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Inhibition of thromboxane synthesis in guinea pig lung and human platelets by clotrimazole and other imidazole antifungals

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Imidazole and a number of its analogs are known to be selective inhibitors of thromboxane A_2 production in different cell types as well as human platelets [1–4]. It was of interest, therefore, to investigate the effects on thromboxane synthesis of imidazole analogs which are used clinically. These analogs include the antimycotics clotrimazole, ketoconazole and miconazole nitrate. Of these, only clotrimazole has been studied previously. Ladd and Lewis [5] demonstrated that in rabbit platelets clotrimazole inhibits thromboxane synthesis while coincidentally stimulating synthesis of other prostaglandins, at least at the one concentration assayed. A selective inhibition of thromboxane production by clotrimazole has been observed in human blood mononuclear cells [6].

In the present study, the effects of clotrimazole on thromboxane synthesis in human platelets and guinea pig lung are compared to those of ketoconazole and miconazole nitrate. This work confirms and extends that reported previously in that additional test systems were used, more complete concentration–response relationships are presented, and the relative effects of these compounds on the production of various arachidonic acid metabolites were studied.

The metabolism of [^{14}C]-arachidonic acid (New England Nuclear Corp., Boston, MA) was assessed in cell-free preparations of guinea pig lung and intact human platelets. Lungs were excised from fed, female guinea pigs and homogenized in 0.92 mM phosphate buffer (pH 7.4), and the homogenates were centrifuged for 15 min at 1000 *g* at 4°. Aliquots (1 ml) of the resulting supernatant fractions containing 10–12 mg protein were used in the incubations. Platelets were obtained from citrated whole blood drawn from healthy human volunteers, free of antiinflammatory medication for the previous week. The platelets were pelleted and resuspended in 0.2 M Tris–HCl buffer (pH 7.4, 0.077 M EDTA). Aliquots (1 ml) of the platelet suspension (1.5 to 3 × 10^5 counts) were incubated.

The cell-free lung preparation and platelet suspensions were incubated with 8 μg [^{14}C-U]-arachidonic acid (0.04 μCi) for 30 min at 37° with the test compound or the vehicle, dimethyl sulfoxide (DMSO) (1%). Reactions were terminated by acidification, the incubation mixtures were extracted into ethyl acetate, and products of the reaction were separated by thin-layer chromatography on silica gel G plates developed in ethyl acetate–acetic acid (99:1, v/v). Radioactivity associated with areas which cochromato-

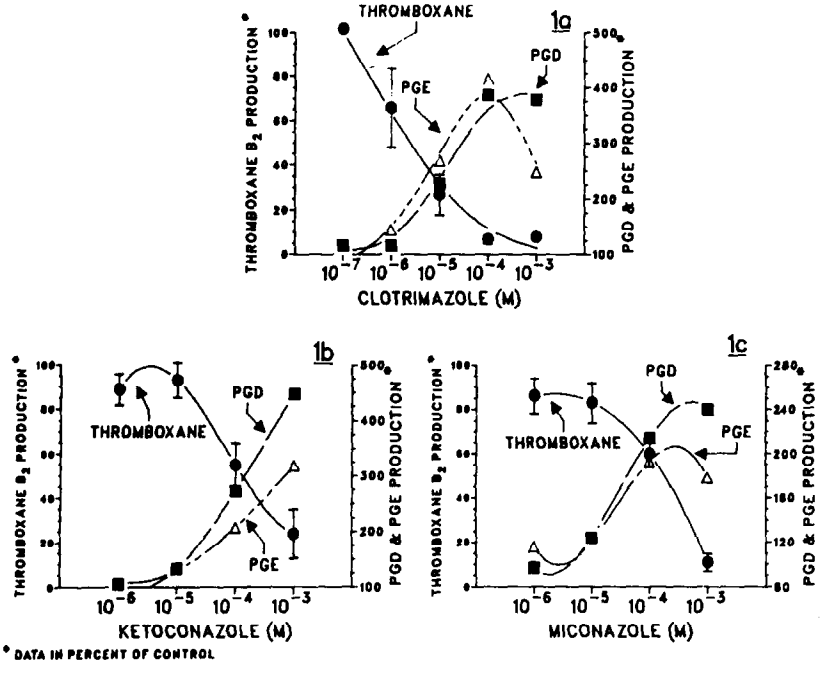

Fig. 1. Effects of imidazole antifungal agents on arachidonic acid metabolism in a cell-free preparation of guinea pig lung. Each point represents the mean of the results from three experiments. Vertical bars represent ± one standard error of the mean. Key: (1a) clotrimazole, (1b) ketoconazole, and (1c) miconazole nitrate.

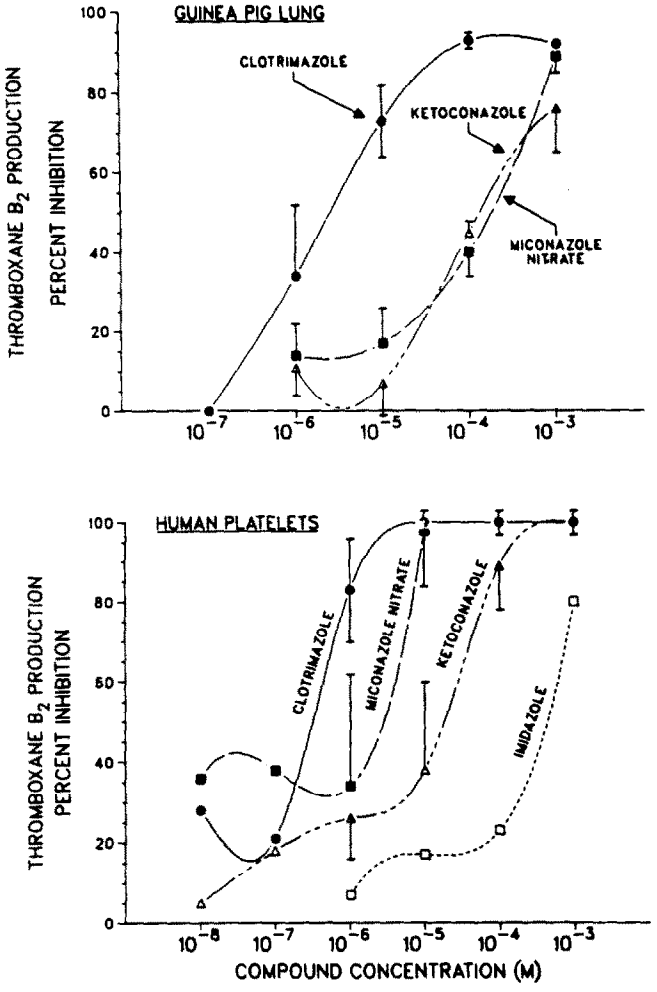

Fig. 2. Inhibition by imidazole antimycotics of the incorporation of [^{14}C] arachidonic acid into thromboxane B_2 in a cell-free preparation of guinea pig lung and in intact washed human platelets. With the exception of the imidazole data (from one experiment), each point represents the average data from two to four individual assays. Vertical bars represent the standard error of the mean.

graphed with standard prostaglandins, thromboxanes and arachidonic acid was counted and compared to total radioactivity extracted from the reaction.

Each point or bar is the average result from two to four individual enzyme preparations, each assayed in duplicate. Experimental errors are presented as standard errors of the mean, and statistical significance was determined by Student's *t*-test.

In the cell-free guinea pig lung preparation, all three imidazole antimycotics produced a concentration-related decrease in the incorporation of [^{14}C]-arachidonic acid into thromboxane B_2 (Fig. 1). Thromboxane represented $21 \pm 2\%$ of the recovered radioactivity under control conditions. Concomitant with the changes in thromboxane synthesis were increases in prostaglandin E_2 (PGE_2) and PGD_2 production. In controls, PGE_2 accounted for $1.9 \pm 0.3\%$ of product, while PGD_2 accounted for $3.9 \pm 0.6\%$. Thus, the 4-fold increases in the production of these products seen at the high compound concentrations (10^{-4} M–10^{-3} M) approximately offset the decrease in thromboxane production. The total utilization of arachidonic acid, therefore, did not change. Clotrimazole was the most potent of the three imidazoles. As seen in Fig. 2, clotrimazole inhibited thromboxane production in the guinea pig lung with an IC_{50} value of about 3×10^{-6} M. The IC_{50} values for ketoconazole and miconazole nitrate were 1 to 2×10^{-4} M.

As measured in this study, thromboxane was the major product of human platelet [^{14}C]-arachidonic acid metabolism. As in the lung, clotrimazole was the most potent inhibitor of thromboxane production of the three imidazole antimycotics tested and exhibited an IC_{50} of 3.7×10^{-7} M (Fig. 2). Miconazole nitrate and ketoconazole were about 1 and 2 orders of magnitude less potent respectively. Imidazole was yet another 10-fold less potent. Again, as imidazole and these imidazole analogs inhibited thromboxane synthesis, they produced a coincident increase in PGE_2 production (Fig. 3).

The results of these experiments extend the work of others who have shown that imidazole and its analogs are selective thromboxane synthesis inhibitors and that several clinically used imidazole analogs possess this same property. In this study, clotrimazole and the other antimycotics appeared to redirect arachidonic acid metabolism away from thromboxane to other prostaglandins in both the

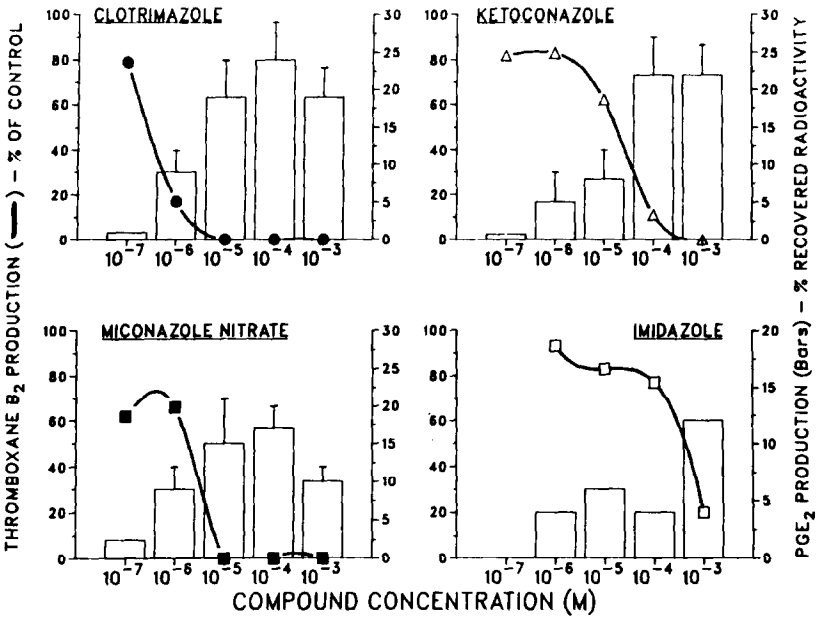

Fig. 3. Effects of imidazole and imidazole antifungal agents on arachidonic acid metabolism in washed human platelets. Thromboxane B_2 production, calculated in percent of control, is represented by the line curves. Prostaglandin E_2 production, as a percent of the total radioactivity recovered, is denoted by the bars.

guinea pig lung and human platelet systems in such a way as not to change the total amount of arachidonic acid being metabolized. This extends the observation of Ladd and Lewis [5] who reported concentration–response data for only clotrimazole in the rabbit platelet and who identified prostaglandin products by bioassay. Using the human peripheral monocyte, Gordon *et al.* [6] found that the increase in PGE_2 production following addition of clotrimazole was too low to account for substrate made available by the inhibition of thromboxane production.

The relationship of our observations, especially in human platelets, to the effects these imidazole antimycotics might have on platelet function in the clinic is not clear. The most potent thromboxane synthesis inhibitor, clotrimazole, is applied only topically. Studies are now underway, however, to determine the effects of clotrimazole and the other antifungals on human platelet aggregation.

In summary, in a cell-free preparation of guinea pig lung and in intact human platelets, clotrimazole and other imidazole antimycotics inhibited selectively the conversion of arachidonic acid to thromboxane, while they produced coincident increases in the production of other arachidonic acid metabolites.

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