fact, that the heating step leads to decarboxylation of any cannabinoid acids present in marijuana, forming the corresponding neutral phenolic cannabinoids. With the RIM test, the localization of cannabinoids is possible using Fast Blue B, a chromogenic reagent for phenols. Using the IFIM test, cannabinoid identification is achieved by induced fluorescence probably caused by the condensation of the neutral phenolic cannabinoids. The formation of highly fluorescent derivatives from the cannabinoid condensation is already documented in literature[19]. To shed some light on cannabinoid fluorescence induced by heating, studies are in progress in our laboratories.

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Heme oxygenase activity is decreased by D-penicillamine in neonates

G. Oroszlán, L. Lakatos, L. Szabó, B. Matkovics and L. Karmazsin

Department of Pediatrics, Debrecen University Medical School, POB 32, H-4012 Debrecen (Hungary), and Biological Isotope Laboratory, 'A.J.' University of Szeged, POB 539, H-6701 Szeged (Hungary), November 12, 1982

Summary. A 3-day D-penicillamine treatment of neonatal rats caused a significant decrease in heme oxygenase activity. This change was not observed in adult rats. The data indicate age-related differences in the effects of D-penicillamine.

Since 1973, D-penicillamine (D-PA) has been used in our department for the treatment of neonatal hyperbilirubinemia[1], and on the basis of more recent experimental and clinical observations for the prevention of retrolental fibroplasia[2]. In our previous animal experiments we found that D-PA enhances the activities of heme-containing enzymes, playing an important role in the defence against oxygen toxicity[3]. Since heme metabolism is a crucial stage in bilirubin production, it was of interest to examine the activity of heme oxygenase (E.C. 1.14.99.3.), the initial and rate-limiting enzyme of heme degradation.

Materials and methods. Experiments were performed on neonatal and on 6-week-old adult male CFY rats. The animals received 1000 mg/kg D-PA i.p. between 08.00 h and 10.00 h in a single dose daily for 3 days. The treatment of the neonatal rats was introduced on the 1st day of their lives. The control group was treated with the same volume of physiological NaCl solution. On the day following the final injection the animals were exsanguinated after decapitation, and the livers were washed with ice-cold phosphate buffer, blotted dry, and weighed. Livers not used immediately were stored at −20 °C.

Heme oxygenase was determined as described by Eaton et al.[4]. Livers were homogenized in 4 vol. of phosphate buffer with a motor-driven Potter-Elvehjem tissue homogenizer. Homogenates were kept on ice until used. The liver homogenate was centrifuged at 15,000 × g for 15 min at 4 °C. A 2-ml aliquot of the supernatant was used for the determination of heme oxygenase activity.

Protein content was measured by the method of Lowry et al.[5]. D-PA (Metalcaptase®) was a gift from Knoll AG, Ludwigshafen/Berlin. The chemicals employed were commercial products of Reanal (Budapest) and Sigma (USA), and were used without further purification.

Results and discussion. The results are given in the table. The 3-day D-PA treatment of the adult animals did not lead to any significant change in the heme oxygenase activity. In contrast, in the neonates a marked reduction in heme oxygenase activity was observed following D-PA treatment.

The rapid degradation of fetal hemoglobin and the oxidation of its heme moiety are contributing factors in the development of postparturition hyperbilirubinemia. It has been shown that in those species in which postparturition hemolysis takes place, the activity of heme oxygenase is enhanced in the newborn period[7]. Heme oxygenase, which constitutes the rate-limiting enzyme in the degradation of heme, utilizes as the substrate not only hemoglobin heme, but the heme moiety of cellular hemoproteins.

Changes in enzyme activities following 3-day D-penicillamine treatment

		Heme oxygenase nmoles bilirubin/mg protein/h
Adult	Control (n = 7)	29.0 ± 9.0
	D-PA (n = 7)	29.9 ± 10.9
Neonate (4-day old)	Control (n = 7)	24.8 ± 9.7
	D-PA (n = 8)	9.5 ± 2.20[a]

[a] $p < 0.01$ (Student's t-test).

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

K. Kawai[1], R. Watanabe, Y. Nozawa, M. Nozaki[2], K. Tsurumi[2] and H. Fujimura[2]

Department of Biochemistry, Gifu University School of Medicine, Tsukasamachi 40, Gifu (Japan 500), June 29, 1982

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

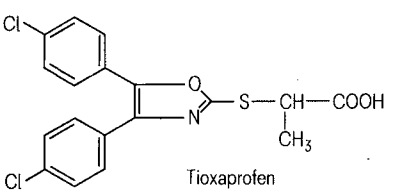

Figure 1. Chemical formula of tioxaprofen.