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Glutathione S-transferase activity during pregnancy in the mouse: effects of *trans*-stilbene oxide pretreatment

Thioether formation catalyzed by glutathione S-transferases (GSHTr, EC 2.5.1.18) represents one of the major mechanisms of *in vivo* detoxication of xenobiotics [1–3]. Recent studies have shown that metabolism of vicinal dihalogens by GSHTr may lead to formation of potent mutagens [4–6]. In mammals, essentially all organs are known to possess this enzyme system [1, 7]. In contrast to the liver, which contains several forms of GSHTr [1], human placenta is reported to possess a single form of this enzyme [8, 9], and its activity is not altered following exposure of pregnant women to the constituents of cigarette smoke [10, 11]. GSHTr activity has also been detected in the placentas of rabbits, guinea pigs [12, 13], rats [14], and monkeys [15]. Data are also available on the changes in the titers of placental GSHTr with gestational age in rabbits and guinea pigs [12, 13]. However, similar information is not available at present for the mouse placental enzyme.

Because of obvious pharmaco-toxicological implications, alterations in the drug-metabolizing enzymes following exposure of pregnant animals to different chemicals have received considerable attention [16–23]. Most of these investigations deal with cytochrome P-450-dependent monooxygenase activity, and little is known about the effects these chemicals have on GSHTr. Earlier, Bell *et al.* [22] reported noninducibility of hepatic GSHTr after treatment of pregnant rats with the classical inducer phenobarbital. However, in subsequent studies by Rouet *et al.* [23], induction of liver GSHTr was observed in pregnant rats and mice exposed to 5,6-benzoflavone. In these reports, possible alteration in the placental GSHTr was not considered. Thus, the question of inducibility of GSHTr

during pregnancy warrants further examination. Although in males of both mice and rats the liver GSHTr are inducible enzymes and a number of chemicals including pesticides and carcinogens have been shown to be inducers, the mouse liver enzymes seem to be more responsive [1, 24, 25]. Therefore, the objectives of the present study were to examine (i) the quantitative changes in the mouse hepatic and placental GSHTr activity due to pregnancy, and (ii) the effects of exposure to a potent inducer of this enzyme system, namely *trans*-stilbene oxide (TSO) [26–28].

Materials and methods

Young virgin mice of the CD_1 strain were purchased from the Charles River Breeding Laboratories and housed for at least 1 week prior to use. Purina 5001 mouse chow and water were given *ad lib*. Animal rooms were on a 12-hr light/dark schedule. The date on which a mating plug was observed was designated as day zero of pregnancy. TSO (Aldrich Chemical Co.) in corn oil was administered intraperitoneally once daily for 3 days on either days 9, 10, and 11 or on days 15, 16 and 17 of gestation at doses of 0 (control), 100, 300, or 500 mg/kg. Animals were killed 24 hr after the last dose (on day 12 or 18 of gestation) by cervical dislocation. Placentas and livers were examined *in situ* for signs of toxicity, removed quickly, weighed, and placed in cold 50 mM Tris buffer, pH 7.4, containing 0.25 M sucrose and 1.0 mM EDTA. Placentas were pooled by litter while maternal livers were processed individually to isolate cytosol by differential centrifugation [25].

Cytosolic GSHTr activity was assayed spectrophotometrically at 37° using 105,000 g supernatant fluid as the

enzyme source and 1 mM 1-chloro-2,4-dinitrobenzene (CDNB) and 1 mM reduced glutathione (GSH) as co-substrates, according to Habig et al. [29].

Results and discussion

The effects of pregnancy on maternal liver GSHTr activity are shown in Fig. 1. Although the values observed in pregnant animals were slightly lower as compared to non-pregnant controls, there was no statistically significant change in GSHTr titers in relation to gestational stage. Polidoro et al. [14] also reported similar observations for liver GSHTr activity towards CDNB and 1,2-dichloro-4-nitrobenzene (DCNB) at mid-pregnancy compared to non-pregnant rats. However, liver cytosols from 19- to 20-day pregnant rats exhibited a significant increase in CDNB and a decrease in DCNB conjugation activity. Their data also indicated that GSHTr activity against p-nitrobenzylchloride did not change while that towards 1,2-epoxy-3-(p-nitrophenoxy) propane increased due to pregnancy. Earlier, Combes and Stakelum [30] observed depression in sulfobromophthalein conjugation with GSH in the livers of pregnant rats. James et al. [13] reported that hepatic GSHTr activity toward styrene oxide measured in pooled samples of pregnant guinea pigs tended to be slightly lower than in comparable samples from adult males. These reports suggest that the changes in liver GSHTr during pregnancy are unpredictable and vary with each substrate in different animal species.

The pretreatment of pregnant mice with TSO resulted in a significant induction of GSHTr in the maternal liver except for the samples examined from 12-day and 18-day pregnant mice receiving 100 mg TSO/kg (Fig. 1). The magnitude of induction was found to be dose dependent and a maximum of about 3.4-fold increase in activity was observed in 18-day pregnant mice receiving 500 mg TSO/kg. Earlier reports [26–28] have shown a 2- to 4-fold induction of liver GSHTr in male rats pretreated with TSO and the induction was believed to be due to TSO itself and not due to its metabolites [27]. Since isozymes of GSHTr from different sources exhibit overlapping substrate specificity and CDNB serves as a substrate for all the forms [1], it remains unknown whether certain or all of the isozymes of pregnant mouse liver GSHTr are induced by TSO pretreatment.

The pregnant mice treated with TSO appeared healthy and active and continued to gain weight. The great majority of fetuses appeared normal when inspected at sacrifice. Occasionally mice receiving 500 mg TSO/kg exhibited some signs of toxicity as evidenced by pale fetuses and discolored placentas. These placentas were not included in enzyme preparations.

Figure 2 shows the changes in the mouse placental GSHTr activity during pregnancy and the effects of TSO pretreatments. At present it is unknown whether the mouse placenta, similar to the human placenta [8, 9], possesses only one form of GSHTr. The data indicate that in untreated mice the GSHTr titers were essentially the same at day 12 and day 18 of gestation. However, using CDNB as a co-substrate, Polidoro et al. [4] reported about a 50% decrease in rat placental GSHTr activity near term relative to mid-pregnancy. It should be noted, however, that only one sample was examined. On the other hand, no such dramatic changes but a relatively small gradual decline has been observed during pregnancy in rabbit and guinea pig placental GSHTr activity toward CDNB [12, 13]. In humans, placental GSHTr activity utilizing styrene oxide as a substrate apparently remains nearly constant between 8 and 25 weeks of gestation followed by about a 50% decline at full term [10]. A similar dramatic drop in GSHTr activity towards styrene oxide was observed in guinea pig but not in rabbit placentas [12, 13] towards the end of gestation. Taken together, these reports tend to suggest that, during pregnancy, substrate as well as species-specific
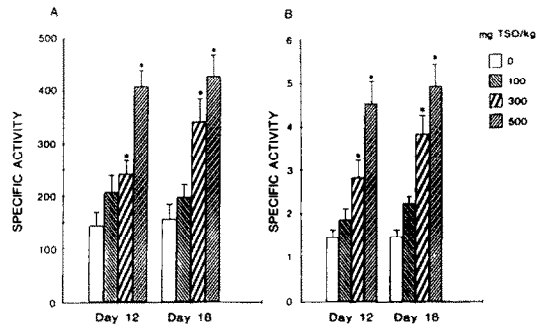

Fig. 1. Maternal liver glutathione S-transferase activity during pregnancy and the effects of trans-stilbene oxide pretreatment. The pretreatment of pregnant mice and the measurement of liver cytosolic enzyme activity were performed as described in Materials and Methods. Each bar represents mean specific activity ± S.E.M. (N = 5–12). The specific activity is expressed as μmoles of CDNB conjugated/min/per g liver (A) or per mg cytosolic protein (B). The specific activity in non-pregnant mice was 1.64 ± 0.16 μmoles/min/mg protein or 216 ± 27 μmoles/min/g liver (N = 5). Key: (*) Significantly different from control (P ≤ 0.001).

differences exist in the titers of placental GSHTr activity. The data given in Fig. 2 also indicate that none of the TSO treatments produced changes in placental GSHTr activities that were significantly different from controls. Comparative data on the TSO-caused alterations in placental GSHTr are not currently available for other animal species. In view of the reported ability of TSO to cause induction of GSHTr in the extrahepatic tissues [28], the observed lack of induction of the mouse placental enzyme was rather unexpected. Although these observations suggest that placental GSHTr, in general, may be refractory to the inductive effects of environmental chemicals, additional data are needed.

In conclusion, the data obtained under the experimental conditions employed indicate the inducibility of liver but not of placental GSHTr in the mouse during pregnancy. Utilizing TSO, the magnitude of induction was dose dependent and is quite comparable to that reported for the male rat.
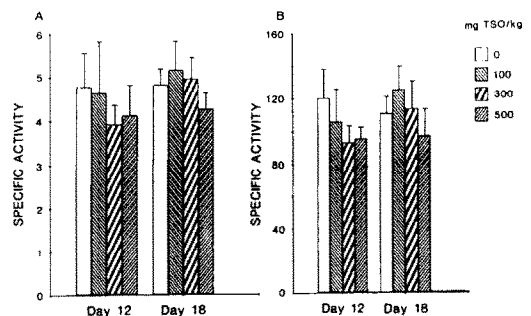
Fig. 2. Placental glutathione S-transferase activity during pregnancy and the effects of trans-stilbene oxide pretreatment. The experimental details are the same as given in the legend for Fig. 1. The specific activity is expressed as μmoles of CDNB conjugated/min per g tissue (A) or nmoles CDNB conjugated/min per mg cytosolic protein (B).

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Evidence for generation of leukotriene B₄ in human polymorphonuclear leukocytes treated with linoleylanilide

In resting human polymorphonuclear leukocytes (PMNs) the free levels of arachidonic acid are low. However, upon stimulation, the levels of arachidonic acid rapidly increase [1–3]. The interest in the study of the mechanisms by which the intracellular levels of arachidonic acid are controlled has grown during the last years (reviewed in [4]). This is due to the finding that arachidonic acid is precursor of the leukotrienes, which are potent mediators of the inflammatory response (reviewed in [5]). We have previously shown that linoleylanilide, a fatty acid anilide supposcly involved in the toxic syndrome in Spain [6, 7], induces the generation of arachidonic acid from human PMNs [3]. Different to other signals, like phagocitic particles or the ionophore A23187 [1, 2], linoleylanilide induces the generation of arachidonic acid without affecting the synthesis of PAF-acether, lysosomal enzymes or O_2^- [3]. This permits us to explore the mechanisms that control arachidonic acid metabolism in human polymorphonuclear leukocytes with relative independence of other processes. In this study we have investigated the following questions: (1) From which phospholipids is arachidonic acid generated in response to linoleylanilide? (2) By which mechanisms is arachidonic acid generated in response to linoleylanilide? (3) Is the arachidonic acid generated in response to linoleylanilide retained intracellularly or is it released to the extracellular medium? and (4) is the arachidonic acid generated in response to linoleylanilide metabolized by the lipoxygenase pathway?

Materials and methods

Cells. Human PMNs were obtained from venous blood of normal volunteers as in [8].

Effect of linoleylanilide on the content in arachidonic acid of phosphatidylcholine and phosphatidylinositol. Human PMNs were labelled with (5,6,8,9,11,12,14,15-[³H])-arachidonic acid (Amersham, 125 Ci/mmole) as previously described [2, 3]. After washing, PMNs (0.6 ml, 1 × 10⁷ cells per ml) were incubated with linoleylanilide (1 mg/ml) for 2 hr at 37°. After extraction, lipids were subjected to TLC in chloroform/methanol/acetic acid/sodium borate 0.1 M