Biochemical Pharmacology, Vol. 22, pp. 543–544. Pergamon Press, 1973. Printed in Great Britain.

SHORT COMMUNICATIONS

Effect of eucalyptol on microsomal enzyme activity of foetal and newborn rats

(*Received* 16 *June* 1972; *accepted* 25 *September* 1972)

IT IS well known that liver microsomal mixed oxygenase enzyme systems, responsible for drug metabolism, show a low activity in foetal and newborn animals.[1–3] It was reported that this activity may be stimulated by treating the mother during different periods of the pregnancy with various inducing agents.[4–6] However, the possibility of stimulating the microsomal enzymes in the pre-natal and neonatal liver tissues is dependent on the placental penetration of the inducers[7] and on the age of the foetus at the time of administration.[6] Previous reports from this laboratory have shown the inducing properties of eucalyptol in various animal species.[8–10] Therefore it was of interest to investigate the possibility of stimulating the drug metabolism in foetal and neonatal periods by treating the mothers with this agent.

Pregnant Sprague–Dawley rats were treated with eucalyptol (500 mg/kg, s.c. daily for 4 days) between days 10 and 14 of pregnancy (experiment A) or during the last 4 days of pregnancy (experiment B) or between the 2nd and the 6th day after delivery (experiment C). Control pregnant rats were given subcutaneously, a corresponding amount of arachis oil (0·2 ml/100 g).

Some suckling rats of control mothers, were given eucalyptol directly (500 mg/kg s.c. daily for 4 days). All the mothers were sacrificed at the same time as their litters, 18 hr after the last treatment. The newborn rats were killed between the first 8 and the 18 hr of life. Foetuses were obtained immediately after mother killing by removing and incising the uterine horns. Livers were immediately removed and frozen on dry ice. Enzymatic activity was measured on the 9000 *g* supernatant fraction of liver homogenates, incubated with cofactors and substrates (aniline or pNO$_2$-anisol) as previously described.[11] Determinations of foetal and newborn enzymatic activity was carried out on pools of six and four livers, respectively.

The results reported in the table show that treatment with eucalyptol greatly enhances the liver microsomal enzyme activity of the mothers during different times of pregnancy and during the first days after delivery. Such treatment also affects the enzyme activity in the foetus livers so that the poor drug metabolizing capacity can be stimulated both in foetal and in newborn rats O-demethylation of pNO$_2$-anisol seems to be more stimulated than the *p*-hydroxylation of aniline. In contrast eucalyptol administration to the nursing mothers, does not induce the microsomal activity of the suckling newborn rats. However, suckling rats directly treated with this inducing agent show an increased liver enzymatic activity. In all cases the protein concentrations of the fraction used for enzymatic assay, and the total liver weights were not significantly modified as previously observed.[10]

The results obtained indicate that eucalyptol probably cannot cross the blood–milk barrier in amounts sufficient to affect the hepatic microsomal enzymes of the offsprings, but it is able to penetrate the placental tissue and to reach a concentration in foetal blood adequate for stimulating the liver enzymatic activity.

This effect is compatible with the high lipid solubility of eucalyptol. In fact, it has been reported that the capacity of stimulating the prenatal liver enzymes is dependent on the placenta penetration of a compound, which is directly related to its lipid solubility.[7]

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Acknowledgement—This work was supported by a grant from N.I.H. No. 1PO1-GM1 837601-PTR.

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TABLE 1.

Treatment	Proteins (mg/g liver)	pNO_2Anisol (nmoles/g/hr)	Aniline (nmoles/g/hr)	Treatment	Proteins (mg/g liver)	pNO_2Anisol (nmoles/g/hr)	Aniline (nmoles/g/hr)
		Mothers				Foetuses	
A Oil	134·5 ± 4·6	325 ± 33	431 ± 50		77·8 ± 2·6	10·2 ± 1·0	29·6 ± 2·7
eucalyptol	149·0 ± 9·0	592 ± 55*	613 ± 31*		85·0 ± 6·6	29·1 ± 5·0*	29·4 ± 0·9
						New-borns	
B Oil	140·3 ± 6·2	449 ± 24	518 ± 59		80·4 ± 3·0	57 ± 5	30·3 ± 4
eucalyptol	142·9 ± 4·6	924 ± 79*	844 ± 67*		87·3 ± 3·0	117 ± 10*	68·0 ± 8*
						Suckling rats	
C Oil	153·0 ± 3·4	482 ± 22	578 ± 33	Oil	106·8 ± 3·0	278 ± 17	395 ± 17
				eucalyptol	129·1 ± 4·0	516 ± 19*	466 ± 36†
eucalyptol	148·6 ± 4·0	789 ± 27*	893 ± 57*	Oil	107·6 ± 2·0	327 ± 13	445 ± 18

The enzymatic activity is expressed as amount (nmoles) of metabolites (pNO_2phenol and pNH_2phenol) formed from the respective substrates (pNO_2anisol and aniline) by 9000 *g* supernatant fraction corresponding to 1 g of fresh liver. Proteins were measured on the same fraction.

Experiment A, Mothers treated between 10–14th day of pregnancy.

Experiment B, Mothers treated during the last 4 days of pregnancy.

Experiment C, Mothers treated between 2nd–6th day after delivery. Suckling rats of control mothers were treated either with arachis oil or with eucalyptol. Suckling rats of eucalyptol treated mothers were only treated with arachis oil.

* $P = 1\%$ vs the corresponding control group.

† $P = 5\%$ vs the corresponding control group.