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Influence of streptozotocin-diabetes on the pharmacokinetics, placental transfer and tissue localization of dexamethasone in rats

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1 The influence of streptozotocin-diabetes on the pharmacokinetics, placental transfer and tissue localization of dexamethasone was determined in Sprague-Dawley rats.

2 Diabetes significantly increased the volume of distribution of dexamethasone in pregnant but not in nonpregnant rats; plasma half-life was not significantly increased.

3 Concentrations of maternally administered dexamethasone in all tissues studied (maternal and foetal serum and livers, foetal lungs and placentas) except the amniotic fluid were lower in diabetic than in control animals.

4 Diabetes did not alter the binding of dexamethasone to maternal or foetal serum proteins.

5 Insulin treatment partially reversed the effects of diabetes on the maternal-foetal exchange of dexamethasone.

6 Diabetes-induced decrease in the foetal localization of dexamethasone appears to be caused by a decrease in the maternal serum levels as well as by an increase in the foetal excretion of the steroid into the amniotic fluid.

7 In so far as the rat model reflects the human situation, the present data suggest that a standard dose of dexamethasone might not be adequate in promoting foetal lung maturation in diabetic pregnancies.

Introduction

Glucocorticoids are used for the prevention of respiratory distress syndrome (RDS) (Liggins & Howie, 1972) because of their ability to promote foetal lung maturation (DeLemos *et al.*, 1970). Dexamethasone is frequently used for this purpose (Collaborative Group on Antenatal Steroid Therapy, 1981). Diabetic pregnancy is associated with a relatively high incidence of RDS (Robert *et al.*, 1976). Antenatal administration of glucocorticoids can be toxic to mother as well as the foetus (Depp *et al.*, 1980). It can be expected that any changes in the pharmacokinetics and maternal-foetal exchange of dexamethasone during diabetes will modify both its therapeutic and toxic effects. However, as far as we know, the influence of diabetes on the transplacental passage and foetal tissue localization of dexamethasone has not been reported. The present study deals with the pharmacokinetics, transplacental passage and maternal as well as foetal tissue localization of dexamethasone in streptozotocin-diabetic rats as the experimental model. The results show that in contrast to the effects of protein-calorie malnutrition presented in the previous publication (Varma & Yue, 1984), diabetes led to a significant decrease in the concentration of dexamethasone in foetal serum, liver and lung.

Methods

Experiments were performed on Sprague-Dawley nonpregnant and pregnant (day 20 of gestation) rats of comparable ages (14–16 weeks old) according to the protocol described in the previous paper (Varma & Yue, 1984); only additional techniques are described below in any detail.

Diabetes in nonpregnant (3 weeks prior to the study) and pregnant (day 1–2 of gestation) rats was induced by intravenous injections of 150 μmol kg^{-1} streptozotocin dissolved freshly in saline-citrate buf-

fer; controls were injected with the buffer (Mulay & Solomon, 1983). Animals had free access to tap water and a normal Purina rat chow instead of the semi-synthetic diet used in the previous study (Varma & Yue, 1984). Urine glucose was measured daily with glucose labsticks (Billilabstix, Miles Laboratory, Rexdale, Ontario, Canada); 0.1 ml blood was collected between 08 h 30 min–09 h 30 min on day 20 of gestation before the start of the experiments for the determination of blood glucose on a Beckman Autoanalyser according to a glucose oxidase method (Kadish *et al.*, 1968). Animals which did not become glucosuric within 48 h following the injection of streptozotocin were excluded from this study. A group of 6 pregnant glucosuric rats were treated with protamine zinc insulin (8 u kg^{-1} once a day between 16 h 00 min–17 h 00 min, s.c.) from day 12 to day 19 of gestation; 2 of the 6 insulin-treated rats remained glucosuric and blood glucose on day 20 of gestation was 26.4 and 31.2 mmol l^{-1}; these 2 animals were excluded from the study. As in the previous study (Varma & Yue, 1984), blood and tissue collections as well as surgical interventions were all done under ether anaesthesia.

Pharmacokinetics of dexamethasone in nonpregnant and pregnant (day 20 of gestation) rats as well as its distribution into different maternal and foetal tissues were determined at 4 μmol kg^{-1} dose level as described previously (Varma & Yue, 1984); however, in these studies the total dose was administered as [^{3}H]-dexamethasone with the required amount of carrier dexamethasone phosphate. In order to determine subcellular distribution, [^{3}H]-dexamethasone (4 nmol kg^{-1}) was injected intravenously; one hour later, maternal and foetal blood and livers, foetal lungs and placentas were removed and nuclear, cytoplasmic and microsomal fractions were prepared as described elsewhere (Varma *et al.*, 1982). Concentrations of dexamethasone in serum and tissues were determined on the basis of the specific activity. The efficiency of the scintillation counter (Intertechnique) for tritium was approximately 30%. Values of dexamethasone determined on the basis of radioactivity were similar to those found with radioimmunoassay (Varma & Yue, 1984).

Serum protein-[^{3}H]-dexamethasone binding was determined by equilibrium dialysis as described previously (Varma & Yue, 1984). Proteins were measured according to Lowry *et al.* (1951). Differences within groups were estimated by an analysis of variance and between two means by Student's *t* test for unpaired data; a probability of less than 0.05 was assumed to denote a significant difference.

Drugs and chemicals

The following agents were purchased: dexamethasone and streptozotocin (Sigma Chemical

Table 1 General effects of streptozotocin-diabetes in pregnant rats on day 20 of gestation.

Parameters	*Control* (a) $n=8-37$	*Diabetic* (b) $n=8-32$	*Diabetic-treated* (c) $n=4$	$P<0.05$
Maternal				
Initial b.wt. (g)	210±4	206±3	205±3	NS
B.wt. increase (%)	56±2	47±4	57±4	a, b; b, c
Serum proteins (g dl^{-1})	7.9±0.3	6.9±0.5	6.9±0.3	NS
Liver weight (% b.wt.)	3.9±0.1	4.4±0.1	4.7±0.1	a, b; a, c
Liver proteins (mg g^{-1})	198±5	233±9	179±5	a, b; b, c
Blood glucose (mmol l^{-1})	4.9±0.2	27.7±0.9	6.1±0.5	a, b; b, c
Foetal				
B.wt. (g)	3.7±0.1	3.0±0.1	3.4±0.1	a, b; b, c
Serum proteins (g dl^{-1})	3.6±0.2	3.1±0.3	3.4±0.1	NS
Liver weight (mg)	294±8	235±6	293±12	a, b; b, c
Liver proteins (mg g^{-1})	134±3	129±4	127±6	NS
Lung weight (mg)	125±4	118±2	130±9	NS
Lung proteins (mg g^{-1})	100±3	86±5	89±1.4	a, b; a, c
Blood glucose (mmol l^{-1})	1.8±0.1	21.1±0.1	not done	a, b
Placental weight (mg)	458±10	510±14	485±42	a, b
Placental proteins (mg g^{-1})	125±5	106±8	118±6	NS
Amniotic fluid (μl)	166±15	355±12	262±37	a, b; a, c; b, c
Litter size (*n*)	11.4±0.5	10.6±0.5	12±0.5	NS

Means ± s.e.

B.wt. = Body weight; NS = not significant. Group (c) received protamine zinc insulin (8 u kg^{-1} day^{-1}, s.c.) from day 12–19 of gestation; dose of streptozotocin was 150 μmol kg^{-1}, i.v.

Table 2 Influence of streptozotocin-diabetes on the pharmacokinetics of dexamethasone in nonpregnant and pregnant (day 20 of gestation) rats

Animals	n	$t_{1/2}$ (h)	V_d (ml kg^{-1})	Cl_p (ml kg^{-1}h^{-1})
Nonpregnant				
Control	9	2.8±0.3	641±49	171±22
Diabetic	8	3.8±0.3	908±50	171±13
Pregnant				
Control	8	3.4±0.5	839±92	172±10
Diabetic	7	3.7±0.5	1029±104*	202±7

Means ± s.e.
V_d = apparent volume of distribution: Cl_p = plasma clearance; dose of dexamethasone was 4 μmol kg^{-1}, i.v.
*Different ($P<0.005$) from control nonpregnant value.

Co., St. Louis, Missouri, U.S.A.); [^{3}H]-dexamethasone (35–50 Ci mmol^{-1}, New England Nuclear, Boston, Massachusetts, U.S.A.); protamine zinc insulin (Connaught Laboratory, Toronto, Ontario, Canada).

Results

General effects of diabetes and insulin treatment (Table 1)

A single intravenous injection of streptozotocin (150 μmol kg^{-1}) caused glucosuria in most animals within 48 h; animals developed polyuria and polydipsia and there was a decrease in maternal body weight gain and liver proteins as well as in foetal body weights, liver weights and lung proteins. Maternal liver weights, maternal and foetal blood glucose levels, placental weights and amniotic fluid volumes were higher in diabetic than in control rats. Injections of protamine zinc insulin (8 u kg^{-1}) once a day from day 12 through day 19 of gestation partially reversed the effects of diabetes.

Influence of diabetes on dexamethasone pharmacokinetics (Table 2)

Both diabetes and pregnancy as independent variables tended to increase the half-life and volume of distribution of dexamethasone; however, these changes were not significant. When both these variables (diabetes and pregnancy) were present concurrently, the volume of distribution of dexamethasone was significantly larger than that in control nonpregnant rats.

Table 3 Influence of streptozotocin-diabetes in rats on the distribution of dexamethasone into maternal (M) and foetal (F) serum and tissues at different time-periods following its injection (4 μmol kg^{-1}, i.v.) on day 20 of gestation

Tissues	3 h			6 h		12 h	
	Control	*Diabetic*	*Diabetic-treated*	*Control*	*Diabetic*	*Control*	*Diabetic*
	(pmol ml^{-1} or pmol g^{-1} wet tissue)						
M serum	2864±275	1777±97*	2192±63	1388±163	1036±64	749±24	485±51*
F serum	570±53	358±57*	451±23	294±29	202±7*	210±11	124±8*
M liver	20853±1652	2267±1810	16067±608	15684±1023	9307±960*	6496±504	3761±399*
F liver	1520±105	927±139*	1260±21	765±86	536±36*	474±10	291±19*
F lung	1377±164	780±93*	785±39*	548±26	411±32*	342±13	238±13*
Placenta	3437±489	2047±276*	2265±183	1400±278	971±35	739±59	482±6*
Amniotic fluid	1387±123	1240±471	1265±177	799±177	1194±261	353±13	387±96

Means ± s.e. ($n=4–12$).
Diabetic-treated group received protamine zinc insulin (8 u kg^{-1} day^{-1}, s.c.) from day 12 through day 19 of gestation.
*Different ($P<0.05$) from the control value at equivalent time period.

Influence of diabetes and insulin treatment on placental transfer of dexamethasone (Table 3)

Following maternal administration, dexamethasone levels in all tissues (maternal and foetal serum and livers, foetal lungs and placentas) with the exception of the amniotic fluid were lower in diabetic than in control animals. Treatment with insulin partially reversed the effects of diabetes on the distribution of dexamethasone into all tissues except the foetal lungs. Despite differences in the absolute concentrations, ratios of foetal to maternal serum dexamethasone concentrations did not differ between control, untreated and insulin-treated diabetic rats.

Influence of diabetes on subcellular distribution of dexamethasone

With the exception of maternal liver cytosol and foetal lung microsomal fractions, concentrations of dexamethasone in different fractions (cytosol, nuclear and microsomal) of maternal and foetal livers and foetal lungs of diabetic rats were significantly lower than those in control tissue fractions 1 h following intravenous injections of [^{3}H]-dexamethasone, 4 nmol kg^{-1}.

Influence of diabetes on serum protein-dexamethasone binding

The binding of [^{3}H]-dexamethasone to any serum sample at three different concentrations studied (0.62, 10.8 and 102.5 nM) did not differ. Studies with 4–6 separate serum samples yielded the following results (mean ± s.e. % bound): control maternal serum 85 ± 1.7, control foetal serum 83 ± 0.9, diabetic maternal serum 80 ± 1.2 and diabetic foetal serum 79 ± 1.3. There was no difference in the binding to maternal and foetal serum within the control or diabetic rats or between the maternal serum from the two groups of animals. However, because dexamethasone binding did not differ up to 2.5 μM concentration, results do not reflect saturable binding.

Discussion

The main objective of this study was to determine the effects of diabetes on the pharmacokinetics, placental transfer and tissue localization of dexamethasone. Streptozotocin-diabetes in rats has been viewed as an appropriate model for studies on foetal and placental development (Farrell *et al.*, 1982). Effects of a moderate dose of streptozotocin used in this study on the mother, foetus and placenta (Table 1) are similar to those reported elsewhere (Mulay & Solomon, 1983). Unlike the previous study (Varma & Yue, 1984), animals in this study were fed a normal laboratory chow instead of a semi-synthetic diet of comparable composition and dexamethasone was quantitated on the basis of radioactivity rather than by radioimmunoassay; these two differences did not appreciably alter the results.

The observed increase in the volume of distribution and a lack of a significant increase in the half-life of dexamethasone in pregnant diabetic rats is in accordance with the reported increase in the volume of distribution of insulin (Navalesi *et al.*, 1978; Kobayashi *et al.*, 1983) and no change in the plasma half-life of tolbutamide (Ueda *et al.*, 1963) in diabetic patients. The exact mechanism for the increase in the volume of distribution of dexamethasone in pregnant diabetic rats is not clear from our studies; it could have been caused by various pregnancy associated physiological changes (Krauer *et al.*, 1980) and diabetic pregnancy associated metabolic disturbance (Pedersen, 1977).

A significant finding of this study was that maternally administered dexamethasone produced significantly lower drug concentrations in the foetal tissues (serum, liver and lung homogenates as well as cytoplasmic and nuclear fractions) of diabetic than of control animals (Table 3). This cannot be attributed to any differences in maternal or foetal serum protein-dexamethasone binding. Streptozotocin-diabetes in rats causes a decrease in maternal and foetal plasma corticosterone levels and either no change or an increase in foetal lung and liver cytoplasmic glucocorticoid receptor levels (Mulay & Solomon, 1983); if anything, both these effects of diabetes will tend to increase rather than decrease the localization of dexamethasone in foetal tissues.

Two observations of this study might explain the observed decrease in the foetal localization of dexamethasone in diabetic rats. First, a low maternal serum dexamethasone level consequent to an increase in its volume of distribution can lead to a low foetal serum concentration of the steroid provided the kinetics of its equilibration in the maternal and foetal serum remain unchanged, which is indicated by similar foetal serum to maternal serum dexamethasone ratios in the two groups of rats. This is different from the effects of protein-calorie malnutrition, which increased ratios of foetal to maternal serum dexamethasone (Varma & Yue, 1984) and salicylate (Varma & Yue, 1983).

A second factor which could have contributed to relatively low foetal dexamethasone levels in diabetic rats is the greater excretion of the drug into the amniotic fluid; this is indicated by the observation that the volume of amniotic fluid in diabetic animals was approximately twice as much as in control rats (Table 1) and yet dexamethasone concentrations in the amniotic fluid of diabetic rats were equal to or

higher than those in controls (Table 3). Because maternally ingested xenobiotics find access into the amniotic fluid mainly via the foetus (Seeds, 1981), a greater net amount of dexamethasone must have been eliminated by the foetuses of diabetic than of control rats. If this is a valid explanation, one could expect that concentration of any drug which is eliminated by glomerular filtration will be relatively low in foetuses of diabetic mothers because hydramnios consequent to foetal hyperglycemia and glucosuria is a common feature of diabetic pregnancy (Pedersen, 1977).

We are not aware of any studies on the influence of insulin treatment on the placental transfer of drugs in Diabetes-induced changes in drug metabolism in rats can be reversed by insulin treatment (Dixon *et al.*, 1963; Reinke *et al.*, 1978). The present data show that effects of diabetes on the maternal-foetal exchange of dexamethasone can also be reversed by insulin. Because these effects of insulin were accompanied by a decrease in the amniotic fluid volume, the data lend support to the argument advanced above that a decrease in dexamethasone levels of diabetic foetuses was partly caused by increased urinary elimination of the steroid.

The observed decrease in foetal tissue including lung concentration of dexamethasone in diabetic rats might be of practical clinical significance. The reported ineffectiveness of antenatal dexamethasone in promoting incorporation of choline into foetal lung phospholipids of diabetic rats (Tsai *et al.*, 1981) could have been partly caused by a decreased localization of the hormone into lung tissue. An extrapolation of the present data to the human situation would imply that a standard dose of dexamethasone might not be adequate in promoting foetal lung maturation in diabetic pregnancy.

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