Biochemical Pharmacology, Vol. 35, No. 5, pp. 761–766, 1986.
Printed in Great Britain.

0006-2952/86 $3.00 + 0.00

EFFECTS ON PROSTANOID FORMATION AND PHARMACOKINETICS OF DAZMEGREL (UK-38,485), A NOVEL THROMBOXANE SYNTHASE INHIBITOR, IN MAN

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(*Received* 24 *June* 1985; *accepted* 2 *September* 1985)

Abstract—The pharmacokinetics of dazmegrel (UK-38,485), a novel selective thromboxane synthase inhibitor, and its effects on *in vivo* prostanoid formation were studied in a 2 weeks, multiple dose, placebo controlled, double blind trial in man. The drug was well tolerated. After dazmegrel 50–200 mg p.o. peak plasma levels of 0.7–3 μ/ml were reached within 1 hr. Elimination was of first order with a half life of 0.88 ± 0.17 hr. Platelet count and bleeding time were unchanged by all regimes of dazmegrel used (100 and 200 mg b.i.d.; 50, 100 and 200 mg t.i.d.). Serum thromboxane (TXB_2) was more than 95% suppressed one hour after all doses studied, but 200 mg t.i.d. were needed to suppress circadian serum TXB_2 profiles more than 90% at all times. Urinary excretion of 2,3-dinor-TXB_2 (TXA_2-M) fell by over 90%. An increase in the excretion of 2,3-dinor-6-keto-$PGF_{1\alpha}$ (PGI_2-M), the major metabolite of prostacyclin, was largely transient and fell short of significance at all times. The ratio of TXA_2-M to PGI_2-M was lowered from about 5.0 to 0.2 and sustained throughout treatment.

Dazmegrel selectively blocks *in vivo* and *ex vivo* TXA_2 formation. Redirection of endoperoxides from total body TXA_2 formation into prostacyclin formation is only minor under basal conditions.

Signs of platelet activation and increased thromboxane (TXA_2)§ formation have been found in conditions like variant [1] and unstable [2] angina, bronchospasm [3], thromboembolism [4], septic shock [5], incipient renal graft rejection [6] and vasoconstriction of hydronephrotic kidney [7]. Moreover, circumstantial evidence like time course, profile of biologic action and experimental models support a pathogenetic role of TXA_2 as an important enhancer of platelet response and vascular tone in some of these settings. Therefore suppression of its production or action is rendered a rational therapeutical goal in these conditions.

Imidazoles selectively block thromboxane synthase, but do not interfere with other enzymes of the prostanoid cascade [8]. Thus, in contrast to long-established cyclooxygenase blockers, this new type of drug preserves formation of prostacyclin, the natural antagonist to TXA_2. Prostacyclin formation may even be enhanced by diversion of endoperoxides away from the blocked thromboxane pathway into the formation of prostacyclin and prostaglandins with a biologic profile of potential benefit in these settings [9]. Some encouraging, although still equivocal evidence with a drug of this type has meanwhile accumulated [10–13]. Dazmegrel (3-(1H-imidazole-1-yl-methyl)-2-methyl-1H-indole-1-propanoic acid; UK-38,485 Pfizer) is a more advanced imidazole compound of higher potency and longer action *in vitro* and *in vivo* [14, 15], low toxicity and protective effects in models of glomerulonephritis [16] and nephrosis [17], and in diabetic nephropathy [18]. As yet, data on the pharmacokinetics and on *in vivo* and *ex vivo* prostanoid formation during chronic administration of this drug are lacking. We therefore studied the effects of dazmegrel on TXA_2 and PGI_2 formation in a multiple dose, placebo controlled, double blind trial in man.

§ Abbreviations used: PG, prostaglandin; TXA_2, thromboxane A_2; TXB_2, thromboxane B_2; TXA_2-M, 2,3-dinor-TXB_2, major metabolite of TXA_2; PGI_2, prostacyclin, prostaglandin I_2; PGI_2-M, 2,3-dinor-6-keto-$PGF_{1\alpha}$, major metabolite of PGI_2; TXSI, thromboxane synthase inhibitor; b.i.d., bis in diem (twice daily); t.i.d., ter in diem (thrice daily).

METHODS

Subjects and protocol

After informed consent had been obtained, 16 healthy males, aged 24 ± 4 years, weighing 70 ± 7.6 kg and free of aspirin-like drugs for at least 2 weeks were entered into the trial and abstained from any medication, smoking and alcohol throughout the 17 day protocol. A detailed history, physical examination and extended routine laboratory check-up was obtained at baseline and at completion of the study. After a 2 days placebo run-in period (day −2, −1), subjects were randomised on day 0 into 4 treatment groups of 4 subjects each and put on dazmegrel 50 mg t.i.d., 100 mg b.i.d., 100 mg t.i.d. or 200 mg b.i.d. for 2 weeks, followed by a placebo run-out on day 15. One subject in each group served as control and remained on a matched placebo throughout the trial. In a second, otherwise identical protocol 18 volunteers aged 26 ± 3 years and weigh-

ing 71 ± 8 kg were randomly allocated on day 0 to either dazmegrel 200 mg t.i.d. or matched placebo.

For twice daily dosing tablets were administered at 7 a.m. and 7 p.m., for thrice daily dosing at 7 am., 2 pm. and 10 p.m.. Supine and upright blood pressure and heart rate were recorded daily. Template bleeding time was assessed before and 1 hr after first dosing on days −2, 0, 2, 7, 14 and 15. After an overnight fast blood for biochemical and hematologic routine parameters was drawn on days −2, 3, 7, 14 and 15. Blood for serum TXB_2 and drug levels was drawn daily before and 1 hr after first dosing. In addition, on days 0 and 14 a detailed 24-hr profile of serum TXB_2 and drug levels was monitored. For 2,3-dinor-TXB_2 (TXA_2-M), 2,3-dinor-6-keto-$PGF_{1\alpha}$ (PGI_2-M) determination and creatinine clearance 24 hr urine samples were collected on days −1, 0, 7 and 14.

Laboratory methods

Assay of serum TXB_2. Five ml of blood were drawn by fresh venepuncture with a 21 gauge 2 in. needle, immediately transferred to a 0.5 in. glass tube, incubated at 37° for 60 min and then spun at 2000 *g* for 10 min. Serum was then stored at −20° until acidic organic extraction and radioimmunoassay in triplicate at three different sample dilutions using a sensitive, specific antibody (gift of Dr L. Levine, Brandeis University, Waltham, Mass.) as described previously [15]. About 5 nCi of ^{3}H-TXB_2 (specific activity 100–150 Ci/mmol) were used per vial. Final diltuion of antiserum was 1 : 190,000, binding of ^{3}H-TXB_2 tracer was 30 ± 4%, the 50% intercept of standard curves was 9 ± 1 pg and the limit of detection was 0.8 ± 0.13 pg per vial (N = 36). Cross reaction of TXB_2 antibody was below 0.01% for 6-keto-$PGF_{1\alpha}$, PGE_2, PGF2α, HHT and 12-HETE, about 0.5% with PGD_2 and 48% with 2,3-dinor-TXB_2.

Assay of urinary 2,3-dinor TXB. Thirty ml of the 24-hr urine specimens were acidified, extracted with Sep-pak C18 cartridges and purified by reversed-phase HPLC as described earlier [15]. Using this solvent system at a flow of 2 ml/min, 2,3-dinor TXB_2 eluted between 7 and 11 min and TXB_2 between 16 and 24 min. Overall recovery was monitored by addition of about 1 nCi of ^{3}H-TXB_2 prior to extraction. For radioimmunoassay of 2,3-dinor TXB_2 in fractions 6 to 12, a TXB_2 antibody with 48% cross-reaction with 2,3-dinor-TXB_2, ^{3}H-TXB_2 tracer and standard 2,3-dinor-TXB_2 was used as described above.

Assay of urinary 2,3-dinor 6-keto-$PGF_{1\alpha}$. To 60 ml of 24-hr urine specimens 25 ng of tetra-deuterated 2,3-dinor-6-keto $PGF_{1\alpha}$ were added as internal standard. After extraction and back-extraction under alkaline and acidic conditions [19], the methoxime, methyl-ester, trimethylsilyl ether derivative was formed and 2,3-dinor-6-keto $PGF_{1\alpha}$ was quantified by combined gas-chromatography mass-spectrometry monitoring ion pairs m/z 570/574 (M^+-31) and m/z 480/484 (M^+-90-31).

Assay of plasma drug levels. After addition of an internal standard, plasma was deproteinized with acetonitrile and the sample analysed by high performance liquid chromatography using a fluorescence detector. The limit of detection of dazmegrel in plasma was 3 ng/ml.

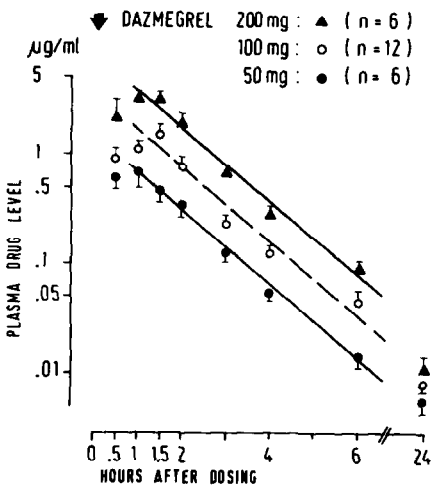

Fig. 1. Plasma drug levels after various oral doses of dazmegrel. Time of administration is indicated by arrow. Data on day 1 and day 14 and for 100 mg b.i.d. and t.i.d. were pooled. Means ± S.E.M.

Routine hematologic and biochemical safety parameters were determined by established standard laboratory methods.

Results are presented as means ± S.D. (tables) or S.E.M. (figures). For groups of less than 6 patients only means are given. As all effects of dazmegrel 200 mg t.i.d. on serum thromboxane, urinary excretion of TXA_2-M and on TXA_2-M/PGI_2-M ratios compared to concurrent placebo controls were highly significant far beyond the $2P < 0.001$ level (Student's *t*-test), no special reference to significance levels is made in the figures.

RESULTS

The time course of dazmegrel plasma levels after oral administration of various doses is plotted in Fig. 1. As no influence of prolonged and multiple administration was evident, plasma levels after the first morning dose on day 0 and day 14 and for the regimes 100 mg b.i.d. and t.i.d. were pooled. Peak plasma drug levels are reached within 1 hr of administration by the oral route. Drug levels are linear dependent on dose, and elimination is exponential with a plasma half life of 0.88 ± 0.17 hr. Early morning predosing drug levels were consistently below 0.02 μg/ml even with 200 mg t.i.d. and no trend in trough levels was evident throughout the 2 weeks treatment. Also in post dosing peak plasma levels no consistent change in the pharmacokinetics of the drug could be detected (Fig. 2). The detailed profiles of plasma drug levels on the first and last day of treatment were virtually identical.

Even at the highest dose used and when assessed before and 1 hour after dosing when peak plasma drug levels are reached, platelet count and bleeding time (233 ± 37 vs 228 ± 34 sec) were not affected by dazmegrel treatment. Serum thromboxane was suppressed more than 95% within 30 min of the first administration of dazmegrel in doses of 50–200 mg

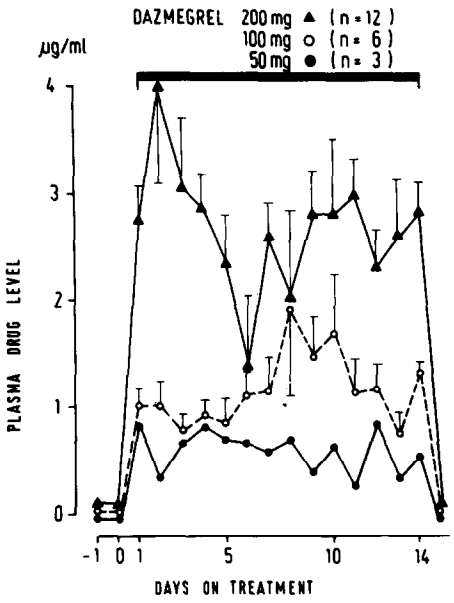

Fig. 2. Time course of peak plasma drug levels 1 hr after the first morning dose during the placebo run-in period, throughout the 2 weeks course of treatment with various regimes of dazmegrel and after withdrawal. Data for 100 mg b.i.d. and t.i.d. were pooled. Means ± S.E.M.

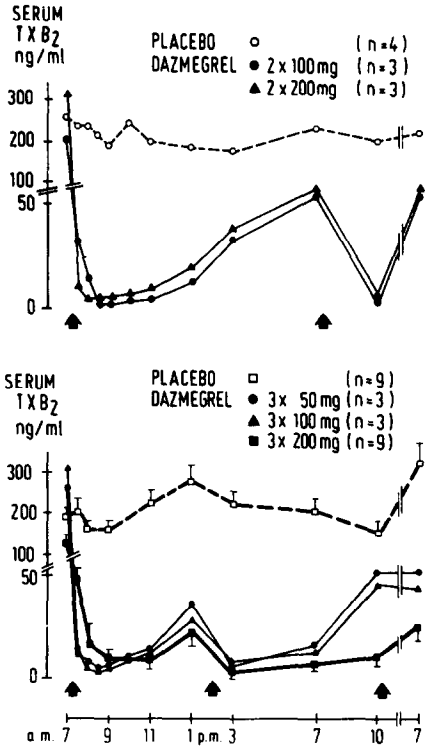

Fig. 3. Profiles of serum thromboxane B_2 on day 1 of treatment with various regimes of dazmegrel. Times of drug administration are indicated by arrows. Regimes 100 and 200 mg b.i.d. are shown on top panel, regimes 50, 100 and 200 mg t.i.d. on bottom panel. Dotted lines indicate placebo controls. Means ± S.E.M.

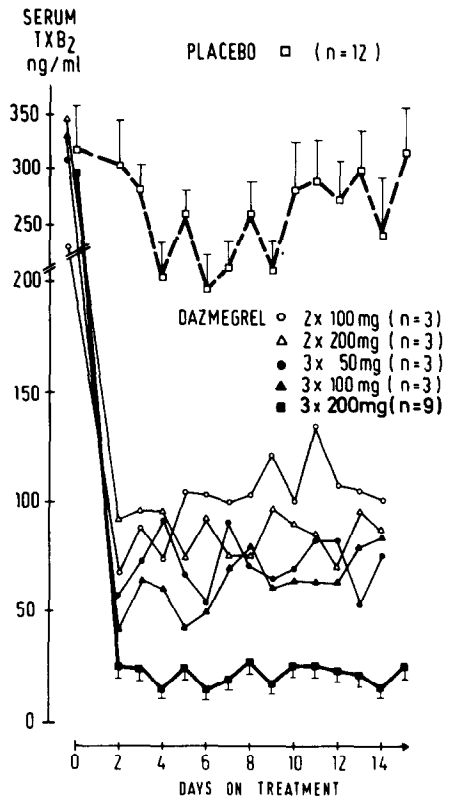

Fig. 4. Time course of early morning pre-dosing serum thromboxane B_2 before and during treatment with various regimes of dazmegrel and after withdrawal. Dotted line indicates placebo control. Means ± S.E.M.

p.o. Six hours after a 50 mg dose there was still more than 80% suppression, 12 hours after a 200 mg dose about 70% and before the first dosing on the next day still more than 60% suppression of serum thromboxane (Fig. 3). Throughout the 2 weeks course of treatment inhibition of peak and trough serum TXB_2 was preserved, that is before (Fig. 4) and 1 hour after (all below 5 ng/ml) first daily dosing respectively.

Urinary excretion of TXA_2-M was more than 90% reduced with all regimes of dazmegrel used and suppression was sustained throughout the treatment period (Fig. 5). Residual TXA_2-M excretion on the first day of treatment was probably due to carry over of the thromboxane load from the pretreatment period. No consistent effect of frequency of administration was evident. In contrast, urinary excretion of PGI_2-M was certainly not reduced. A trend towards increased excretion of PGI_2-M on day 1 was consistently observed at all regimes of dazmegrel used but had largely worn off on days 7 and 14 of treatment. In the second study using the highest dose of dazmegrel in a larger group the trend towards increased PGI_2-M excretion on dazmegrel was less pronounced on day 1 and fell short of significance throughout the trial (Fig. 6). Accordingly, the dramatic reduction of the ratio of TXA_2-M to PGI_2-M excretion was in the first line due to decreased TXA_2-M excretion. It was sustained throughout the trial and similar with all regimes of dazmegrel used (Table 1).

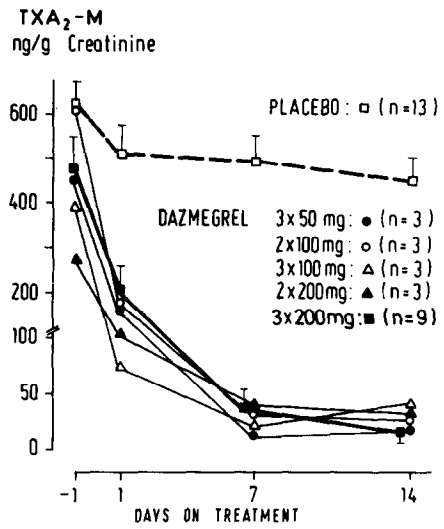

Fig. 5. Time course of 24 hr urinary excretion of major thromboxane A_2 metabolite (TXA_2-M) before and during treatment with various regimes of dazmegrel. Dotted line indicates placebo control. Means ± S.E.M.

The drug was well tolerated and especially no effects on heart rate, blood pressure and orthostatic regulation were observed. In routine laboratory and hematological checks no side effects were evident.

DISCUSSION

The antagonism between thromboxane and prostacyclin offers an intriguing challenge for directed pharmacological intervention. The feasibility of selective thromboxane synthase inhibition will largely depend on whether endoperoxides with their intrinsic thromboxane-like activity can quickly and effectively be divertd into prostacyclin or, for example, PG D_2 formation, at least at sites of pathological platelet vessel wall interaction. If so, dazmegrel certainly is an attractive pharmacological candidate. Dazmegrel is rapidly absorbed, peak levels are proportional to dose and elimination follows a first order mechanism with a plasma half life of less than 1 hr.

Table 1. TXA_2-M/PGI_2-M ratio during treatment with various doses of dazmegrel

Days on treatment	0	1	7	14
Placebo (N = 13)	5.1 ± 2.9	5.1 ± 3.2	5.7 ± 2.8	5.2 ± 1.9
Dazmegrel 3 × 50 mg/d	4.3	1.3	0.14	0.12
2 × 100 mg/d	6.0	0.93	0.24	0.25
3 × 100 mg/d	4.3	0.29	0.16	0.32
2 × 200 mg/d	3.4	0.59	0.09	0.22
3 × 200 mg/d (n = 9)	3.8 ± 1.8	1.9 ± 0.8	0.15 ± 0.22	0.21 ± 0.19

Detailed pharmacokinetic monitoring throughout a 2 weeks course of treatment at various doses did not reveal any change in resorption or evidence for drug accumulation or enzyme induction. Dazmegrel was well tolerated and no hemodynamic side effects were detected.

Dazmegrel effectively and reversibly blocked *ex vivo* serum thromboxane formation, which is a way to assess the capacity of circulating cells to form prostanoids on a maximal stimulus. Some dependence of serum thromboxane formation on drug dosage was seen in early morning trough drug levels, but less clear-cut in the detailed circadian profiles. The biologic effect somewhat outlasted the plasma drug levels with still more than 70% inhibition of thromboxane formation in the interval at plasma drug levels well below 0.01 μg/ml. In human platelet microsomal thromboxane synthase the IC_{50} of dazmegrel was found to be about 0.005 μg/ml, but the IC_{50} for collagen stimulated aggregation of PRP *in vitro* was about 8.5 μg/ml [14]. This is probably due to the intrinsic activity of endoperoxides on the platelet TXA_2 receptor [20]. Accordingly, for acetylsalicylic acid IC_{50} for cyclooxygenase and aggregation inhibition are less dissociate [20, 21]. Once the steady state was reached, no accumulation or waning of the action of dazmegrel was evident. Nevertheless thrice daily dosing rather than an increased single dose was required to smoothly suppress serum TXB_2 formation in circadian profiles.

The mode least prone to artifacts to assess spontaneous *in vivo* TXA_2 and PGI_2 formation certainly is the measurement of the major urinary metabolites of TXA_2 and PGI_2, 2,3-dinor-TXB_2 (TXA_2-M) and 2,3-dinor-6-keto-$PGF_{1\alpha}$ (PGI_2-M). Dazmegrel effectively suppressed this basal thromboxane formation over 90% in all regimes tested. Residual TXA_2-M excretion was not further suppressed by higher or

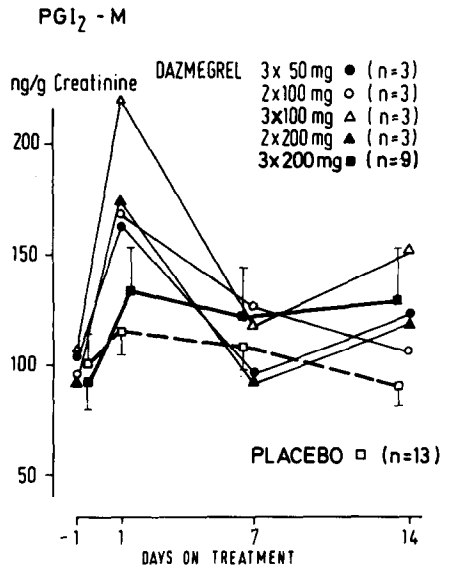

Fig. 6. Time course of 24 hr urinary excretion of major prostacyclin metabolite (PGI_2-M) before and during treatment with various regimes of dazmegrel. Dotted line indicates placebo control. Means ± S.E.M.

more frequent dosing. The effective suppression by a thromboxane synthase inhibitor, the concordance with the normal range reported elsewhere [22] and in the placebo group the constance of TXA_2-M excretion over time conversely lend support to the relevance and validity of this parameter of basal total body thromboxane formation.

The specificity of dazmegrel is demonstrated by the simultaneous preservation of basal PGI_2 formation as evidenced by the excretion of its major metabolite. An inhibition of cyclooxygenase or prostacyclin synthase by dazmegrel can therefore be excluded. Actually, a transient increase of PGI_2-M was observed in all regimes of the dose finding study on day one of treatment. It was most pronounced with t.i.d. and higher dosing, but largely waned later on. Statistical evaluation was precluded by the small number of subjects in these groups. In the second trial, launched thereafter with 200 mg t.i.d. in a larger group, this increase of PGI_2-M by dazmegrel was only minor and fell short of significance at all times, but appeared as a consistent trend. In a single dose study with a different thromboxane synthase inhibitor (TXSI) somewhat higher, but short lived increases of PGI_2-M have previously been reported [23]. Therefore shunting of endoperoxides from the TXA_2 to the PGI_2 pathway seems to occur only to a minor extent at least in the long run under basal conditions *in vivo*, and this contrasts to some but not all findings with various *in vitro* systems and to findings *ex vivo* [9, 23–25]. It may depend on the method applied and may be different in patients with increased PGI_2-M excretion thought to reflect increased platelet activation by diseased vessels [27] or septicemia [5, 28]. Accordingly, the ratio of TXA_2-M to PGI_2-M excretion was not further lowered by higher dosing of dazmegrel. The validity of PGI_2-M as a parameter of basal prostacyclin formation is supported by its constancy in the placebo group within a range reported in the literature and its suppression by cyclooxygenase inhibitors reported previously and confirmed also in our laboratory [22].

Endoperoxides formed in platelets exposed to selective thromboxane inhibitors may themselves activate the thromboxane receptor or may shunt into the PGD_2, E_2 or $F_{2\alpha}$ pathways that are available within the same cell [26] rather than shunting into the PGI_2 pathway available only in the anatomically separated vessel wall or in white cells. This could explain the lack of effects on bleeding time by dazmegrel in our study and fits well with at most minor effects on bleeding time in studies with other TXSIs compared to cyclooxygenase inhibitors. Some of these TXSIs may in addition block the TXA_2 receptor [29].

Despite dramatic biochemical effects of TXSIs, their effects on *ex vivo* platelet aggregation have been minor compared to cyclooxygenase inhibitors and, in fact, are more of a delay and slowing of the aggregation response to some stimuli rather than an actual prevention [23]. The effects by TXSIs have also largely depended on the test system used, the parameter monitored and the inclusion of a source of prostacyclin synthase [14, 22, 24, 28]. Inevitably, the direct activation of the thromboxane receptor by endoperoxides or the increased formation of pro-aggregatory PG E_2 are inherent drawbacks of TXSIs over cyclooxygenase inhibitors or thromboxane receptor blockers [29, 30]. However, at least in some infarct models [31, 32] TXSIs were superior to cyclooxygenase inhibitors, which may be due to preserved local PGI_2 formation, increased sensitivity to PGI_2 of platelets exposed to TXSIs [31, 34] or shunting of endoperoxides to antiaggregatory PGD_2 within the platelet. Ultimately the place of TXSIs will have to be defined by clinical studies. They could be more beneficial in conditions with increased TXA_2 formation at other sites than the platelet like the kidney [16, 17] or the lung [4, 35], where the enzymatic apparatus for PGI_2 formation may be anatomically linked to thromboxane synthase and easier to access for the endoperoxides piled up.

Acknowledgement—The expert help of Dr. U. Fenzel and Miss E. Schmidt is gratefully acknowledged. Dazmegrel was kindly provided by Dr. H. M. Tyler, Pfizer Central Research, Sandwich, U.K. This work was supported by Deutsche Forschungs Gemeinschaft.

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