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Masahiro Moriyama[*] Haruyo Domoto[†] Syoichi Yamashita[‡]

Katsushi Furuno[**] Ryozo Oishi[††]

Hiromu Kawasaki[‡‡] Yutaka Gomita[§]

[*]Okayama University,

[†]Okayama University,

[‡]Okayama University,

[**]Okayama University,

[††]Kyushu University,

[‡‡]Okayama University, kawasaki@pheasant.pharm.okayama-u.ac.jp

[§]Okayama University,

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## Abstract

We examined the pharmacokinetics of phenobarbital before and during pregnancy in rats. Animals were divided into four groups: (a) control, (b) pregnant, (c) phenobarbital-treated, and (d) phenobarbital-treated pregnant groups. The increase in body weight of nonpregnant or pregnant rats was not influenced by long-term phenobarbital treatment. Plasma phenobarbital concentrations during the period of long-term phenobarbital treatment with a fixed dosage by body weight were not significantly affected by pregnancy. Furthermore, pregnancy did not affect pharmacokinetic parameters of phenobarbital between 0.25 and 24h after administration. These results suggest that pregnancy does not influence on the pharmacokinetics of long-term phenobarbital treatment at a fixed dosage by body weight.

**KEYWORDS:** phenobarbital, pharmacokinetics, pregnancy, plasma concentrations

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# Effect of Pregnancy on Plasma Phenobarbital Concentrations in Rats

Masahiro MORIYAMA, Haruyo DOMOTO, Syoichi YAMASHITA, Katsushi FURUNO, Ryozo OISHI[a], Hiromu KAWASAKI and Yutaka GOMITA*

Department of Hospital Pharmacy, Okayama University Medical School, Okayama 700, Japan and [a]Department of Hospital Pharmacy, Faculty of Medical Sciences, Kyushu University, Fukuoka 812, Japan

We examined the pharmacokinetics of phenobarbital before and during pregnancy in rats. Animals were divided into four groups: (a) control, (b) pregnant, (c) phenobarbital-treated, and (d) phenobarbital-treated pregnant groups. The increase in body weight of nonpregnant or pregnant rats was not influenced by long-term phenobarbital treatment. Plasma phenobarbital concentrations during the period of long-term phenobarbital treatment with a fixed dosage by body weight were not significantly affected by pregnancy. Furthermore, pregnancy did not affect pharmacokinetic parameters of phenobarbital between 0.25 and 24 h after administration. These results suggest that pregnancy does not influence on the pharmacokinetics of long-term phenobarbital treatment at a fixed dosage by body weight.

**Key word:** phenobarbital, pharmacokinetics, pregnancy, plasma concentrations

The pharmacokinetics of various antiepileptic drugs are well known, and therapeutic monitoring of these drugs is very useful for the treatment of epilepsy. With advances in the drug treatment of epilepsy, some patients with epilepsy have been able to become pregnant and have given birth. However, few studies have been done on the pharmacokinetics of antiepileptic drugs in pregnant patients (1).

Many investigators have reported that the pregnancy causes various changes (increase, decrease and no effect) in seizure frequency (2-5). In our hospital, therapeutic monitoring of antiepileptic drugs has also been done in some pregnant patients. However, many important factors, such as differences in age and body weight of patients, dose, compliance, and time of blood sampling make it difficult to clarify the effect of pregnancy on the pharmacokinetics of antiepileptic drugs.

We have established a method for determining the plasma concentrations phenobarbital (PB), an antiepileptic agent, in rats using only 60 $\mu$l of blood (6) which makes repeated determinations of plasma concentrations possible. In the present study, therefore, we examined the pharmacokinetics of PB during pregnancy in rats.

## Materials and Methods

***Materials.*** PB was purchased from Sigma Chemical Co. (St. Louis, MO, USA). For oral administration to the rat, PB was suspended in 0.5 % sodium carboxymethylcellulose (CMC). Acetanilide (Waco Pure Chemical Industries, Osaka, Japan) was used as the internal standard (IS) and was dissolved in 2 $\mu$g/ml in 50 % methanol. All other reagents used were of reagent grade.

***Animals.*** Female Wistar rats weighing 195-280 g at the beginning of the study were obtained from Charles River Japan, (Atsugi, Japan). They were housed in plastic cages (26 × 36 × 25 cm) in a room maintained on a 12-h light/dark cycle (lights on from 7:00 to 19:00) and at 22-24 °C and 60 % relative humidity.

***Determination of plasma PB concentration.*** After centrifugation at 12,000 rpm for 3 min in a hematocrit centrifuge (Compur M 1100, Compur Electronic, Munich, Germany), 20 $\mu$l of plasma and 0.1 $\mu$g of IS were passed through a Bond Elut cartridge C-18 solid-phase extraction column (1 ml volume, No. 1210-2001, Varian SPP), which had previously been washed twice with 1 ml of methanol and with 1 ml of 0.01 M $KH_2PO_4$. After the column was washed twice with 1 ml 0.01 M $KH_2PO_4$, the samples to be measured were eluted

* To whom correspondence should be addressed.

with 250 $\mu$l of methanol. The eluate (20 $\mu$l) was applied to a high-performance liquid chromatograph system, which was composed of a pump (Type 510, Waters-Millipore, MA, USA), an automatic sample processor (Type 710B), a ultraviolet monitor (Type 481), and a data module (Type 730). The analytical column was a Li-ChroCART RP-18e (4 $\mu$m particle size, 4 × 125 mm, Cica-MERCK Co., Tokyo, Japan). The mobile phase was a mixture of acetonitrile/0.01 M $KH_2PO_4$ (25/75, v/v), the flow rate was 0.8 ml/min and absorbance was measured at 210 nm. The retention times of IS and PB were 4.6 min and 7.8 min, respectively.

***Experimental procedure.*** Animals were divided into four groups: (a) control, (b) pregnant, (c) PB-treated and (d) PB-treated pregnant groups. Body weights were measured and PB (20 mg/kg) was administered by gavage to rats of groups c and d twice (at 7:00 and 19:00) every day. To the rats of groups a and b, 0.5 % CMC solution (vehicle) was administered orally. When the plasma concentrations of PB reached the steady state (7–10 days after starting administration), rats were mated with male rats for 5 days. Pregnancy of the rat was confirmed by observing the rat shape in the late period of pregnancy. Before the administration of PB every morning, blood was collected from a tail vein into 60-$\mu$l capillaries.

During the late period of pregnancy (5–7 days before delivery), plasma PB concentrations were determined from 0.25 to 24 h after oral administration of PB in all groups. In the case of the experiment of time-course changes in plasma PB concentration, food was withheld from the animals 12 h before and during the experiment.

***Pharmacokinetic analysis.*** Pharmacokinetic parameters were obtained from the PB plasma concentration-time data for each animal, using a personal computer program for nonlinear least squares regression analysis (MULTI) (7). Time to maximal concentration (Tmax), maximal plasma concentration (Cmax), area under the plasma concentration-time curve ($AUC_{0-12}$) and mean residence time (MRT) were calculated by standard linear trapezoidal integration.

***Statistics.*** Results were statistically evaluated with the two-tailed Student's $t$-test.

## Results

***Changes in body weight.*** Fig. 1 shows changes in body weight in the 4 experimental groups before and during pregnancy. Rats became pregnant about 21 days before delivery. All groups showed gradual increases in body weight. There was no significant difference in body weight between the PB-treated groups and the untreated groups.

***Daily plasma PB concentrations during the administration period in pregnant or nonpregnant rats.*** Fig. 2 shows the changes in

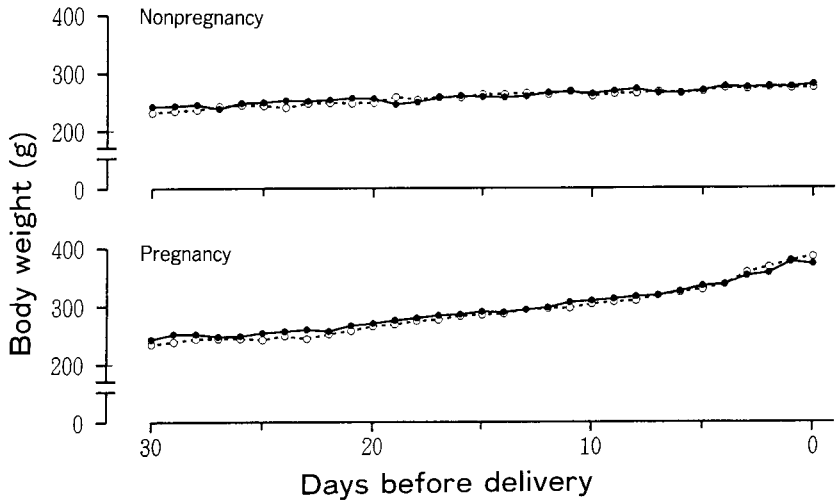


Fig. 1 Changes in body weight during pregnancy and nonpregnancy in phenobarbital (PB)-untreated and PB-treated rats. Each point indicates the mean value from 3 to 5 experiments. ○ PB-untreated, ● PB-treated

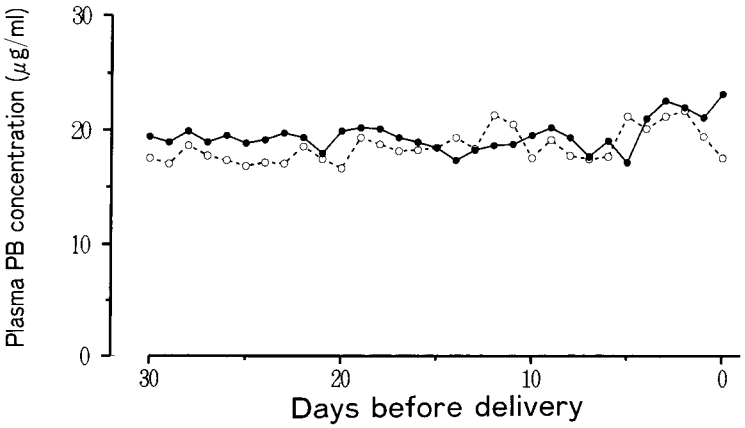


**Fig. 2** Effect of pregnancy on PB plasma concentrations in rats. Each point indicates the mean value. ○ PB-treated nonpregnant (n = 5), ● PB-treated pregnant (n = 4). PB: See Fig. 1.

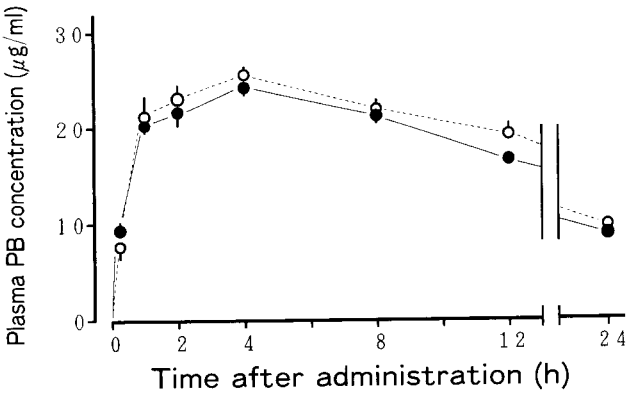


**Fig. 3** Effect of pregnancy on changes in plasma PB concentrations after oral administration in PB-untreated rats. Each point indicates the mean value. ○ control (n = 4), ● pregnant (n = 3). Vertical bar indicates the standard error of the mean value. PB: See Fig. 1.

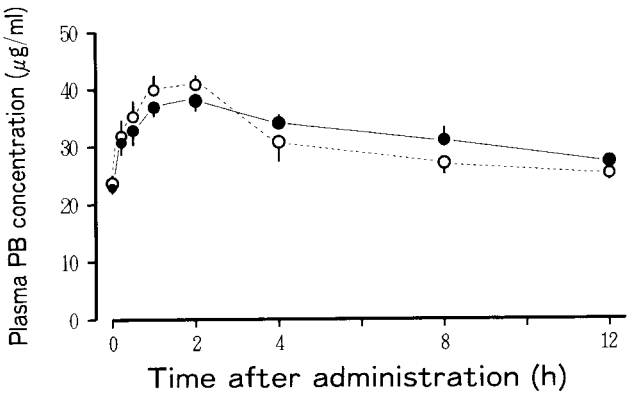


**Fig. 4** Effect of pregnancy on changes in plasma PB concentrations in PB-treated rats. Each point indicates the mean value. ○ PB-treated nonpregnant (n = 5), ● PB-treated pregnant (n = 4). Vertical bar indicates the standard error of the mean value. PB: See Fig. 1.

plasma PB concentrations in pregnant and nonpregnant rats chronically treated with PB. Plasma PB concentrations reached a steady-state 7 to 10 days after the start of treatment with 20 mg/kg of PB orally, twice a day. There was no significant variation in plasma PB concentrations during the 30 days before delivery in either pregnant or nonpregnant rats. There was no significant difference in plasma PB concentrations between pregnant and nonpregnant rats.

***Effect of pregnancy on plasma PB concentrations.*** Fig. 3 shows the effect of pregnancy on plasma PB concentrations after a single dose of PB in PB-untreated pregnant and nonpregnant groups. In nonpregnant and pregnant rats, plasma PB concentrations rapidly increased to reach a maximum of approximately 26.5 and 25.2 μg/ml at 3.0 and 2.4 h after the oral administration, respectively, and then gradually decreased. However, there was no significant difference between both groups. The pharmacokinetic parameters also showed no significant difference (Table 1).

In PB-treated nonpregnant and pregnant rats, plasma PB concentrations rapidly increased to reach a maximum

**Table I** Effect of pregnancy on pharmacokinetic parameters

| Experimental groups | Tmax (h) | | Cmax ($\mu$g/ml) | | $AUC_{0-12}$ ($\mu$g·h/ml) | | MRT (h) | |
|---|---|---|---|---|---|---|---|---|
| Control | 3.0 | *** | 26.5 | *** | 256.5 | * | 6.1 | ** |
| Pregnancy | 2.4 | | 25.2 | | 246.2 | | 5.9 | |
| PB | 1.4 | * | 41.9 | ** | 425.3 | ** | 4.1 | *** |
| PB + Pregnancy | 1.4 | | 38.7 | | 404.2 | | 4.9 | |

Each data indicates the mean value. control (n = 4), pregnancy (n = 3), phenobarbital (PB) (n = 5), PB + pregnancy (n = 4). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

of approximately 41.9 and 38.7 $\mu$g/ml at 1.4h after the oral administration, respectively, and then gradually decreased to the steady state concentration (Fig. 4). However, pregnancy did not significantly affect plasma PB concentrations or pharmacokinetic parameters at any time point examined (Table 1).

When the pharmacokinetic parameters in the PB-treated groups were compared with those in untreated groups, Tmax and MRT were significantly lower, and Cmax and $AUC_{0-12}$ were significantly higher in the PB-treated groups.

## Discussion

The data obtained in the present study showed that (a) chronic PB treatment does not affect the increase in body weight in pregnant or nonpregnant rats, (b) plasma PB concentrations during the period of chronic PB treatment is not affected by pregnancy, if the fixed dosage of PB per body weight is administered, and (c) both Tmax and MRT decrease with the duration of PB treatment in both pregnant and nonpregnant rats.

These results suggest that pregnancy does not influence the pharmacokinetics of chronic PB administration. The decreases in Tmax and MRT by long-term PB treatment may be due to enzyme induction. However, no significant influence by pregnancy on these parameters was noted.

Most pregnant women with epilepsy worry about the influence of antiepileptic drugs on the fetus. However, epilepsy itself carries a risk of fetal defects, and abrupt discontinuation of medication induces a definite risk of status epileptics and its associated hazards for the fetus and mother. It has been reported that the frequency of seizures tends to increase during late pregnancy (8). Although the precise reasons for this phenomenon are not clear, some possible factors, such as psychological changes, decrease in compliance with prescribed drugs regimen, changes in the pharmacokinetics of antiepileptic drugs, and hormonal and metabolic changes, have been considered.

Bardy *et al.* (1) reported the pharmacokinetics of PB before and during the pregnancy in 23 patients, but have not shown consistent results. A report by Battino *et al.* (9) indicated a tendency for plasma PB to decrease in 6 patients who received a constant dosage of PB. In the present study, when the fixed dose of PB by body weight was administered to pregnant rats, plasma PB concentrations were not different from those of nonpregnant rats, which suggests that an increase in the dosage of PB with increasing body weight can maintain steady-state plasma PB concentrations in pregnant women to prevent seizures.

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