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Effects of benzodiazepines on digoxin tissue concentrations and plasma protein binding

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We have previously shown that the half-life of digoxin in rats is longer when diazepam and chlorazepate are present (Castillo & Carmona 1981). It was also reported that, in healthy volunteers, digoxin half-life and plasma concentrations increased and urinary excretion of digoxin decreased, after diazepam administration (Castillo et al 1980).

In order to ascertain the mechanism of this interaction we have studied digoxin binding to plasma protein in-vitro and its tissue levels after administration of different benzodiazepines (BZD) in dogs.

Methods

Bound and unbound digoxin plasma levels were determined in-vitro, in a control human plasma containing $2{\cdot}5 \times 10^{-6}$ M (2 ng ml^{-1}) of digoxin to which 1 and 3 μg ml^{-1} were added of each BZD. Molar equivalence ($\times 10^{-3}$M) of these concentrations are as follow: diazepam 3·5 and 10·5; chlorazepate 3·0 and 9·02; chlordiazepoxide 3·34 and 10·0; clonazepam 3·17 and 9·5; flunitrazepam 3·19 and 9·58; nitrazepam 3·55 and 10·7; flurazepam 2·58 and 7·73; and clobazam 3·32 and 9·98 respectively. Free digoxin was separated by the technique described by Shah et al (1974) where normal human plasma to which digoxin and BZD were added, was incubated for 4 h at 37 °C. Free and bound fractions were afterwards separated through a Visking membrane by centrifugation at 2200 rev min^{-1} for 25 min at 25 °C.

The in-vivo experiments were in dogs, 15 to 22 kg, and aimed to ascertain any possible modification of tissue concentration of digoxin. Digoxin was given at 50 g kg^{-1} i.v. after i.v. administration of diazepam (0·15 mg kg^{-1}) or chlorazepate (0·72 mg kg^{-1}) to three different groups of animals. Anaesthesia was induced by thiopentone sodium i.v. (25 mg kg^{-1}) and maintained by N_2O (60–65%), O_2 (25–30%) and chloroform (5–15%). The heart rate, e.c.g. and blood pressure were monitored. Digoxin and BZD were injected as soon as the animals were anaesthetized. The digoxin tissue concentrations were determined at 8 h periods after drug administration (Güllner et al 1974) by taking samples from right and left papillary and myocardial muscle, liver, kidney and psoas muscle.

Digoxin determinations were performed by radioimmunoassay (Kit Digoctk-125 from C.I.S.).

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Affinity constants were calculated (Davison 1971) according to the equation:

$$\text{Ka}\,(\text{M}^{-1}) = \frac{\text{PD}}{(\text{P}_t - \text{PD}) \times \text{D}}$$

PD: bound digoxin; D: free digoxin; P_t: total protein.

Data were analysed by the Student's *t*-test for group comparison. A *P* value of less than 0·05 was considered to indicate statistical significance.

Results

Table 1 shows in-vitro results. With the exception of clonazepam and chlorazepate, the BZD tested induced a statistically significant increase in the binding affinity constant of digoxin to protein (Ka) and at 1 μg ml^{-1} reduced the digoxin-free fraction. At the concentration of 3 μg ml^{-1} there were no modifications of the digoxin-free fraction when compared with the control value.

In-vivo, diazepam and chlorazepate significantly decreased the tissue concentrations of digoxin (Fig. 1).

Discussion

There is an equilibrium between bound and unbound drug concentrations but it is only the free fraction that crosses membranes and becomes available for metabolism and distribution (Bickel & Gerny 1980; Koch-Weser & Sellers 1976a,b).

Four different binding loci have been described in the albumin molecule (Kragh-Hansen 1981; Sjöholm et al 1979; Sjöholm 1980; Wollert et al 1980). Fatty acids can modify the binding of some drugs like digitoxin or warfarin to plasma albumin either increasing or decreasing it (Madsen & Ellis 1981; Nielsen et al 1977; Storstein 1976).

Table 1. Effect of benzodiazepines on plasma protein binding of digoxin *$P<0{\cdot}05$.

	Ka ($\times 10^3$ M^{-1})		
Digoxin plus	Control	+ BDZ 3 μg ml^{-1}	+ BDZ 1 μg ml^{-1}
0	0·916	—	—
Diazepam	—	0·500	2·314*
Chlorazepate	—	0·562	0·115*
Chlordiazepoxide	—	0·932	1·339
Clonazepam	—	0·999	0·739
Flunitrazepam	—	0·884	1·897*
Nitrazepam	—	0·749	2·863*
Flurazepam	—	1·035	3·010*
Clobazam	—	0·593	3·791*

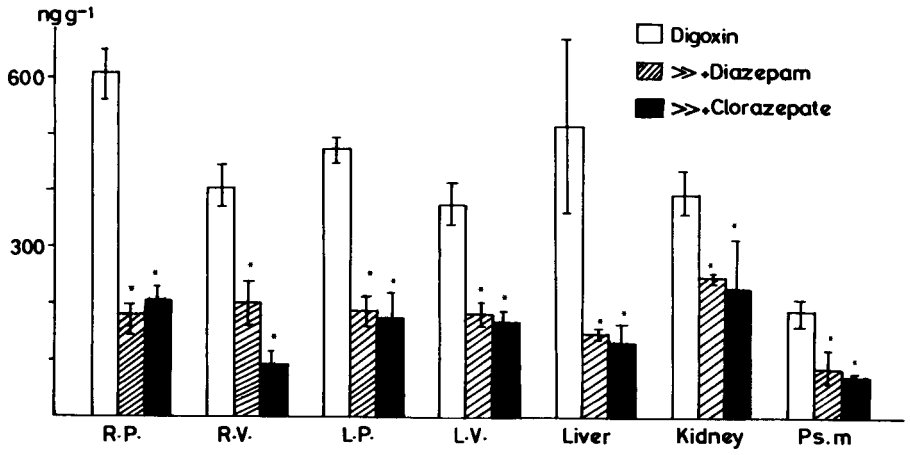

FIG. 1. Effect of diazepam and chlorazepate on digoxin tissue concentration. Bars indicate mean ± s.e. of 4 experiments in each group. Right and left papillary (R.P., L.P.), right and left myocardial muscle (R.V. and L.V.), psoas muscle (Ps.m.). $*P < 0.05$.

Our results suggest that BZD (1 μg ml^{-1}) bind to some locus in the albumin molecule, inducing a cooperative effect on digoxin binding. At BZD concentrations of 3 μg ml^{-1} however, there is no such cooperative effect. Thus, reduction of digoxin-free fraction can account for the lower tissue concentrations in-vivo.

The lower protein binding of clonazepam as well as its higher apparent volume of distribution may explain the absence of interaction with digoxin at protein binding sites (Berlin & Dahlstrom 1975; Müller & Wollert 1973). The lack of interaction between clonazepam and dixoxin might be advantageous when such association is needed in clinical situations.

The difference between the in-vitro and in-vivo results of chlorazepate could be explained because it is biotransformed to an active metabolite (Ochs et al 1979). This is why in-vivo it would be possible to find an interaction that does not occur in-vitro.

The cooperative effect between digoxin and the different BZD studied could be responsible for the longer half-life of digoxin as well as its increased plasma concentrations previously reported.

Such effect might lead to clinical failure in spite of higher plasma concentrations of digoxin, like in the interaction digoxin-quinidine (Belz et al 1982). Further studies in patients once steady-state has been reached are needed to assess the clinical importance of the interaction described.

REFERENCES

Belz, G., Doerin, W., Aust, P. E., Heinz, M., Matthew, S. J., Schneider, B. (1982) Clin. Pharmacol. Ther. 31: 548–554

Berlin, A., Dahlstrom, H. (1975) Eur. J. Clin. Pharmacol. 9: 155–159

Bickel, M. H., Gerny, R. (1980) J. Pharm. Pharmacol. 32: 669–674

Castillo-Ferrando, J. R., Carmona, J. (1981) J. Pharmacol. 12: 74

Castillo-Ferrando, J. R., Garcia, M., Carmona, J. (1980) The Lancet 2: 368

Davison, C. (1971) in: La Du B. N., Mandel, H. G., Way, E. L. (eds) Fundamentals of Drug Metabolism and Drug Disposition. The Williams & Wilkins Company, Baltimore, pp 63–75

Güllner, H. G., Stinson, E. B., Harrison, D. C., Kalman, S. M. (1974) Circulation 50: 653–655

Koch-Weser, J., Sellers, E. M. (1976a) New Engl. J. Med. 294: 311–315

Koch-Weser, J., Sellers, E. M. (1976b) Ibid. 294: 526–531

Kragh-Hansen, U. (1981) Pharmacol. Rev. 33: 17–53

Madsen, B. W., Ellis, G. H. (1981) Biochem. Pharmacol. 30: 1169–73

Müller, W., Wollert, U. (1973) Naunyn-Schmiedeberg's Arch. Pharmacol. 280: 229–237

Nielsen, O. G., Storstein L., Jacobsen, S. (1977) Biochem. Pharmacol. 26: 229–235

Ochs, H. R., Greenblatt, D. J., Allen, M. D., Harmatz, S. J., Shader, R. I., Rodem, G. (1979) Clin. Pharmacol. Ther. 26: 449–456

Shah, V. P., Wallace, S. M., Riegelman, S. (1974) J. Pharm. Sci. 63: 1364–1367

Sjöholm, I., Ekman, B., Kober, A., Ljungstedt-Pahlman, I., Seiving, B., Sjödin, T. (1979) Mol. Pharmacol. 16: 767–777

Sjöholm, I. (1980) Acta Pharm. Suec. 17: 76–77

Storstein, L. (1976) Clin. Pharmacol. Ther. 20: 15

Wollert, V., Feshke, K. H., Müller, W. E. (1980) Acta Pharm. Suec. 17: 80–81