ESTRADIOL INDUCED VAGINAL CORNIFICATION IN SPAYED RATS : A MODEL TO STUDY HEPATIC MICROSOMAL ENZYME INDUCTION

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Summary : Utilizing vaginal cornification as a response for bioassay, a study was conducted to observe the variation in the median cornification dose (cED_{50}) of estradiol, a hepatic-first-pass candidate, given either ip or sc in spayed rats, with or without enzyme induction by rifampin.

Comparisons within and between the groups showed that after enzyme induction cED_{50} was increased fourfold and cED_{50} ip/cED_{50} sc ratio was doubled.

The findings clearly demonstrate that this animal model faithfully reflects alterations in hepatic enzyme activity and could serve as an alternate for conventional hexobarbitone sleeping time test to study enzyme induction.

Key words :   estradiol   enzyme-induction   rifampin   vaginal-cornification

INTRODUCTION

Many drugs and chemicals are known to induce hepatic microsomal enzymes. Enzyme inducing property of drug has not only clinical implications such as those in drug interactions and development of tolerance (1), but it can also vitiate pre-clinical evaluation of a new drug (which itself could be an inducer), particularly in chronic toxicity test (2). It is therefore, desirable that any drug undergoing preclinical tests be also investigated for its enzyme inducing potentiality.

Since inducing property has no relation to chemical structure (3) one cannot predict such an activity on the basis of chemical structure. Hence, *in vivo* or *in vitro* biological methods are needed to investigate enzyme inducing property. Coney (4) has summarised various methods for the purpose. Of these, ip hexobarbitone sleeping time is often used because of its simplicity (5). Recently, Graff *et al.* (5) have opined that hexobarbitone sleeping time may not reflect well the hepatic microsomal

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enzyme activity. Hence, a simple, inexpensive and reliable alternative in vivo test model is desirable.

In such a model (as now being reported) (a) the indicator drug (i.e., an alternative for hexobarbitone as enzyme substrate) must undergo extensive first pass (hepatic) metabolism by inducible enzymes but by itself should not be an inducer, (b) the pharmacological action monitored should be specific and quantifiable, (c) the same animals could be recycled without fear of tolerance and (d) the test should be simple, inexpensive and practicable.

MATERIALS AND METHODS

In this model estradiol was chosen as indicator substrate since it undergoes extensive first pass elimination (6) and is not reported to be an inducer. Cornification of vaginal epithelium, a sensitive and specific response to estrogen (7) used for its bioassay (8) was chosen for monitoring. Spayed rats were employed to avoid interference by endogenous estrogens.

Female rats weighing 150-200 g and showing normal estrous cycles were spayed by the conventional method. Absence of any estrous in these spayed animals was taken as a proof of absence of endogenous estrogen.

A stock solution of estradiol (0.1%) was prepared in absolute alcohol as described by Burn et al (8). A suitable dilution was made by adding distilled water to provide desired dose in 0.1 ml of diluted solution.

Based on pilot studies, estradiol in doses of 25, 50 and 100 ng/100 g body weight was given either ip or sc to 20-30 spayed rats. The number and spacing of estradiol injections and monitoring of animals for vaginal cornification was as per Burn *et al.* (8). This was carried out before and after enzyme induction.

Enzyme induction was achieved by oral administration of rifampin (160 mg/kg) daily for 6 days. This schedule was adopted on the basis of a report by Miguet *et al* (9). after computing the dose for

rats according to Paget and Barnes (10). The reliability of the schedule was tested by pilot studies where enzyme induction was confirmed by noting an increase in 24 hr uninary excretion of ascorbic acid, which is a biochemical indicator of enzyme induction in rats and mice (8). Percent of animals showing vaginal cornification with each dose and each of the routes was calculated.

Dose response curves (DRC; Log dose vs probit) were constructed from the data. The median cornification doses (ED_{50}) for each route and their relative potency ratio were estimated by the method of Litchfield and Wilcoxon (11).

RESULTS

The DRCs, ip and sc administration of same doses of estradiol before and after rifampin-induced enzyme induction, are shown in Figure 1. It is clear that DRCs of ip route are far to the right of corresponding DRCs of sc route, indicating that estradiol by ip route is far less potent than it is by sc administration.
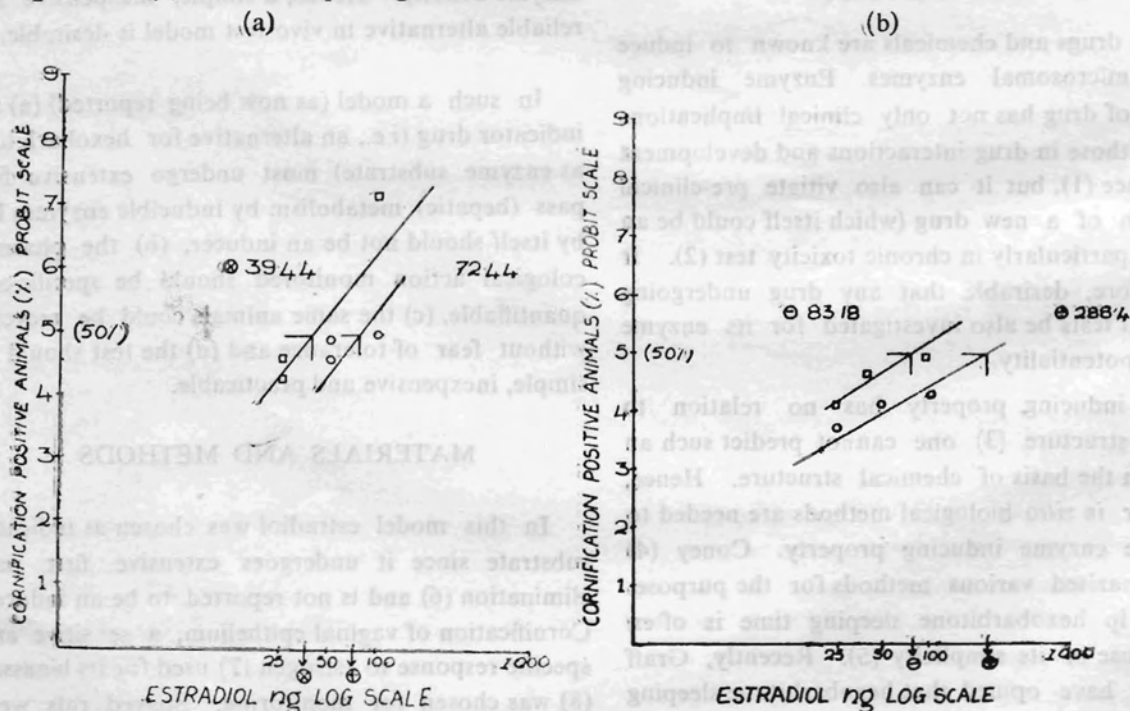
(a)

(b)

Fig. 1 : Median cornification dose for IP (o—o) and SC (□—□) route before (a) and after (b) enzyme induction by rifampin (160 mg/kg for 6 days)

The calculated ED_{50} and the ratio of ED_{50} ip ED_{50} sc are shown in the Table I. It is evident that this ratio is greater than unity before as well as after enzyme induction and the value of this ratio is almost doubled after enzyme induction. Further, after enzyme induction the ED_{50} (ip) increased four fold while it just doubled in case of sc route.

TABLE I : ED_{50} values and their ratios Vaginal cornification in rate by estrogen.

ED_{50} ng/100 g		ED_{50} ratios			
IP	SC	b/a	d/c	a/c	b/d
a) 72.44 (49.95-105)	c) 39.44 (29.48-53.73)				
		3.98	2.08	1.82	3.46
b) 288.4 (144.2-576.8)	d) 83.18 (46.21-149.72)				

(a) and (c) before, and (b) and (d) after rifampin respectively

DISCUSSION

The basis of this test model is that : (i) ratio between ED_{50} ip and ED_{50} for other parenteral route will be greater than unity for a drug undergoing extensive first pass hepatic metabolism and (ii) the value of this ratio will significantly increase if hepatic enzymes are induced.

The findings of the study substantiate the assumptions; the ratio ED_{50} ip ED_{50} sc was found to be greater than unity before and after enzyme induction. It is therefore, reasonable to infer that this in vivo model is sensitive and provides quantifiable data to investigate enzyme inducing property of drugs and chemicals. Further more, ED_{50} for both ip and sc route were increased after rifampin again suggesting that the model effectively detects enzyme induction. The increase in ED_{50} after rifampin was four fold and two fold by ip and sc routes respectively; this fact is yet another indication of model's sensitivity.

The use of estradiol as a substrate for hepatic enzyme activity has following merits : (i) by itself it is not an enzyme inducer, hence it is not likely to vitiate results of screening of drugs for enzyme inducing property, (ii) cornification of vaginal epithelium is specific and sensitive : (iii) tolerance does not develop to this action and (iv) the pharmacological action, vaginal cornification, chosen for monitoring is less vulnerable for pharmacodynamic interferences by the actions of drugs being investigated for enzyme induction, except when the drug possesses estrogenic or antiestrogenic activity. This is unlike the hexobarbitone sleeping time test because the sleeping time test monitored by righting reflex could be interfered with if drugs under test affect CNS or skeletal muscle.

The test model is fairly simple, does not require sophisticated equipment and is inexpensive. The spayed rats can be recycled (and have been, in our study); the only need being practice of ovarectomy and of correctly identifying cornified cells in vaginal smear. An important limitation of this method is that it is not as fast as hexobarbitone sleeping time test.

In conclusion, this test is simple, economical, easy, and sensitive but some-what time consuming. It could serve as an alternative for hexobarbitone sleeping time test.

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