UTEROTROPIC EFFECT OF CLOFIBRATE AND PHENYLBUTAZONE IN IMMATURE FEMALE RATS

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Summary : The uterotropic effect of clofibrate and phenylbutazone was studied in immature female rats A significant increase in the uterine wet weight was observed following clofibrate and phenylbutazone administration. Clofibrate but not phenylbutazone synergized with the uterotropic effect of ethinyl-oestradiol. Phenylbutazone pretreatment significantly decreased the pentobarbitone sleeping time. The uterotropic effect of clofibrate and phenylbutazone might involve displacement of 17-β oestradiol (or ethinyl oestradiol) from plasma albumin binding sites in rats.

Key words : clofibrate, phenylbutazone, synergism, sleeping time, uterotropic effect, ethinyl-oestradiol

INTRODUCTION

Clofibrate, the hypolipidaemic drug, is reported to cause untoward effects like tenderness of the breast and decreased libido (4) in males as with oestrogen therapy. An oestrogenic effect of clofibrate has so far not been reported; hence clofibrate was investigated in the present study for a possible oestrogenic effect using the uterine wet weight of immature female rats as a criterion. Clofibrate has also been shown to induce hepatic microsomal enzymes (5, 6) and binds to plasma albumin (4). In view of this, drug-induced–alteration in pentobarbitone sleeping timg in adult female rats was also investigated. Since enzyme induction and binding to albumin were thought to be involved in clofibrate action, the effect of phenylbutazone, a drug which produces both these changes, was also studied for comparison.

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MATERIALS AND METHODS

Uterotropic effect - Immature (19–21 days old) female rats weighing between 15 to 25 g were used. They were pretreated orally twice a day for 3 days with either 0.9% sodium chloride (saline), alkaline saline (pH. 10–11) or arachis oil (vehicles control groups) or with clofibrate (I.C.I.) in arachis oil (2 *mg/kg*) or phenylbutazone (S.G. Chemicals) in alkaline saline (50 *mg/kg*) as described by Calhoun *et al.* (2). On 4th day half of the animals of group receiving clofibrate or phenylbutazone and some control rats were administered oral single 10 *µg* dose of ethinyl oestradiol (Indian Schering) suspended in arachis oil and the animals were killed 6 hr after ehtinyl oestradiol treatment instead of 4 hr as suggested by Astwood (1) in order to achieve the peak uterotropic response. The uterine wet weight was expressed in *mg* per 100 *g* of body weight.

Pentobarbitone sleeping time - Adult female rats weighing between 100 – 150 g were used. Pentobarbitone sodium (40 *mg/kg*, ip) was injected in control rats and in rats pretreated with either clofibrate, or phenylbutazone or their respective vehicles as described above. The time between loss and reappearance of righting reflex for each rat was recorded on 4th day as described by Turner (7).

The significance of results was analysed using Student's t–test.

RESULTS

Uterotropic effect of clofibrate and phenylbutazone - No significant difference in uterine wet weights could be observed between the groups receiving the three different vehicles (Table I). Oral treatment for 3 days with clofibrate and phenylbutazone or once with ethinyl oestradiol significantly increased the uterine wet weight. Further, clofibrate pretreatment significantly enhanced the effect of ethinyl oestradiol treatment ($P<0.02$). This potentiating effect was not observed with phenylbutazone pretreatment. However, significant increase in uterotropic effects was observed in the group pretreated with phenylbutazone followed by ethinyl oestradiol as compared to the group treated with phenylbutazone alone.

TABLE I : Effect of orally administered clofibrate, phenylbutazone and ethinyl oestradiol on Uterine wet weight of immature female rats.

Group No.	Oral treatment		Wet uterine weight (mg/100 g ± SEM)	Comparison groups
	On day, 1, 2 & 3	On day 4		Nos. P value*
1	Normal saline (8)	—	59.5 ± 1.19	
2	Alk. saline (6)	—	57.0 ± 3.88	
3	Arachis oil (15)	—	59.7 ± 3.38	
4	—	Ethinyl oestradiol 10 μg (15)	105.6 ± 5.42	3, 4 < 0.001
5	Clofibrate 2 mg/kg (10) in arachis oil	—	87.0 ± 4.74	3, 5 < 0.001 4, 5 < 0.05
6	Phenylbutazone (17) 50 mg/kg in alk. saline	—	75.8 ± 4.19	2, 6 < 0.01
7	Clofibrate (9) as in group 5	Ethinyl oestradiol 10 μg	128.3 ± 6.54	4, 7 < 0.02 5, 7 < 0.001
8	Phenylbutazone (8) as in group 6	Ethinyl oestradiol 10 μg	98.5 ± 4.57	2, 8 < 0.001 6, 8 < 0.01 4, 8 N.S.

Numbers in parentheses denote the number of animals.

*Groups were compared by 't' test.

Pentobarbitone sleeping time - In a preliminary study, no significant change was observed in sleeping time between the groups pretreated with saline, alkaline saline and arachis oil. Pretreatment with clofibrate did not produce any significant change in pentobarbitone induced sleeping time, while the rats pretreated with phenylbutazone showed a significant ($P<0.001$) decrease in sleeping time as compared to their respective controls (Table II).

TABLE II : Effect of orally administered clofibrate and phenylbutazone on pentobarbitone (40 *mg/kg*, ip) sleeping time (minutes, mean ± SEM) in female rats.

Saline	*Alkaline saline*	*Arachis oil*	*Clofibrate 2 mg/kg* b.d. *for 3 days in arachis oil*	*Phenylbutazone 50 mg/kg* b.d. *for 3 days in alkaline saline*
(11)	(5)	(7)	(14)	(8)
137.0±9.43	134.5±7.56	155.3±16.57	158.3±13.21	51.9±3.02*

Numbers in parentheses denote the number of animals used.
*The value significantly differs from control group ($P<0.001$).

DISCUSSION

Our observations suggest that clofibrate and phenylbutazone exhibit oestrogenic activity in immature female rats. Donovan and Van der Worff Ten Bosch (3) had shown that in the second week of life, female rats secrete 17–β oestradiol. The oestrogenic effect of clofibrate and phenylbutazone could be caused by an increase in free circulating oestrogen, either due to a displacement from binding sites in plasma, or because of suppression of hepatic microsomal enzymes and as a consequence reduced oestrogen metabolism.

Both natural and synthetic oestrogens circulate in the blood in association with albumin and sex hormone–binding globulin. Clofibrate (4) and phenylbutazone also bind with albumin and might have displaced the 17–β oestradiol or ethinyl oestradiol and increased the free circulating oestrogen levels. However, Calhoun *et al.* (2) did not observe any significant change in uterine weight with intraperitoneal phenylbutazone.

Hess *et al.* (5) and Murad and Gilman (6) reported induction of hepatomegaly associated with increase in mitochondrial glycerol-phosphate dehydrogenase, catalase, cytochrome-oxidase and urate oxidase with clofibrate in rats. In the present study clofibrate did not produce any significant change in pentobarbitone sleeping time indicating that clofibrate did not alter the microsomal enzymes which interact with pentobarbitone. The significant decrease in the sleeping time with phenylbutazone suggests induction of such hepatic microsomal enzymes. The failure of phenylbutazone to enhance the oestrogenic

effect of ethinyl oestradiol, an oestrogen preparation metabolised slowly by the liver, is not clarified by our study. However, it may be due to the induction of oestrogen metabolizing enzymes during the later period of pretreatment with phenylbutazone that did not allow to synergize the action of ethinyl oestradiol.

In conclusion, we assume that the uterotropic effect of clofibrate and phenylbutazone might be due to displacement of 17–β oestradiol from the albumin binding sites of oestrogen rather than inhibition of metabolism of natural oestrogens.

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