It was observed in the present experiments that a decrease in heme oxygenase activity occurs only in the neonates; there was no change in the activity of the enzyme in adult animals. This characteristic, age-dependent difference in the effect of D-PA administration was also detected in our earlier studies. In parallel experiments on neonate and adult animals it was found that in the neonatal period D-PA significantly reduced the hexobarbital sleeping-time[6], enhanced the activities of catalase and peroxidases[3], and exerted a marked radiation-protective effect[8]. Similar phenomena were not observed in adult animals. Our earlier and present data are in accordance with the experimental results of Maines and Kappas[7] and with their conceptions of the characteristic neonatal state of heme metabolism. At the same time the findings may also give a common explanation of our clinical observation, mentioned in the introduction, that D-PA can be employed to advantage for the treatment of neonatal hyperbilirubinemia and similarly for the prevention of retrolental fibroplasia developing as a consequence of hyperoxia in infants with very low birth-weights.

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A new anti-mycotic drug tioxaprofen and its uncoupling effect on isolated mitochondria

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Summary. An oxazole compound, tioxaprofen, exerted a strong anti-mycotic activity against *Trichophyton mentagrophytes* and *T. rubrum*, which were major dermatophytes from patients. It was found that tioxaprofen was a potent uncoupling agent of mitochondrial respiration.

An oxazole compound, tioxaprofen (fig. 1) synthesized for a non-steroid anti-inflammatory drug[3], was found to exhibit a strong anti-mycotic activity against *Trichophyton mentagrophytes, T. rubrum*, and *Microsporum canis* at concentrations below 10 μg per ml. In order to investigate the mode of action on biomembranes, the effect of the drug on mitochondrial reactions was studied first using isolated rat liver mitochondria, whose molecular mechanism for oxidative phosphorylation is known in detail. Imidazole anti-mycotic drugs, which have been demonstrated to attack mainly plasma membranes in fungi and yeasts (Yamaguchi[4] and references cited therein), have been shown to disturb the ATP synthesis in mitochondria; this is accompanied by a drastic swelling of the mitochondria (Kawai, unpublished data), which suggests that mitochondria can also be a target of these anti-mycotic compounds. Tioxaprofen, too, was found to uncouple the oxidative phosphorylation in isolated mitochondria. In this preliminary communication, the effect of tioxaprofen on some mitochondrial reactions is described.

Materials and methods. Tioxaprofen was a gift of E. Merk, Japan. Tris, ADP, and bovine serum albumin (BSA, fraction V) were products of Sigma Chemical Co. Other reagents were of the purest grade commercially available. *Microsporum canis* 8022 was supplied by Dr S. Watanabe (Department of Dermatology, Shiga Medical College). *Trichophyton rubrum* and *T. mentagrophytes* were supplied by Dr Y. Kitajima (Department of Dermatology, Gifu University School of Medicine). Anti-mycotic activity of tioxaprofen was measured by an assay with Sabouraud broth to which tioxaprofen dimethylformamide solution was added in graded concentrations after sterilization; fungi were then inoculated in a final volume of 5 ml. The culture broth was kept at room temperature (24–27 °C) for 2 weeks without shaking.

Rat liver mitochondria were prepared according to the procedure of Schneider[5] using 0.25 M sucrose solution which contained 0.5 mM EDTA and 10 mM Tris-Cl, pH 7.4. Mitochondrial respiration was measured by means of a Galvani type oxygen electrode (Sensanics Japan Co.). RC and P/O indexes were calculated by the method of Chance and Williams[6]. Reaction medium was composed of 0.225 M sucrose, 10 mM KCl, 5 mM $MgCl_2$, 5 mM phosphorus, 0.5 mM EDTA, and 20 mM Tris-Cl, pH 7.4, in a final volume of 3 ml. Mitochondrial swelling was measured by monitoring the absorbance decrease at 550 nm according to the finding by Tedeschi and Harris[7] using a Hitachi 320-S recording spectrophotometer. Mitochondrial protein was determined by the method of Lowry et al.[8] using BSA as a standard protein.

Results and discussion. The effect of tioxaprofen on growth of dermatophytes was examined by a dilution method. The minimum inhibitory concentration (MIC) of tioxaprofen was found to be around 8 μg (22 nmoles) per ml for *Microsporum canis, Trichophyton mentagrophytes,* and

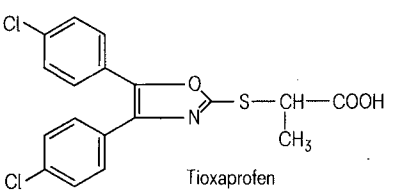

Figure 1. Chemical formula of tioxaprofen.

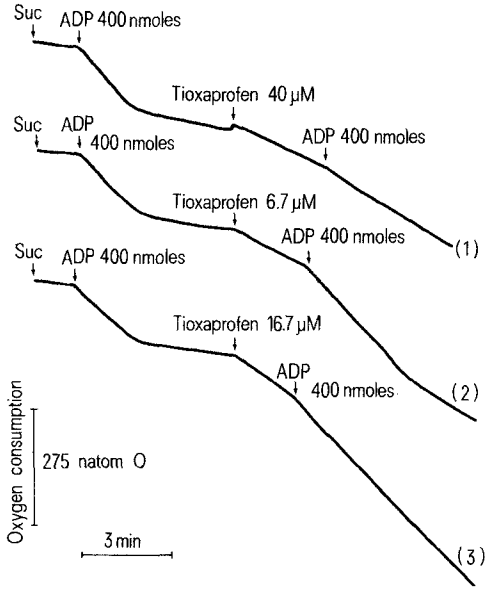

Figure 2. Effect of tioxaprofen on respiration of mitochondria oxidizing succinate. Reaction mixture contained 675 µmoles sucrose, 30 µmoles KCl, 15 µmoles $MgCl_2$, 15 µmoles phosphorus, 1.5 µmoles EDTA, 3 mg of mitochondrial protein, and 60 µmoles Tris-Cl, pH 7.4, in a final volume of 3 ml. Suc, succinate (15 µmoles).

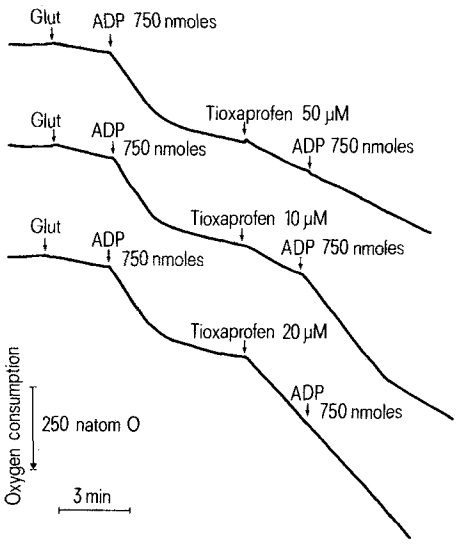

Figure 3. Effect of tioxaprofen on respiration of mitochondria oxidizing L-glutamate. Reaction conditions were the same as figure 2. Mitochondrial protein was 3.6 mg. Glut, L-glutamate (15 µmoles).

T. rubrum. *Candida albicans* showed a lesser sensitivity to the drug; MIC was larger than 40 µg per ml.

Figure 2 shows an oxygram of respiration in isolated rat liver mitochondria, oxidizing succinate as a substrate. Freshly prepared mitochondria exhibited an RC index of 5.6–6.0 and a P/O index near to 2.0. The addition of tioxaprofen caused a release of state 4 respiration, which was accompanied by decrease of RC and P/O indexes (curve 2). At concentrations higher than 15 µM, mitochondrial respiration was completely uncoupled (curve 3), and the respiratory control disappeared. It was observed that state 3 respiration was progressively depressed by the drug

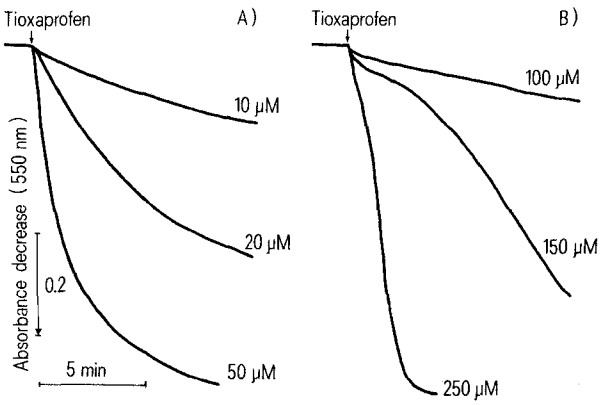

Figure 4. Tioxaprofen-induced swelling of mitochondria. *A* Reaction mixture was composed of 450 µmoles KCl, 1.5 µmoles EDTA, 0.7 mg mitochondrial protein and 60 µmoles Tris-Cl, pH 7.4, in a final volume of 2.0 ml. *B* Reaction mixture was composed of 750 µmoles sucrose, 1.5 µmoles EDTA, 0.7 mg mitochondrial protein and 60 µmoles Tris-Cl, pH 7.4, in a final volume of 2.0 ml.

according to its increase in concentration (curve 1). As depicted in figure 3, respiration in mitochondria oxidizing L-glutamate was also strongly affected by tioxaprofen, exhibiting the release of respiratory control and inhibition of state 3 respiration. The effect of tioxaprofen on latent ATPase activity of mitochondria has not been studied yet. The latent ATPase activity of isolated mitochondria may be markedly enhanced by the drug due to its strong uncoupling effect on oxidative phosphorylation in mitochondria, as is generally observed in experiments with uncoupling agents (Myers and Slater[9] and references cited therein).

Figure 4 shows tioxaprofen-induced swelling of mitochondria. It was found that tioxaprofen was able to induce large amplitude swelling of mitochondria in an isotonic solution of alkali metal ions (A) and even in 1 isotonic sucrose solution (B). In curve A, the swelling process in isotonic KCl solution is shown. The addition of tioxaprofen caused a decrease in absorbance at 550 nm, which indicates the swelling of mitochondria[7]. The swelling was also observed in isotonic NaCl solution. Though apparently a higher concentration was required, tioxaprofen induced drastic swelling of mitochondria in isotonic sucrose solution, where pseudo-energized swelling is inhibited[10]. The induction of mitochondrial swelling in sucrose solution might be elicited by a detergent-like action of the drug, which suggests that a lytic activity of tioxaprofen on biomembranes such as plasma membranes can be expected, like the lytic actions by imidazole anti-mycotic drugs[4].

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