Table 2. Mass spectral analysis of cyproheptadine and carbamazepine photoirradiation products.

Compound	Peaks *m/z* (relative abundance)
Cyproheptadine	288 (100%), 273 (26%), 215 (64%), 96 (41%), 70 (19%)
Cyproheptadine 10,11-epoxide	304 (32%), 288 (47%), 274 (28%), 202 (100%) 96 (5·0%)
Photo product I	304 (53%), 288 (100%), 274 (26%), 202 (22%), 96 (16%)
Photoproduct II	288 (100%), 273 (13%), 215 (57%), 96 (34%), 70 (11%)
Carbamazepine	237 (33%), 194 (100%)
Carbamazepine 10,11-epoxide	253 (29%), 237 (100%), 224 (31%), 181 (25%)
Photo product I	253 (3%), 237 (100%), 224 (15%), 181 (8%)
Photoproduct II	237 (100%), 194 (64%)

After 2 h a white crystalline precipitate (30 mg) was produced. This was filtered off and dried under vacuum. Photo-irradiation of the solution was continued. After 16 h a colourless solution was obtained which was extracted with chloroform. Tlc examination of the chloroform extract showed the presence of two bands. The R_F values and uv spectral characteristics of these separated bands compared with those of reference compounds indicated that the composition of these bands was: band 1, carbamazepine 10,11-epoxide and band 2, unchanged carbamazepine (Table 1). These findings were confirmed by a comparison of the chemical ionisation mass spectra of the isolated bands with those for the appropriate reference compounds (Table 2). The white crystalline precipitate was identified as the cyclobutyl dimer of carbamazepine on the following evidence: Melting point (capillary), 370 °C, Kricka et al (1974) give 367–370 °C, uv λ_{max} (methanol) 208 nm, chemical ionization ms $M^+ + 1$ peak *m/z* 473 (29%), and fragmentation peaks at *m/z* 237 (100) and *m/z* 194 (54), pmr spectrum (CF_3COOD) 7·3 δ multiplet, (16H), aromatic protons, 4·1 δ singlet, (4H), cyclobutyl protons, 0·9 δ singlet (4H) exchangeable with D_2O, NH_2 protons.

Thus, it can be concluded that the major photoirradiation product of both cyproheptadine and carbamazepine under these conditions is the cyclobutyl dimer. Also produced are small amounts of the respective 10,11-epoxides.

In view of the ease with which protriptyline, cyproheptadine and carbamazepine form these cyclobutyl dimers one might speculate that the mechanism of photoinduced toxicity with these compounds might be by formation of cyclobutyl adducts with the pyrimidine bases of DNA in an analogous way to the furanocoumarins (Murajo et al 1967).

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On the usefulness of ultrafiltration in drug-protein binding studies

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Severe non-specific adsorption of verapamil, nifedipine, prazosin and nadolol was observed during ultrafiltration of the drug solutions through the Centriflo CF 50A, YMT, YMB and Visking membranes. The results question the adequacy of the ultrafiltration procedure for the protein binding assay of the tested drugs.

Protein binding of drugs in plasma and its role in pharmacokinetics and the dynamics of the pharmacological response have attracted attention in recent years (McNamara et al 1979; Øie et al 1980; Levy 1980). An essential methodological aspect of these studies is choice of the assay procedure, device and materials. Equilibrium dialysis is most frequently used for these purposes, however it is time-consuming and leads to sample dilution. Attempts have been made to achieve more rapid separation of free and protein-bound drugs using various modifications of ultrafiltration. During our pharmacokinetic studies of cardiovascular drugs, we turned to ultrafiltration for the determination of the protein binding of verapamil, nifedipine, prazosin and nadolol in serum of patients treated with these drugs. In the preliminary experiments reported here we have tested the binding of the drugs to the membrane filters routinely used for utlrafiltration. The results question the adequacy of the ultrafiltration procedure for the protein binding assay.

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Materials and methods

Membranes of four types were tested by means of centrifugal ultrafiltration: Centriflo CF 50A conical membrane filters, YMT and YMB membranes for the MPS 1 micro partition system (Amicon, USA) and Visking dialysis tubing (type 8/32, Serva, FRG). In the last case, the method of Heckman & van Ginneken (1982) was used. Drug solutions were prepared in

Krebs-Ringer bicarbonate buffer (pH 7·4) or in the primary ultrafiltrate of serum pooled from several healthy subjects. The primary ultrafiltrate was obtained with the use of the Centriflo CF 50A membrane cones.

Drugs used were: verapamil, 0·25% solution for injection (Isoptin, Lek, SFRJ); prazosin, nadolol and nifedipine–kindly provided by Orion (Finland), Squibb (England) and VEB Germed (GDR), respectively.

The ultrafiltration of 1 ml samples of the drug solutions was in a T 23 centrifuge (Janetzki, GDR) at 1000–2000*g* for the time required to obtain 0·1–1 ml of the filtrate (10–60 min, depending on the membrane type) at room temperature (20 °C). Each experiment was repeated five times. The assays of verapamil, prazosin and nadolol were carried out by hplc as described by Piotrovskii et al (1983a, b). Nifedipine was determined according to Hamman & McAllister (1983) using Tracor 570 gas chromatograph (Tracor, USA). The minimal detectable levels of the drugs were (ng/ml): verapamil, 5; prazosin, 0·2; nifedipine 2; nadolol, 1.

Results and discussion

It is obvious that in the absence of a non-specific binding the concentrations of the drugs in the filtrates should be equal to the initial concentrations. The results presented in Table 1 indicate severe binding of the drugs to the membranes at the concentrations used. The greatest retention was observed for verapamil, prazosin and nifedipine. These drugs were almost completely retarded by the YMT, YMB and CF 50A membranes at concentrations close to or even exceeding the therapeutic concentrations of free drugs in plasma. Pretreatment of the membranes with the primary protein-free serum ultrafiltrate (in accordance with the manufacturer's recommendations) did not decrease the non-specific binding (drug concentrations in these ultrafiltrates were not detectable). No retention was found only for nadolol when the YMB membrane was used.

Table 1. Concentrations of drug solutions before and after ultrafiltration.

	Initial concentrations ($ng\,ml^{-1}$)	Concentrations after ultrafiltration ($ng\,ml^{-1}$; mean ± s.e.m.; n = 5)			
		Visking	YMB	YMT	CF 50A
Verapamil	2500	1460 ± 80	NT	NT	NT
	5000	NT	ND	ND	ND
Prazosin	20	NT	ND	ND	ND
	200	NT	ND	ND	ND
Nifedipine	10	NT	ND	ND	ND
	30	NT	ND	ND	ND
Nadolol	500	NT	503 ± 20	415 ± 18	448 ± 17

NT, not tested.
ND, not detectable, see *Materials and methods* for the minimal detectable concentrations.

The observed dramatic non-specific binding of verapamil, prazosin, nifedipine and nadolol to the ultrafiltration membranes and/or devices prohibited the use of this technique for the determination of the protein binding of these drugs in serum at their therapeutic concentrations which usually do not exceed 1 $\mu g\,ml^{-1}$. The usefulness of such systems in the studies of the interactions of these drugs with macromolecular components of biofluids is also doubtful.

Similar results have been obtained by Hinderling et al (1974) for disopyramide and its metabolites with the Centriflo conical membranes. The binding of theophylline (Franconi et al 1976), diazepam, ibuprofen and quinidine sulphate (Whitlam & Brown 1981) has been reported for PTGC membranes (Millipore). Tasker & Nakatsu (1982) have observed the adsorption of theophylline, acetaminophen and warfarin on PM10 filters (Amicon). Some of the cited authors have attempted to evaluate the capacities of the membranes for the tested drugs or to presaturate the filters with the drugs and introduce appropriate corrections. The reliability of such corrections in the analysis of plasma or serum samples with unknown drug concentrations is however doubtful. The use of the membranes presaturated with the drugs is also open to criticism since the possibility cannot be excluded of uncontrolled desorption or displacement of the adsorbed drug by component(s) of plasma or serum which would cause the overestimation of the free drug concentration. Moreover, all the above modifications deprive ultrafiltration of its main advantage, i.e. rapidity. Therefore we believe that, at least for the drugs tested in the present work, the ultrafiltration cannot substitute for the equilibrium dialysis in the protein binding studies. The latter method does not suffer from non-specific adsorption on the membrane due to the "reservoir effect" of the protein-drug complex. The binding of the drug, if any, will shift the equilibrium of the drug-protein complex formation but will not affect the binding constant.

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