SERUM PROTEIN BINDING OF DIAZEPAM IN MATERNAL AND FOETAL SERUM DURING PREGNANCY

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- 1 The serum binding capacity for diazepam was significantly lower in pregnancy and there was a linear correlation with gestational age.
- 2 The binding of diazepam was not correlated to albumin during pregnancy.
- 3 In cord sera there was a significantly reduced binding capacity for diazepam with albumin levels of less than 40 g/l.

Introduction

Diazepam is one of the most commonly prescribed drugs in the management of pre-eclampsia and eclampsia. A number of studies have indicated that it can rapidly cross the placenta (Mandelli *et al.*, 1975; Mirkin & Singh, 1976; Haram & Bakke, 1980) and reach steady-state concentrations in the foetus similar to (Cree *et al.*, 1973) or higher (Gamble *et al.*, 1977) than in the mother. The drug may persist in the circulation of the neonate for up to 1 week (Gamble *et al.*, 1977). In non-pregnant women, circulating diazepam is largely bound to albumin (Klotz *et al.*, 1975; Kaplan, 1980) and is metabolised in the liver (Klotz *et al.*, 1976).

Normal physiological changes in pregnancy can modify the pharmacokinetics of some drugs (Krauer & Krauer, 1977), as reflected by an alteration of circulating levels (Eadie *et al.*, 1977). Among these changes, the fall in serum albumin (Hyttén & Leitch, 1971) which occurs during pregnancy is likely to affect the distribution of diazepam by reducing the bound fraction in blood. In the present study we have used an ultrafiltration method to examine the protein binding of diazepam in maternal and foetal serum during pregnancy.

Methods

Serum samples from non-pregnant women (15 subjects), normal women between 13 and 40 weeks of pregnancy (54 subjects), and umbilical cord blood from normal term neonates (15 subjects) were collected and stored at -20°C until assay. None of the subjects were taking diazepam and the pregnant women received iron and vitamin supplements only. Diazepam binding to serum protein was measured by ultrafiltration (Monks *et al.*, 1978). Briefly [^{14}C]-diazepam (approximately $5000\text{ d min}^{-1}\text{ ml}^{-1}$, radiochemical purity greater than 97%; Radiochemical Centre, Amersham, UK) and unlabelled diazepam (Hoffman-La Roche Company, UK) were added to each sample to give a concentration of about 300 ng/ml (approximately therapeutic concentrations).

Following incubation for 30 min at room temperature, 2 ml of serum was placed in a vialing dialysis tube (6 mm diameter). After centrifugation for 8 min at 900 g/min to remove excess water, these bags were centrifuged again for a further 30 min. The volume of this ultrafiltrate was about 150 μl . The binding of all samples was measured in duplicate. The within-assay coefficient of variation was 2%.

Results

The binding capacity of serum for diazepam was lower in the second and third trimesters of pregnancy than in non-pregnant women (Table 1) and seemed to have a linear relationship with the stage of gestation (Figure 1). In the umbilical cord blood, binding of diazepam was similar to that in the mother (Table 1). Serum albumin was reduced throughout pregnancy but did not follow a linear relation to the binding

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Table 1 Free diazepam fraction and serum albumin concentration in sera of pregnancy and non-pregnant women, and umbilical cord blood

Group	Weeks of gestation	Number of cases	Free diazepam fraction (%)* (Mean $\pm$ s.d.)	Albumin (g/l)** (Mean $\pm$ s.d.)
Normal non-pregnant women		15	2.15 $\pm$ 0.29	44.2 $\pm$ 3.58
Pregnant women	13-20	21	2.34 $\pm$ 0.21	40.6 $\pm$ 2.74
	21-34	16	2.58 $\pm$ 0.41	38.4 $\pm$ 4.48
	>35	17	3.00 $\pm$ 0.46	39.7 $\pm$ 3.42
Umbilical cord blood		15	2.72 $\pm$ 0.57	39.9 $\pm$ 4.49

* (1) Analysis of variance of overall differences between the various groups: $P < 0.005$

(2) Miller (sequential) range tests: $P < 0.05$ for 3 means apart, $P < 0.01$ for 4 or 5 means apart

** (1) Analysis of variance of overall differences between the various groups: $P < 0.005$

(2) Tukey's test: $P < 0.05$

Figure 1 The correlation of diazepam free fractions and gestational age in pregnancy ($y = 1.86 + 0.03x$, $r = 0.58$, $P < 0.001$).

Figure 2 The correlation of diazepam free fractions and maternal serum albumin concentrations during pregnancy ($r = 0.04$, not significant).

capacity for diazepam (Figure 2). However, there was a significant negative correlation between albumin concentration and the free fraction of diazepam in cord blood (Figure 3) and binding capacity for diazepam was significantly reduced in cord sera with albumin levels less than 40 g/l (Figure 4).

Discussion

The measurement of free drug concentration is a more direct measurement of the therapeutically active form than the total level. Since the elimination rate for diazepam is poor or restricted, factors which can increase the free fraction in serum will usually result in a corresponding increase in total clearance (Sellers, 1979).

Thus, alterations of drug binding to plasma proteins may change the intensity and duration of the pharmacological effect of the drug (Levy, 1976). Decreased protein binding of diazepam may be caused by: (1) sex difference or age (Abel *et al.*, 1979;

Figure 3 The correlation of diazepam free fractions and serum albumin concentrations in umbilical cord blood ($y = 5.24 - 0.06x$, $r = -0.534$, $P < 0.05$).

Figure 4 The relation between diazepam free fractions and serum albumin concentrations in umbilical cord blood (the bar indicates mean, Student's *t*-test: $t = 3.53$, $P < 0.01$).

Routledge *et al.*, 1981; Macleod *et al.*, 1979); (2) hypo-albuminaemia due to liver cirrhosis (Andreasen *et al.*, 1976); (3) renal insufficiency (Reidenberg, 1977; Greenblatt *et al.*, 1978); (4) the presence of

other drugs such as tryptophan (Muller & Wollert, 1975); and (5) an increase of endogenous inhibitors of diazepam binding such as some fatty acids (i.e. oleic acid and palmitic acid) (Wong & Sellers, 1979) which are known to be increased during pregnancy (Rebound *et al.*, 1963).

In the present study diazepam binding capacity decreased from the second to the third trimester of pregnancy, while the albumin concentration itself remained unchanged; there was no obvious correlation between the serum albumin and binding capacity of diazepam. In contrast, another group reported that a correlation existed (Dean *et al.*, 1980). The similarity in protein binding capacity of diazepam between the mother near term and the newborn is consistent with the observations of Krasner & Yaffe (1975). Kanto and his colleagues, however, reported that there was significantly higher binding of diazepam in maternal than in cord plasma (Kanto *et al.*, 1973). The positive correlation between cord serum albumin levels and binding capacity of diazepam, in the absence of a similar correlation in the mother, suggests that there is a different mechanism of binding in the foetus. For example, other and as yet unspecified binding proteins or inhibitors may be involved in the material compartment.

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References

- ABEL, J.G., SELLERS, E.M., NARANJO, C.A., SHAW, J., KADAR, D. & ROMACH, M.K. (1979). Inter- and intra-subject variation in diazepam free fraction. *Clin. Pharmac. Ther.*, **26**, 247-255.
- ANDREASEN, P.B., HENDEL, J., FREISEN, G. & HVIDBERG, E.G. (1976). Pharmacokinetics of diazepam in disordered liver function. *Eur. J. clin. Pharmac.*, **10**, 115-120.
- CREE, J.E., MEYER, J. & HAILEY, D.M. (1973). Diazepam in labour: its metabolism and effect on the clinical condition and thermogenesis of the newborn. *Br. med. J.*, **4**, 251-255.
- DEAN, M., STOCK, B., PATTERSON, R.J. & LEVY, G. (1980). Serum protein binding of drugs during and after pregnancy in humans. *Clin. Pharmac. Ther.*, **28**, 253-261.
- EADIE, M.J., LANDER, C.M. & TYRER, J.H. (1977). Plasma drug level monitoring in pregnancy. *Clin. Pharmacokin.*, **2**, 427-436.
- GAMBLE, J.A.S., MOORE, J., LAMKI, H. & HOWARD, P.J. (1977). A study of plasma diazepam levels in mother and infant. *Br. J. Obstet. Gynaecol.*, **84**, 588-591.
- GREENBLATT, D.J., HARMATZ, J.S. & SHADER, R.I. (1978). Sex differences in diazepam protein binding in patients with renal insufficiency. *Pharmacology*, **16**, 26-29.
- HARAM, K. & BAKKE, O.M. (1980). Diazepam as an induction agent for Caesarean section: A clinical and pharmacokinetic study of fetal drug exposure. *Br. J. Obstet. Gynaecol.*, **87**, 506-512.
- HYTTEN, F.E. & LEITCH, I. (1971). *The physiology of human pregnancy*. 2nd edition. Oxford: Blackwell Scientific Publications.
- KANTO, J., ERKKOLA, R. & SELLMAN, R. (1973). Accumulation of diazepam and *N*-desmethyldiazepam in the fetal blood during labour. *Ann. Clin. Res.*, **5**, 375-379.
- KAPLAN, S.A. (1980). Pharmacokinetics of Benzodiazepines. In *Benzodiazepines Today and Tomorrow*, eds Priest, R.G., Vianna Filho, U., Amrein, R. & Skreta, M., pp. 47-60. Lancaster: MTP Press Limited.
- KLOTZ, U., ANTONIN, K.H. & BEICK, P.R. (1976). Pharmacokinetics and plasma binding of diazepam in man, dog, rabbit, guinea pig and rat. *J. Pharmac. exp. Ther.*, **199**, 67-73.
- KLOTZ, U., AVANT, G.R., HOYUMPA, A., SCHENKER, S. & WILKINSON, G.R. (1975). The effects of age and liver disease on the disposition and elimination of diazepam in adult man. *J. clin. Invest.*, **55**, 347-359.
- KRASNER, J. & YAFFE, S.J. (1975). In *Basic and Therapeutic Aspects of Perinatal Pharmacology*, eds Morselli, P.L., Grattini, S. & Sereni, F., p. 357. New York: Raven Press.

- KRAUER, B. & KRAUER, F. (1977). Drug kinetics in pregnancy. *Clin. Pharmacokin.*, **2**, 167-181.
- LEVY, G. (1976). Effect of plasma protein binding of drugs on duration and intensity of pharmacologic activity. *J. pharm. Sci.*, **65**, 1264-1265.
- MACLEOD, S.M., GILES, H.G., BENGERT, B., LIU, F.F. & SELLERS, E.M. (1979). Age and gender-related differences in diazepam pharmacokinetics. *J. clin. Pharmac.*, **19**, 15-19.
- MANDELLI, M., MÖRSELLI, P.L., NORDIO, S., PARDI, G., PRINCIPI, N., SERENI, F. & TOGNONI, G. (1975). Placental transfer of diazepam and its disposition in the newborn. *Clin. Pharmac. Ther.*, **17**, 564-572.
- MIRKIN, B.L. & SINGH, S. (1976). Placental transfer of pharmacologically active molecules. In *Perinatal Pharmacology and Therapeutics*, ed. Mirkin, B.L., pp 1-69. New York: Academic Press Inc.
- MONKS, A., BOOBIS, S., WADSWORTH, J. & RICHENS, A. (1978). Plasma protein binding interaction between phenytoin and valproic acid *in vitro*. *Br. J. clin. Pharmac.*, **6**, 487-492.
- MULLER, W.E. & WOLLERT, U. (1975). Benzodiazepines: Specific competitors for the binding of L-tryptophan to human serum albumin. *Naunyn Schmiedebergs Arch. Pharmac.*, **288**, 17-27.
- REBOUND, P., GROULADE, J., GROSLAMBERT, P. & COLOMB, M. (1963). The influence of normal pregnancy and the postpartum state on plasma proteins and lipids. *Am. J. Obstet. Gynecol.*, **86**, 820-828.
- REIDENBERG, M.M. (1977). The binding of drugs to plasma proteins and the interpretation of measurements of plasma concentration of drugs in patients with poor renal function. *Am. J. Med.*, **62**, 466.
- ROUTLEDGE, P.A., STARGEL, W.W., KITCHELL, B.B., BARCHOWSKY, A. & SHAND, D.G. (1981). Sex-related differences in the plasma protein binding of lignocaine and diazepam. *Br. J. clin. Pharmac.*, **11**, 245-250.
- SELLERS, E.M. (1979). In *Advances in Pharmacology and Therapeutics*. Vol. 7, ed. Tilement, J.P., p. 153. Oxford: Pergamon Pres.
- WONG, G.B. & SELLERS, E.N. (1979). Intravascular factors affecting diazepam binding to human serum albumin. *Biochem. Pharmac.*, **28**, 3265-3270.

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