UNALTERED DIAZEPAM PLASMA BINDING USING INDWELLING HEPARINIZED CANNULAE FOR SAMPLING

The extent of plasma binding of diazepam is one of the determinants of its elimination and changes in plasma diazepam free fraction may induce alterations in its disposition (Klotz, Antonin & Bieck, 1976). A recent report has suggested that the results of pharmacokinetic studies may be seriously biased because of altered drug plasma binding associated with the use of indwelling heparinized cannulae (Wood, Shand & Wood, 1979). The authors reported an increase in propranolol free fraction after the use of 50 i.u. of heparin to flush the cannula and a significant correlation between the change in free fraction and the log of the cumulative dose of heparin. However, it is possible that the acute effects attributed to heparin could be confounded by the influence of a 'light breakfast' given before the samples were taken. We studied two groups of patients to assess the influence of acute and

cumulative administration of heparin and the frequency of sampling through a heparin lock, on diazepam free fraction.

Both *acute* and *cumulative* effects of heparin on plasma diazepam free fractions were studied in ten fasting healthy subjects (five males and five females, aged 21 to 32 years), who received diazepam (10 mg i.v.) and who were sampled, every 3 or 5 min, through an indwelling heparinized cannula. The cannula was flushed after every sample with 40 U.S.P. units of heparin (1 ml) up to a total of 320 U.S.P. units over 30 or 40 min. The *cumulative* effects were also assessed in six other healthy subjects (five males, one female, aged 18 to 27 years) who received diazepam (10 mg i.v.) and who were sampled hourly or two-hourly over 36 h through the heparin lock. They received a total of 1280 U.S.P. units of heparin. The doses of heparin administered allowed a cumulative dose-response curve to be constructed. Diazepam free fractions were determined in duplicate by equilibrium dialysis (Abel, Sellers, Naranjo, Shaw, Kadar & Romach, 1979). [^{14}C]-diazepam (specific activity 26 $\mu\text{Ci}/\text{mg}$) was added to 0.5 ml aliquots of plasma as a tracer and was dialysed at 37°C for 6 h to equilibrium against a physiological 0.124 M phosphate saline buffer pH 7.4. The coefficient of variation of duplicate diazepam free fraction determinations was 2.0%.

Figure 1 shows that a single injection of 40 U.S.P. units of heparin did not have any consistent effect on diazepam free fraction (paired *t*-test = 0.11, $P > 0.90$). The lack of influence of cumulative doses of heparin on diazepam free fraction is shown in Figure 2a and b. There was no correlation between the log of the cumulative dose of heparin and the changes in diazepam free fraction in subjects sampled every 3 or 5 min ($r = 0.13$, NS) and in those sampled hourly or two-hourly through the heparin lock ($r = 0.17$, NS). In addition, two-way analysis of variance, confirmed the large interindividual variation for both the group of subjects shown in Figure 2a ($F = 32.04$, $P < 0.001$) and 2b ($F = 8.9$, $P < 0.001$); and the lack of effect of heparin ($F = 2.30$, NS and $F = 0.81$, NS, respectively).

This study shows that frequent sampling with the use of indwelling heparinized cannulae did not have an effect on plasma diazepam binding. The proposed mechanism of the heparin-induced changes in drug free fraction is an increase in free fatty acids (FFA) caused primarily by the activation of lipoprotein lipase (Krauss, Levy & Fredrickson, 1974). Diazepam is displaced from albumin by oleic and lauric acid (Tsutsumi, Inaba, Mahon & Kalow, 1975; Sjodin, 1977; Colburn & Gilbaldi, 1978) and although an

Figure 1 Plasma diazepam free fraction (%) in ten fasting subjects sampled before and after receiving 40 U.S.P. units of heparin. Males (○), females (●).

Figure 2 (a) Change in diazepam free fraction (%) in plasma in relation to log of the cumulative dose of heparin (U.S.P. units) in ten subjects sampled every 3 or 5 min through indwelling heparinized cannulae. (b) Change in diazepam free fraction (%) in plasma in relation to log of the cumulative dose of heparin in six subjects sampled hourly or 2-hourly through indwelling heparinized cannulae.

effect of heparin on diazepam binding to plasma albumin might be expected it did not occur. This suggests that under the conditions of our experiment the activation of plasma lipolytic activity was not sufficient to increase the FFA/albumin molar ratio to displace diazepam. Postheparin plasma lipolytic activity is a heterogeneous enzymatic system and thus likely to be very dependent on the dose of heparin and the frequency of sampling. For example, after an injection of 10 u./kg of heparin, lipase activity was maximal between 10 to 20 min and then declined rapidly (Krauss *et al.*, 1974). Since plasma samples are taken before the heparin administration, the residual effect of heparin will depend on the time course of its effect in a given individual. It is important to note that we were unable to detect any significant change in diazepam free fraction even in subjects sampled every 3 or 5 min for 30 or 40 min.

Pharmacokinetic and metabolic studies using the

heparin lock, generally employ doses similar to those used in our study. It is necessary to await studies on individual drugs before generalizing the concept that drug free fraction is altered by the use of heparin locks. Such changes may happen for some drugs, but are more likely to occur when very large doses of heparin are used, such as those given in cardiopulmonary bypass surgery or in haemodialysis procedures, in which several thousand units of heparin are given in a few hours.

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