and longer lasting than inhibition by azapropazone. Although the capacity of TXB_2 biosynthesis in clotting whole human blood does not correlate with bleeding-time [16], these data correlate well with inhibition of collagen-induced platelet aggregation by both drugs. In the aggregation experiments azapropazone also had only short-lasting effects which were less than those of rac-ketorolac. Using a sigmoidal $E_{\max}$ model the serum concentrations of azapropazone and rac-ketorolac were related to the biological effects. Both drugs injected in commonly used analgesic doses reach serum concentrations which affect TXB_2 biosynthesis to a different extent, as confirmed by the *in vitro* experiments.

Our biochemical and *ex vivo* platelet data suggest that the moderate inhibition of platelet function by azapropazone as compared with rac-ketorolac might be an advantage with regard to its use as a post-operative analgesic. However, further studies will be needed to confirm this.

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