Biochemical Pharmacology, Vol. 30, No. 20, pp. 2773–2776, 1981.
Printed in Great Britain.

0006-2952/81/202773-04 $02.00/0

SULPHINPYRAZONE PREVENTS *IN VIVO* THE INHIBITORY EFFECT OF ASPIRIN ON RAT PLATELET CYCLO-OXYGENASE ACTIVITY

GRAZYNA RAJTAR*, CHIARA CERLETTI†, MANUELA LIVIO‡ and GIOVANNI DE GAETANO
Laboratory of Cardiovascular Clinical Pharmacology, Instituto di Ricerche Farmacologiche "Mario Negri", Via Eritrea, 62–20157 Milan, Italy

(*Received* 1 *April* 1981; *accepted* 23 *April* 1981)

Abstract—Sulphinpyrazone reportedly inhibits *in vitro* platelet cyclo-oxygenase activity. This study shows that sulphinpyrazone administration (200 mg/kg) to rats was followed by long lasting inhibition of platelet cyclo-oxygenase, as measured by malondialdehyde generation by sodium arachidonate. The inhibition was apparently competitive and could in fact be ascribed to supposed metabolites of the drug. When given 30 min–6 hours before aspirin (5–25 mg/kg), sulphinpyrazone and to a considerable extent its metabolites significantly prevented permanent inhibition of platelet cyclo-oxygenase activity normally produced *in vivo* by aspirin. Sulphinpyrazone at 100 mg/kg was unable by itself to modify platelet malondialdehyde or thromboxane B_2 generation, yet if effectively interfered with aspirin activity. This suggests that sulphinpyrazone and its metabolites may interact with a binding site on cyclo-oxygenase not directly involved with the enzyme activity. Interaction of aspirin with this binding site would be a prerequisite for its inhibitory effect on the enzyme active site.

The clinical implications of this study include a reappraisal of the pharmacological basis for the association of sulphinpyrazone and aspirin in thrombosis prevention trials.

INTRODUCTION

Sulphinpyrazone, originally developed as a uricosuric drug, was subsequently found to normalise shortened platelet survival and to modify platelet function [1]. *In vitro* this drug has been reported to inhibit platelet cyclo-oxygenase activity [2]. In this respect it is similar to aspirin, although the mechanisms involved in aspirin's effect are more complex. However, at the usual therapeutic doses, sulphinpyrazone does not affect bleeding time whereas aspirin does, and clinical trials of the two drugs in patients with myocardial infarction or stroke have given conflicting results [3]. Whether this is due to differences in the pharmacological actions of sulphinpyrazone and aspirin or to differences between the experimental designs has not been established. When given together to patients with threatened stroke, no overall synergism or antagonism was observed between sulphinpyrazone and aspirin, although the combination therapy seemed to give somewhat better results [4].

Recent *in vitro* studies have suggested that sulphinpyrazone may prevent aspirin inhibiting platelet cyclo-oxygenase activity [5]. We administered these two drugs to rats in order to investigate their interaction as regards cyclo-oxygenase activity. Since the *in vivo* inhibitory effect of sulphinpyrazone on platelet arachidonic acid metabolism has been ascribed to some of its metabolites [6–8], aspirin was administered at various intervals after sulphinpyrazone. The results show that sulphinpyrazone and—to a considerable extent—its supposed metabolites interfere with aspirin, preventing the permanent inhibition of platelet cyclo-oxygenase activity normally produced *in vivo* by this drug.

MATERIALS AND METHODS

Animals. Male CD-COBS rats (Charles River, Calco, Italy), 250–300 g body weight were used.

Drug treatment. Animals were given a single oral dose of either sulphinpyrazone (Ciba-Geigy, Italy) or its suspending vehicle (hydroxypropyl cellulose, 0.3% solution) and killed from 30 min to 24 hr later by ether anaesthesia. In the interaction studies, animals were given aspirin intraperitoneally, as its soluble lysine salt (Flectadol, Maggioni, Italy) at different intervals (30 min–6 hr) after sulphinpyrazone or its vehicle. All these animals were killed 24 hr after the first treatment.

Platelet malondialdehyde and thromboxane B_2 generation. Blood was collected and malondialdehyde (MDA) formation was measured by a modification [9] of the spectrophotometric assay described by Smith *et al.* [10], after stimulation of unstirred platelet-rich plasma (PRP) with 0.4 and 0.8 mM sodium arachidonate (Sigma Chemical Co., St. Louis, MO) at 37° for 15 min.

In selected experiments an aliquot of PRP was pipetted off after 10 min incubation, diluted in an equal volume of cold ethanol, spun and the supernatant was stored at −20° for thromboxane B_2 (TxB_2) analysis. TxB_2 was measured by radioimmunoassay

* On leave of absence from the Department of Pharmacology, Lublin University, Medical School, Lublin, Poland.
† To whom reprint requests should be addressed.
‡ Research Fellow of the Italian National Research Council (C.N.R.).

according to Lewy *et al.* [11], using specific antibodies provided by J. B. Smith (Thomas Jefferson University, Philadelphia, PA).

Statistical analysis. Data were statistically analysed by Duncan's multiple range test [12]. For each experiment, the results obtained in treated animals were expressed as per cent inhibition in respect to the mean values obtained in control rats.

RESULTS

Time course of sulphinpyrazone's effect on platelet MDA formation. Figure 1 shows the time course of the inhibitory effect on platelet MDA formation of 200 mg/kg of sulphinpyrazone. Maximum inhibition was reached 6 hr after drug administration and lasted at least 24 hr. This inhibitory effect was evident only when platelets were stimulated with 0.4 mM sodium arachidonate, whereas almost no effect appeared with 0.8 mM sodium arachidonate (data not shown), suggesting a competitive type of inhibition. A lower dose of sulphinpyrazone (100 mg/kg) had no effect on platelet MDA formation 3 and 24 hr after administration in the experimental conditions used (data not shown).

Sulphinpyrazone–aspirin interaction. Figure 2 depicts the inhibition of platelet MDA formation in rats given sulphinpyrazone (200 mg/kg) or aspirin (5 or 25 mg/kg), alone or in combination. MDA was determined 24 hr after sulphinpyrazone or its vehicle. As can be seen in the upper panel, MDA produced in response to platelet stimulation with 0.4 mM sodium arachidonate was still inhibited by about 50% in rats given sulphinpyrazone alone and by about 70% in rats given aspirin (5 mg/kg) alone. In combination experiments MDA was inhibited about 80% in rats given sulphinpyrazone 30 min before aspirin. The inhibition was reduced to about 50% when aspirin was administered either 3 or 6 hr after sulphinpyrazone.

When the same platelet samples were challenged with 0.8 mM sodium arachidonate, sulphinpyrazone alone appeared to be almost inactive. Pretreatment with sulphinpyrazone reduced to about 20% the inhibitory effect of aspirin administered 30 min later, and completely abolished it when the interval between the two treatments was 6 hr. Similar results were obtained with 25 mg/kg aspirin, a dose totally inhibiting by itself MDA production, when given 6 hr after sulphinpyrazone.

Figure 3 shows that 100 mg/kg of sulphinpyrazone given 6 hr before 5 mg/kg of aspirin also prevented its inhibitory effect, though it was inactive by itself.

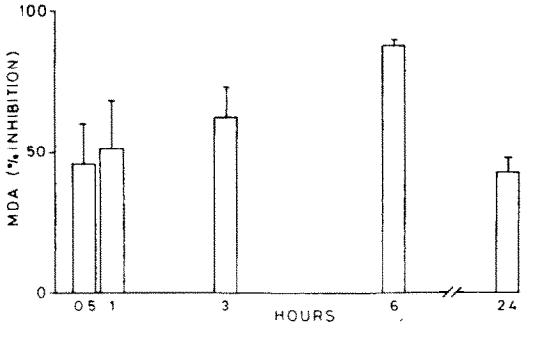

Fig. 1. Time course of inhibition of platelet MDA formation after a single dose of sulphinpyrazone (200 mg/kg, p.o.). The results (mean + S.E.M.) are expressed as percentages of the mean control value (1.18 ± 0.07 nmole/10^9 platelets/15 min) obtained from 10 animals. Each group consisted of 3 animals.

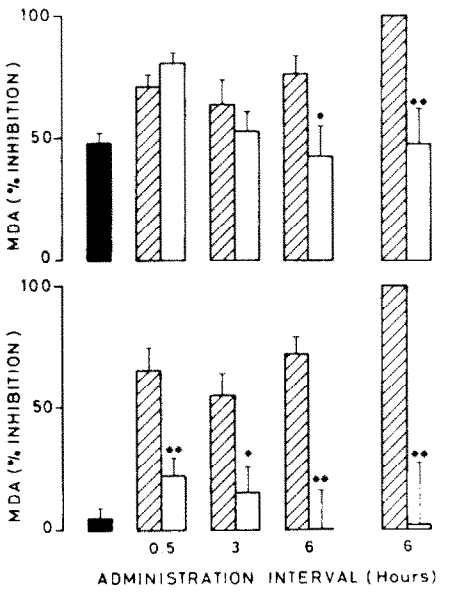

Fig. 2. Inhibition of platelet MDA formation measured after administration of either sulphinpyrazone, 200 mg/kg p.o. (black columns) or aspirin, 5 mg/kg (shaded columns) or a combination of both drugs (white colums). The interval between administration of the two drugs is indicated. Each group consisted of 3–6 animals. Upper and lower panels report the results with 0.4 mM and 0.8 mM sodium arachidonate, respectively. The last two columns in each panel represent the results when 25 mg/kg i.p. of aspirin was administered 6 hr after sulphinpyrazone (white columns) or its vehicle (shaded columns). *P<0.05; **P<0.01 compared to corresponding aspirin group. For further details see legend to Fig. 1 and Materials and Methods.

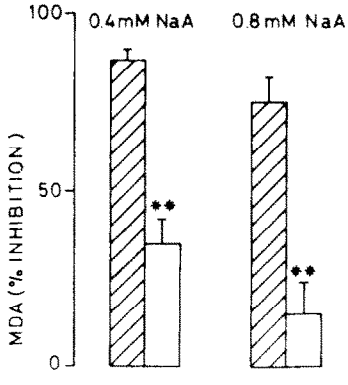

Fig. 3. Inhibition of platelet MDA formation by 5 mg/kg i.p. aspirin given 6 hr after sulphinpyrazone, 100 mg/kg p.o. (white columns) or its vehicle (shaded columns). The concentrations of sodium arachidonate (NaA) used, are indicated. Each group consisted of 4 animals. **P<0.01 compared to corresponding aspirin group. For further details see legend to previous figures.

Figure 4 shows that the interaction observed measuring platelet MDA production was also apparent when TxB_2 generation was determined.

DISCUSSION

Sulphinpyrazone administration to rats is followed by long-lasting inhibition of platelet prostaglandin generation triggered by sodium arachidonate. These results confirm the concept that the metabolism of sulphinpyrazone probably plays a major role in the drug's platelet inhibitory effect [6–8]. This effect was observed using low concentrations of arachidonate, but was overcome by increasing the concentration of the stimulus, thus extending to an *in vivo* condition previous *in vitro* observations of a competitive inhibitory activity of sulphinpyrazone on platelet cyclo-oxygenase [2].

The inhibitory effect of sulphinpyrazone was seen after a single dose of 200 mg/kg, but not after 100 mg/kg. However, the latter dose prevented the inhibitory effect of aspirin (5 mg/kg) given 6 hr after sulphinpyrazone. Experiments using 200 mg/kg of sulphinpyrazone and 5 mg/kg of aspirin showed that an interaction was already apparent when the two drugs were given 30 min apart and reached a maximum when the interval was 6 hr.

Prevention of aspirin activity by sulphinpyrazone was clearly apparent when platelets were stimulated with the higher concentration of sodium arachidonate since—as already mentioned—no inhibitory effect of sulphinpyrazone itself could be detected. In contrast, with the lower concentration of sodium arachidonate, about 50% inhibition of platelet MDA formation was still detectable in the group given sulphinpyrazone alone, making the interaction less readily apparent. Raising the dose of aspirin fivefold did not counteract the preventing effect of sulphinpyrazone (200 mg/kg).

As far as we know this is the first demonstration of an *in vivo* interaction between sulphinpyrazone and aspirin. Though indirectly, the present study also indicates that metabolites of sulphinpyrazone may have even more effect than the parent molecule on platelet cyclo-oxygenase activity, and also in interfering with aspirin. In this context it should be mentioned that two metabolites of sulphinpyrazone have recently been identified in rabbit and human plasma [13].

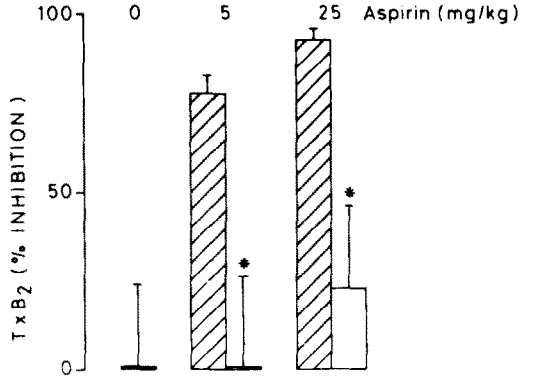

Fig. 4. Inhibition of platelet TxB_2 formation induced by 0.8 mM sodium arachidonate after administration of either sulphinpyrazone, 200 mg/kg p.o. (black column) or aspirin 5 or 25 mg/kg i.p. (shaded columns) or the combination of both drugs, 6 hr apart (white columns). The results (mean + S.E.M.) are expressed as percentages of the corresponding control values (1.98 ± 0.29 nmole/10^9 platelets/10 min). *$P<0.05$ compared to corresponding aspirin group. Each group consisted of 3 animals. For further details see legend to Fig. 2.

The possibility that the interaction observed occurred at a level different from cyclo-oxygenase is unlikely since a similar interaction was shown in suspensions of washed lysed platelets [5]. Moreover the intraperitoneal route of administration of aspirin used in the present study rules out the possibility of altered aspirin absorption in the gastrointestinal tract, and any binding of sulphinpyrazone (or its metabolites) to plasma proteins would have eventually resulted in increased availability of aspirin for the platelet enzyme.

It has recently been proposed that drugs inhibiting cyclo-oxygenase interact first with a binding site different from the active site [14, 15]. This would explain the observation that sodium salicylate, though inactive as an inhibitor of the enzymatic activity, prevents the effect of aspirin [5, 16, 17]. The finding reported here that a dose of sulphinpyrazone which did not block platelet cyclo-oxygenase activity did prevent aspirin inhibition supports the concept of a binding site not directly involved with cyclo-oxygenase activity and suggests that aspirin does not interact with the enzyme solely by acetylating its active site [15, 17, 18].

Whatever the exact mechanism of this interaction, its clinical implications merit some comments. If the goal is to block platelet arachidonic acid metabolism more effectively, then the association of sulphinpyrazone and aspirin seems not to be the best pharmacological approach. Though 24 hr after aspirin administration platelet MDA and TxB_2 formation was still blocked, it was nearly normal when sulphinpyrazone was associated with aspirin treatment. Sulphinpyrazone (200 mg q.i.d.) did not modify the risk of ischaemic attack, stroke or death in patients with threatened stroke, whereas aspirin (325 mg q.i.d.) significantly reduced it [4]. The fact that when combined with aspirin sulphinpyrazone did not prevent the beneficial effect of the latter drug, but even tended to favour it, suggests that either no pharmacological interaction similar to that described here occurred at the doses used in that study, or that aspirin exerted its antithrombotic effect through mechanisms different from cyclo-oxygenase inhibition.

The Canadian Cooperative Study Group [4] proposed a formal study to test the possibility that the combination of aspirin and sulphinpyrazone is the treatment of choice in threatened stroke. The results presented here should be considered before embarking on such an enterprise.

Acknowledgements—This work was supported by the Italian National Research Council (CNR 80.010.86.83, Progetto Finalizzato Medicina Preventiva, Aterosclerosi). Judith Baggott, Gigliola Brambilla, Vanna Pistotti, and Felice and Vincenzo de Ceglie helped prepare the manuscript.

REFERENCES

1. J. F. Mustard, H. C. Roswell, H. A. Smythe, A. Senyi and E. A. Murphy, *Blood* **29**, 859 (1967).
2. M. Ali and J. W. D. McDonald, *J. Lab. clin. Med.* **89**, 868 (1977).
3. E. Braunwald, *New Engl. J. Med.* **302**, 290 (1980).
4. Canadian Cooperative Study Group, *New Engl. J. Med.* **299**, 53 (1978).
5. M. Ali and J. W. D. McDonald, *Prostaglandis Medicine* **3**, 327 (1979).
6. M. R. Buchanan, J. Rosenfeld and J. Hirsh, *Thromb. Res.* **13**, 883 (1978).
7. M. Livio, S. Villa and G. de Gaetano, *J. Pharm. Pharmac.* **32**, 718 (1980).
8. A. Kirstein Pedersen and P. Jakobsen, *Thromb. Res.* **16**, 871 (1979).
9. G. Rajtar, M. Livio, J. Merino and G. de Gaetano, *Thromb. Haemost.* **44**, 49 (1980).
10. J. B. Smith, C. M. Ingerman and M. J. Silver, *J. Lab. clin. Med.* **88**, 167 (1976).
11. R. I. Lewy, J. B. Smith, M. J. Silver, Y. Saia, P. Walinsky and L. Wiener. *Prostaglandis Medicine* **5**, 243 (1979).
12. C. Y. Kramer, *Biometrics* **12**, 307 (1956).
13. P. Jakobsen and A. Kirstein Pedersen, *J. Pharm. Pharmac.* **33**, 89 (1981).
14. J. L. Humes, C. A. Winter, S. J. Sadowski and F. A. Kuehl Jr., *Proc. natn. Acad. Sci. U.S.A.* (1981) in press.
15. M. Livio, G. Rajtar, J. Merino and G. de Gaetano, *Thromb. Haemost.* **44**, 52 (1980).
16. B. B. Vargaftig, *Eur. J. Pharmac.* **50**, 231 (1978).
17. J. Merino, M. Livio, G. Rajtar and G. de Gaetano, *Biochem. pharmac.* **29**, 1093 (1980).
18. C. Cerletti, M. Livio, G. Rajtar and G. de Gaetano, *Thromb. Haemost.* **46**, 25 (1981).