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The use of CD spectroscopy for preliminary detection of drug bioavailability and interaction

GIULIANO SILIGARDI AND ROHANAH HUSSAIN

Drug Interaction-Activity-Screening (DIAS) Unit, Department. of Pharmacy, King's College London, Manresa Road, London SW3 6LX

Pharmacokinetics, bioavailability and interactions of pharmaceuticals are important features for the formulation and drug dosage determination. Bioavailability of a free drug *in vivo* is vital for its therapeutic and/or prophylactic actions. Once administered into the body the drug is partitioned to various biological system compartments and is also bound to circulating carrier proteins. One of the major carrier proteins in the body is human serum albumin, HSA. Drugs bound to HSA are thus not available to reach the target site hence affecting the efficacy of the drugs in their pharmacological action. This is especially critical for a drug with a very small therapeutic margin such as cytotoxic drugs for chemotherapy or hypoalbuminemic patients. The amount of free drugs available *in vivo* is also affected by other ligands competing on the same binding sites influencing the drug's efficacy. A model drug, diazepam, an antidepressant, and a synthetic lipopeptide PKC inhibitor, (2B) (K(Pm)FARKGALRQKNK(Pm); Pm, palmitoyl) binding to HSA are explored and discussed below.

Interaction of non-chiral drugs to proteins is readily achieved in solution by circular dichroism (CD). The screening of binding properties of non-chiral ligands in solution is facilitated by the fact that only the ligand bound species show induced CD. The ordinary UV spectrum of the ligand-host complex will have contributions from both free and bound ligand species. The CD, on the other hand, will be induced only for the ligand species bound to the host chiral binding sites. Induced CD is thus a powerful means of identifying, in solution, non-chiral ligands bound to chiral hosts.

Here we present the induced CD of diazepam (DZ) bound to fatty acid free human serum albumin (HSAff) (Fig. 1). Above 320nm, HSA has no CD contribution therefore the CD profile of HSA+DZ (1:1) mixture is due only to the bound DZ (Fig. 1).

HSA is a lipid carrier. NaCaprylate (CA) is normally used to stabilise HSA. CA is transparent in the near UV region (250-350nm). On adding CA to the HSA-DZ [1:1] complex, an overall decreased intensity of the induced CD of DZ was observed indicative of DZ displacement by CA (Fig. 1).

The competition study also provided the means of assessing the number of DZ bound species to HSA and other model drugs such as diclofenac. The fact that the profile of the induced CD of DZ, a negative band at about 320nm and two positive bands at about 290nm and 260nm, decreased co-operatively with the same rate upon additions of CA was interpreted to be the evidence for only one single bound species of diazepam. Similar observation was obtained when palmitic acid (PA) was added to HSAff:DZ mixtures at various molar ratios of PA (Fig. 1). A reduction of bound DZ was also detected when 2B was added to the HSAff-DZ mixture (Fig. 1) indicating the Pm moiety of the 2B is competing with DZ on the same HSA:DZ binding site thus affecting the amount of free DZ and 2B.

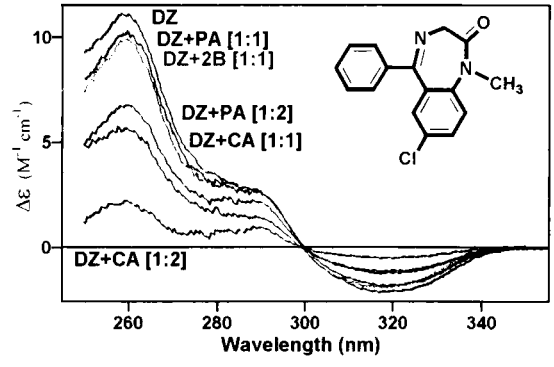

Figure 1. Induced CD of bound DZ to HSAff obtained by subtracting the CD spectrum of HSAff from the HSAff+DZ mixture with equivalent molar ratio of 2B and different molar ratios of PA and CA.

Thus, the amount of fatty acids and fatty acid containing drugs bound to HSA will affect the bioavailability of the free drug hence influencing the efficacy of the drug. Less bound drug means more free drug in circulation that can reach the target site. CD is an ideal tool in determining the preliminary drug interaction properties, biostability and properties of excipients in drug formulations.