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Investigation of the maternal to foetal serum concentration gradient of dexamethasone in the rat

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1 The basis of the maternal to foetal serum concentration gradient of dexamethasone was investigated in the rat on day 20 of gestation.

2 The pregnant rat was assumed to behave as a two-compartment open model with one maternal and one foetal pool exchanging with each other and each to outside.

3 Bolus injections of unlabelled dexamethasone were made into the mother and of [^{3}H]-dexamethasone into its foetuses and serial maternal and foetal blood samples were collected a number of times. The monitoring of both forms of the drug in each sample permitted the estimation by a non-linear least square approach of two placental and two non-placental clearances.

4 After both maternal and foetal injections of dexamethasone, its concentrations were higher in the maternal than in the foetal serum. Placental clearance of dexamethasone from the foetus to the mother was 824 ± 40 ml kg^{-1} h^{-1} and 8.5 ± 1 times greater than the placental clearance from the mother to foetus (103 ± 2 ml kg^{-1} h^{-1}). Foetal non-placental clearance was zero.

5 It is suggested that a maternal to foetal serum gradient of dexamethasone is caused by its active transfer from the foetal to maternal side.

Introduction

The placenta is not an absolute barrier to passage of most drugs (Ginsburg, 1971). Therefore the ratio of foetal to maternal blood levels of xenobiotics should attain a value of 1 or greater than 1 at some time (Dawes, 1973) unless some specific mechanism prevents such equilibration. Concentrations of several drugs, however, remain lower in the foetal than in the maternal circulation for long periods of time (see, Szeto, 1982). A persistent maternal to foetal gradient of a xenobiotic, which cannot be accounted for by differences in protein binding and ionization in the maternal and foetal blood is possible if the drug is eliminated from the foetus by non-placental routes (Szeto, 1982) or is actively eliminated by the foetus into the mother against a concentration gradient (Szeto *et al.*, 1978).

Foetal concentration of maternally administered dexamethasone remains significantly lower than its maternal concentration for long periods of time (Bayard *et al.*, 1972; Funkhouser *et al.*, 1978; Anderson *et al.*, 1979; Kream *et al.*, 1983; Mulay & Varma, 1984; Varma & Yue, 1984). Dexamethasone is highly non-polar. The binding of dexamethasone to maternal and foetal serum proteins does not differ significantly (Funkhouser *et al.*, 1978; Anderson *et al.*, 1979; Mulay & Varma, 1984; Varma & Yue, 1984) and it is not appreciably metabolized by the placenta or the foetus (Blanford & Murphy, 1977; Varma & Yue, 1984). These observations suggest that a maternal to foetal gradient of dexamethasone is not maintained by foetal non-placental elimination but could be achieved by its active elimination from the foetal to maternal side. The present studies were done to examine this possibility using a rat model which permitted the determination of maternal to foetal and foetal to maternal placental clearances as independent parameters (Varma & Ramakrishnan, 1985).

Methods

The details of the procedure used in these studies have been described previously (Varma & Ramakrishnan, 1985). Experiments were done on Sprague-Dawley rats (Charles River, St. Constant, Quebec, Canada) on day 20 of gestation and the presence of sperms in the vaginal washings was designated as day 0 of pregnancy. Animals had free access to laboratory rat chow and tap water. All surgical interventions including intravenous injections and blood collections were

done under brief periods (2–3 min) of ether anaesthesia. During the remaining duration of an experiment which lasted for 9 to 12 h, animals were conscious and moved freely inside the cages.

Determination of transplacental kinetics

The pregnant rat was assumed to behave as a two-compartment open model with one maternal and one foetal pool exchanging with each other and each to outside (metabolism and renal elimination in the case of the mother and metabolism and net loss into amniotic fluid in the case of its foetuses). In order to determine the two placental and non-placental clearances as independent parameters, unlabelled dexamethasone was injected into the mother and [^{3}H]-dexamethasone into its foetuses.

The drug mass transported between the pools or to outside as a function of time can be estimated by multiplying the drug concentration, C, by the clearance, CL (volume of serum totally cleared of the drug per unit time, expressed as ml h^{-1} kg^{-1} maternal body weight). Therefore maternal serum drug concentration (C_m) and foetal serum drug concentration (C_f) at any time can be described by Equations (1) and (2) (Varma & Ramakrishnan, 1985):

$$\frac{dC_m}{dt} = \frac{CL_{fm}C_f - CL_mC_m}{V_m} \quad (1)$$

$$\frac{dC_f}{dt} = \frac{CL_{mf}C_m - CL_fC_f}{V_f} \quad (2)$$

where CL_{fm} is the foetal placental clearance, CL_{mf} is the maternal placental clearance, CL_m is the sum of maternal placental and non-placental clearances, CL_f is the sum of foetal placental and non-placental clearances, V_m is the maternal volume of distribution, and V_f is the foetal volume of distribution.

It can be solved mathematically and has been shown to hold experimentally (Varma & Ramakrishnan, 1985) that:

$$\frac{CL_{fm}}{CL_{mf}} = \frac{C_m^{\,f}}{C_f^{\,m}} \quad \frac{\chi}{j} \quad (3)$$

where $C_m^{\,f}$ is the maternal concentration after foetal injection of dose j and $C_f^{\,m}$ is the foetal concentration after maternal injection of dose χ.

Experimental protocol

Because the maternal and foetal doses differed by nearly 1,000 fold, the model described above could only be used if serum concentrations increased proportionally to the dose. In order to test this, dexamethasone was injected intravenously into 4 non-pregnant rats at 1, 2,000, 8,000 and 16,000 nmol kg^{-1} at weekly intervals and blood samples were collected at 1, 3, 6, 9 and 12 h. Serum dexamethasone concentration at time zero, the area under the concentration-time curve as well as the volume of distribution, half-life and serum clearance were determined at each dose level.

In order to estimate transplacental kinetics of dexamethasone, native dexamethasone (2 μmol kg^{-1}) was injected into the tail vein of the mother. The maternal abdomen was opened by a longitudinal incision and [^{3}H]-dexamethasone (approximately 2 nmol kg^{-1} maternal body weight) was injected into the peritoneal cavities of all its foetuses (50 pmol into each foetus) through the uterine wall. Maternal and foetal blood samples were collected serially at 1, 3, 6 and 9 h. The maternal blood (0.1 to 0.2 ml) was collected from the tail artery. For the collection of the foetal blood, the maternal abdomen was again incised and three to four foetuses were removed starting from the ovarian end. The cut end of the uterus was ligated and the abdomen was closed. Foetuses were decapitated and blood was allowed to drip into 0.5 ml polyethylene tubes. Blood was left to clot and serum was separated by centrifugation.

Unlabelled dexamethasone was quantitated by radioimmunoassay (Varma & Mulay, 1980) and labelled dexamethasone by scintillation spectrometry. It was established in preliminary experiments that the radioactivity in the serum samples originating from foetal injections was infinitely small compared with unlabelled dexamethasone and did not interfere with radioimmunoassays and so no correction was needed. In other experiments, [^{3}H]-dexamethasone was injected intravenously into 3 non-pregnant rats and intrafoetally into 3 pregnant rats. Blood samples from the non-pregnant and pregnant animals were collected 3 h and 6 h after and from all the foetuses 3 h after the injections. The serum from these blood samples was first diluted in approximately 10 volumes of saline and then extracted in 5 ml methylene chloride. The methylene chloride extract was dried to a smaller volume under nitrogen and then chromatographed on a silica gel G plate using a benzene:acetone (7:3, v/v) solvent system. The radioactivity was monitored on a Packard scanner and the chromatographic pattern of native dexamethasone was identified under u.v. light. Greater than 97% of the radioactivity in serum samples appeared as single peaks corresponding to [^{3}H]- and native dexamethasone.

Desired parameters were estimated by fitting the solution of the model to the observed data using a non-linear least square approach with a computer programme which can solve a general pool model with multiple injections (Ramakrishnan *et al.*, 1984). The goodness-of-fit of these curves was indicated by a lack of

Table 1 Dose-serum concentration relationship of dexamethasone in non-pregnant female rats

Parameters	*Dexamethasone dose* (nmol kg^{-1})			
	1	*2,000*	*8,000*	*16,000*
C_o (pmol ml^{-1})	1.16 ± 0.05	2215 ± 50	10122 ± 327	19013 ± 460
AUC (arbitrary units)	0.27 ± 0.02	513 ± 24	2628 ± 241	4449 ± 264
V_d (ml kg^{-1})	865 ± 41	903 ± 19	791 ± 25	842 ± 20
$t_{\frac{1}{2}}$ (h)	3 ± 0.2	3 ± 0.1	2.7 ± 0.2	3 ± 0.1
Clearance (ml kg^{-1}h^{-1})	197 ± 18	208 ± 8	203 ± 7	197 ± 13

Data shown are means ± s.e., $n = 4$. The same 4 animals were used for different dose levels at weekly intervals.

significant deviations in plus and minus signs in relation to the curve on multiple runs and runs of the residuals and the lack of significant weighted residual errors. Also the model fitted the data better than simpler models and there was no improvement if the model was made more complex. The iterative process used by the computer programme terminated when no further improvement was observed in the last 3 iterations. It was assumed that the absorption from the foetal peritoneal cavities into the circulation was instantaneous and complete. We have previously observed (unpublished data) that, at least from 15 min after intravenous injection of dexamethasone into pregnant rats, the decline in plasma concentration is monoexponential and this had constituted the basis of the conventional pharmacokinetic analysis according to one-compartment open model (Varma & Yue, 1984; Mulay & Varma, 1984).

Differences between two means were compared by Student's t test and a probability of less than 0.05 was assumed to denote a significant difference. Data are presented as means ± s.e.

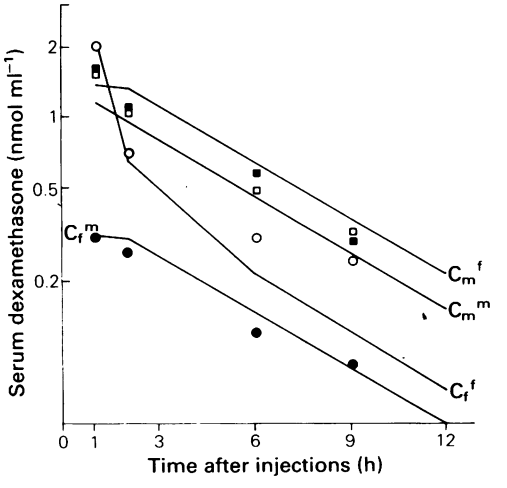

Figure 1 A plot of the maternal and foetal serum dexamethasone concentrations after injection into the mother of dexamethasone (2 μmol kg^{-1}) and into the foetuses [^{3}H]-dexamethasone (1.5 nmol kg^{-1} maternal body weight). C_m^f (■) – maternal concentration after foetal injection; C_m^m (□) – maternal concentrations after maternal injection; C_f^f (○) – foetal concentration after foetal injection; C_f^m (●) – foetal concentration after maternal injection. Concentrations after foetal injections have been multiplied by the ratio of the maternal to foetal dose.

Chemicals

Antisera against dexamethasone-3-carboxymethyl-oxime-bovine serum albumin conjugate was kindly provided by Dr S. Solomon, Endocrine Laboratory, Royal Victoria Hospital, Montreal, Canada; dexamethasone phosphate was a gift from Merck Frosst Laboratories, Dorval, Quebec, Canada. [6,7-^{3}H]-dexamethasone (50 Ci mmol^{-1}) was purchased from New England Nuclear, Boston, Massachusetts, U.S.A.

Results

Dose-concentration relationship

Concentrations of dexamethasone at time 0 and the area under the serum concentration-time curves after injections of 1, 2,000, 8,000 and 16,000 nmol kg^{-1} dexamethasone were proportional to the dose whereas the volume of distribution, serum half-life and clearance values were not dose-dependent (Table 1).

Transplacental kinetics

The concentration of dexamethasone was higher in the maternal serum than in the foetal serum from 1 to 9 h after maternal as well as foetal injections. Consistent with the mathematical solution of the model (Varma & Ramakrishnan, 1985), the relationship between the maternal concentration of dexamethasone after foetal injection to foetal concentration following maternal injection remained relatively constant during the course of an experiment. Figure 1 depicts the com-

Table 2 Maternal and foetal serum dexamethasone concentrations after simultaneous maternal and foetal injections on day 20 of gestation in rats

Parameters	*Time* (h)			
	1	*3*	*6*	*9*
C_m^m (pmol ml^{-1})	1603 ± 118	1141 ± 103	534 ± 45	302 ± 29
C_f^m (pmol ml^{-1})	271 ± 19	234 ± 23	116 ± 11	82 ± 8
C_m^f (pmol ml^{-1})	2101 ± 90	1575 ± 51	841 ± 58	534 ± 42
C_f^f (pmol ml^{-1})	1953 ± 302	836 ± 78	596 ± 64	357 ± 13
C_m^f/C_f^m	7.9 ± 0.5	7.0 ± 0.6	7.4 ± 0.7	7.2 ± 1.2

Data shown are means ± s.e., $n = 7$. Abbreviations: C_m^m – maternal concentration after maternal injection; C_f^m – foetal concentration after maternal injection; C_m^f – maternal concentration after foetal injection; C_f^f – foetal concentration after foetal injection. Concentrations in the table after foetal injections are the observed concentrations times the ratio of maternal to foetal dose (approximately 1,000 fold). The maternal dose of dexamethasone was 2 μmol kg^{-1} and the foetal dose of [^{3}H]-dexamethasone was approximately 2 nmol kg^{-1} maternal body weight.

puter plot of one experiment which yielded values closer to mean values for all the experiments. The data from all the animals and the manually calculated values are presented in Table 2.

The placental clearance of dexamethasone from the foetal to maternal side was greater than that from the maternal to foetal side and the mean ratio of the former to the latter based on non-linear regression was 8.5 ± 1. The non-placental foetal clearance was zero (Table 3).

Table 3 Transplacental kinetics of dexamethasone in rats on day 20 of gestation

Parameters	
Maternal V_m (ml kg^{-1})	1577 ± 148
Foetal V_f (ml kg^{-1})	178 ± 16*
CL_{mf} (ml kg^{-1}h^{-1})	103 ± 2
CL_{fm} (ml kg^{-1}h^{-1})	824 ± 40*
CL_{mo} (ml kg^{-1}h^{-1})	434 ± 65
CL_{fo} (ml kg^{-1}h^{-1})	0
CL_{fm}/CL_{mf}	8.5 ± 1

Data shown are means ± s.e., $n = 7$. Abbreviations: CL_{mf} – placental clearance from mother to foetus; CL_{fm} – placental clearance from foetus to mother; CL_{mo} – maternal non-placental clearance; CL_{fo} – foetal non-placental clearance. Volumes of distribution (V_m and V_f) and clearances are in terms of maternal body weights. Doses of unlabelled dexamethasone into the mother and of [^{3}H]-dexamethasone into its foetuses were 2 μmol kg^{-1} and approximately 2 nmol kg^{-1} maternal body weight, respectively. *Significantly ($P < 0.05$) different from the immediate top value.

Discussion

The main purpose of these studies was to determine if a maternal to foetal gradient of dexamethasone is maintained by its active transfer across the placenta from the foetal to maternal side against a concentration gradient. It has been previously shown that despite the invasive experimental protocol, the model yields reproducible data (Varma & Ramakrishnan, 1985). No animal died during the course of these studies. Moreover, relative maternal and foetal serum dexamethasone levels determined in these studies are comparable to values when blood samples were collected only once from each animals (Mulay & Varma, 1984; Varma & Yue, 1984) as well as to those of Funkhouser *et al.* (1978) who collected serial maternal and foetal blood samples under prolonged anaesthesia. Dexamethasone accounted for more than 97% of the total radioactivity in the serum and we have previously shown (Varma & Yue, 1984) that its concentration, based on the specific activity, is comparable to that determined by radioimmunoassay. Thus the computation based on concentrations of dexamethasone estimated on the basis of the radioactivity and radioimmunoassay in both the maternal and foetal serum is reasonable. However, the lack of sufficient data points especially during the first hour after the injections conferred definite constraints on fitting the data so that the computation of absolute clearance values may not be as accurate as the ratios of foetal to maternal placental clearances.

The existence of a maternal to foetal gradient for dexamethasone, concurrent with its greater placental clearance from the foetal to maternal side than in the reverse direction, satisfies the kinetic criterion of active biological transport according to which the ratio of fluxes and the ratio of solute concentrations in two compartments separated by biologoical membranes must not be equal (Christensen, 1975). The existence

of a maternal to foetal gradient of dexamethasone in the absence of its non-placental foetal elimination again suggests its active placental elimination (Szeto *et al.*, 1978). The persistence of a lower concentration of dexamethasone in the foetal than in the maternal blood has been interpreted by other workers (Funkhouser *et al.*, 1978) to imply its active placental elimination. Greater placental clearances of dexamethasone (Bayard *et al.*, 1972) and triamterene (McNay & Dayton, 1970) from the foetal to maternal than from the maternal to foetal side has also been demonstrated in the sheep.

On the other hand, a maternal to foetal gradient of dexamethasone has been attributed to placental diffusion limitation (Anderson *et al.*, 1979). However, the derivation of the placental diffusion *a priori* excludes the existence of any active transport and assumes that placental clearances in both directions are equal but does not establish this by measuring these as independent parameters. In contrast, we measured placental clearances in both directions as independent parameters by adapting for bolus injection the technique developed for steady-state conditions (Szeto, 1982).

An important limitation of this study is that the associated features of an active biological transport process, namely, the energy-dependence, saturability and competitive inhibition were not characterized. Although the energy-dependence may not be possible to demonstrate under *in vivo* conditions, it will be of interest to study if a significant increase in the dose of dexamethasone and concomitant administration of other synthetic glucocorticoids will decrease the ratio of foetal to maternal placental clearance indicating respectively, saturability and competitive inhibition of the transport process. It may be pointed out, however, that the mere existence of a foetal to maternal gradient, opposite to that found for dexamethasone, has been treated as sufficient evidence for the active placental transfer of many essential nutrients from the maternal to foetal side (Faber & Thornburg, 1983).

In conclusion, the present study provides evidence of an active placental elimination of dexamethasone from the foetal to maternal side in the rat. Clinical reports of lower concentrations of dexamethasone (Kream *et al.*, 1983), methylprednisolone (Anderson *et al.*, 1981), betamethasone (Ballard *et al.*, 1975) and certain antibiotics (see, Duignan *et al.*, 1973) in the cord than in the maternal blood could also be indicative of a similar active placental transfer mechanism, provided these concentration differences are not reflections of relatively lower protein binding in the foetal blood. If an active placental transfer mechanism does exist in humans, it can be a potential site of interaction between certain types of drugs.

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