β-Lipoproteins: Possible Plasma Transport Proteins for Basic Drugs

J. J. VALLNER [x] **and L. CHEN**

Abstract □ Various drugs were screened for possible interaction with β-lipoprotein. The results suggest that β-lipoproteins may act in conjunction with albumin in the plasma transport of basic or cationic drugs.

Keyphrases □ β-Lipoproteins—interactions with various acidic and basic drugs screened □ Drug–biomolecule interactions—various acidic and basic drugs with β-lipoproteins □ Interactions, drug–biomolecule—various acidic and basic drugs with β-lipoproteins □ Plasma transport proteins—β-lipoprotein interactions with various acidic and basic drugs screened

Numerous literature reports deal with the binding of drugs to human or bovine serum albumin, and it is generally agreed that albumin is the main transport protein for many anionic drug molecules. In contrast, there have been few reports of albumin binding of positively charged species.

When the binding of three sulfonylureas to serum proteins was examined (1), almost no binding to lipoproteins could be demonstrated. The largest portion of each hypoglycemic was bound to the albumin fraction. However, in an examination of the binding of the basic drugs chlorpromazine and imipramine (2), the interaction with lipoproteins was as avid as that with albumin or red cells. In this same study, the binding of an anionic agent, salicylic acid, to lipoprotein could not be shown. In another study, lipoproteins demonstrated an affinity for the basic drug quinidine almost equal to that of serum albumin (3). The high affinity quinidine site on lipoprotein (low and high density) was about 2×10^5.

This work was undertaken to test whether lipoproteins have a greater affinity for basic drugs than other agents. β-Lipoproteins may serve as plasma transporters of basic drugs as albumin does of primarily anionic species. To examine this possibility, numerous acidic and basic drugs were screened. The method of absorbance difference spectrophotometry was employed because of its capability for detecting small changes should binding occur between the drug and lipoprotein.

EXPERIMENTAL

The drugs were used as received from the manufacturers without further purification. All other materials were USP grade. The β-lipoprotein[1] was used as supplied, except that, in some experiments, it was dialyzed against 10 volumes of buffer for 5 hr prior to the drug binding examination. Sorenson phosphate buffer, adjusted to pH 7.4, was used throughout this study.

The drug–macromolecule interaction was assessed on a spectrophotometer[2] using the tandem cell technique (4). As in a previous study employing the difference spectrophotometric method, the concentrations of drug and protein had to be kept constant in both reference and sample beams throughout the titration of the macromolecule (5). All reported results are the averages of at least 3-μl aliquot titrations of the β-lipoprotein with the drug.

[1] Cohn fraction III-0, lot 4351, Nutritional Biochemical Corp., Cleveland, Ohio.
[2] Cary model 118C, Cary Instruments, Monrovia, Calif.

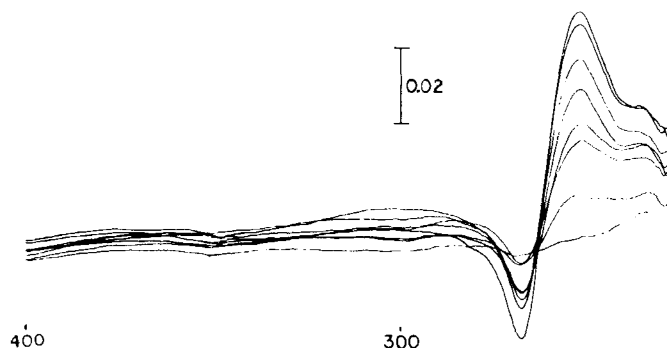

Figure 1—*Example of a difference spectrophotometric titration of β-lipoprotein (0.02%) with trifluoperazine dihydrochloride (0.104 mg/ml). The lower curve is the baseline, and each subsequent curve was generated by the addition of 50 μl of drug as described in the text.*

RESULTS AND DISCUSSION

Interactions between β-lipoprotein and various drugs were examined by difference spectrophotometry. Typical difference absorption spectra for the association of a drug with lipoprotein in phosphate buffer are shown in Fig. 1. A family of curves was generated by the incremental addition of a drug to a protein solution of constant concentration; the solutions ranged from 0.01 to 0.8% in lipoprotein. Drug solutions employed in the titrations ranged from 0.005 to 0.5 mg/ml.

Inspection of these difference absorbance spectra suggests that more than a single class of sites exists on the β-lipoprotein. A distinct isosbestic point was not maintained throughout the titration with any drug. However, the phenomenon of increased difference absorption spectra in the presence of increased amounts of β-lipoprotein was upheld throughout the range of lipoprotein employed.

Table I presents the results of the screening of drugs for binding to

Table I—Results of Drug Screening for Binding Interaction with β-Lipoprotein

Drug	Character	Absorbance Difference[a]	Drug–Protein Ratio
Trifluoperazine hydrochloride	Basic	0.089	0.08
Pyridostigmine bromide	Basic	0.103	1.25
Tolbutamide sodium	Acidic	0[b]	—
Tolazoline hydrochloride	Basic	0	—
Decamethonium bromide	Basic	0.010	5.00
Chlorpromazine hydrochloride	Basic	0.041	0.15
Phenylbutazone sodium	Acidic	0	—
Edrophonium chloride	Basic	0.051	3.07
Isopropamide iodide	Basic	0	—
Propranolol hydrochloride	Basic	0.027	1.67
Warfarin sodium	Acidic	0	—
Clofibrate sodium	Acidic	0.020	2.50
Prednisone	Neutral	0.068	1.10
Phenobarbital sodium	Acidic	0	—
Phenoxybenzamine hydrochloride	Basic	0.017	3.33
Hexamethonium chloride	Basic	0	—

[a] The absorbance difference is measured from a peak (or trough) to the adjacent trough (or peak). The drug to protein ratio at which this absorbance difference occurs is given in the succeeding column. [b] If the absorbance difference is zero, the drug–protein ratio is not given. However, with most drugs, a ratio greater than 8 was tried.

β-lipoproteins. A large number of the basic drugs examined resulted in a perturbation of the macromolecule baseline, reflecting that an interaction had taken place. Most of the cationic drugs showing a perturbation had a large hydrophobic portion in the molecule. Perhaps the β-lipoprotein, in order to bind these basic drugs, requires not only the center of deficient electron density but also a hydrophobic area. With the two aliphatic, yet basic, drugs tested, decamethonium and hexamethonium, only a very weak interaction resulted from the larger drug, decamethonium.

The description of the interaction was based on measuring a suitable absorbance difference from at least three titrations and also providing the drug–lipoprotein ratio of such absorbance diferences. In a number of aliquot titrations of the protein, saturation was not reached. In these instances, the results in Table I are the largest absorbance differences recorded at the ratio causing the perturbation.

Considering the results of Refs. 1–3 and those of this preliminary work, it is possible that cationic drugs may be preferentially transported by β-lipoproteins as well as other plasma proteins. If so, then research can be directed toward examining drug–β-lipoprotein interactions when new basic drugs are introduced. The β-lipoprotein can be assessed as to the strength of binding and the numbers of binding sites if some work is done on purification and exact molecular weight determination. Competitive displacement of drugs by concurrently administered agents may be an area in need of further examination if β-lipoproteins have a high affinity for specific basic drugs.

REFERENCES

(1) J. Judis, *J. Pharm. Sci.*, **61**, 89 (1972).
(2) M. H. Bickel, *J. Pharm. Pharmacol.*, **27**, 733 (1975).
(3) O. G. Nilsen and S. Jacobsen, *Biochem. Pharmacol.*, **24**, 995 (1975).
(4) A. S. Brill and H. E. Sandberg, *Biophys. J.*, **8**, 664 (1968).
(5) J. J. Vallner, L. A. Sternson, and D. L. Parsons, *J. Pharm. Sci.*, **65**, 873 (1976).

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[x] To whom inquiries should be directed.

GLC Determination of Opium Alkaloids in Papaveretum

G. FISHER [x] and R. GILLARD

Abstract □ A GLC method for the determination of the opium alkaloids in papaveretum, based on the formation of the acetyl derivatives of morphine and codeine, is described. The analytical results are compared with those obtained by the official method. The proposed method is fast and accurate and is particularly suited to the analysis of the raw material.

Keyphrases □ Opium alkaloids—GLC analysis in papaveretum, prepared samples □ Papaveretum—GLC analysis of opium alkaloids, prepared samples □ GLC—analysis, opium alkaloids in papaveretum, prepared samples □ Narcotics—GLC analysis of opium alkaloids in papaveretum, prepared samples

Papaveretum raw material (1) is a synthetic mixture of the hydrochlorides of the opium alkaloids, containing the equivalent of anhydrous morphine (47.5–52.5%), anhydrous codeine (2.5–5.0%), noscapine (16.0–22.0%), and papaverine (2.5–7.0%). It has the analgesic and narcotic properties of morphine but is claimed to produce fewer side effects and may be used in all cases where morphine or opium is indicated (2).

The official method of analysis (1, p. 346), involves a titrimetric method for morphine and gravimetric methods for the other alkaloids. Gravimetric analyses, due to their very nature (3), are prone to error, and the many operations required in the official procedure make it tedious and time consuming. For example, the method involves some 30 extractions, more than 40 washings (*i.e.*, of filter papers and solvent layers), and more than 20 actual transfers of the substances to be determined. A skilled and experienced analyst would require about 2 working days to complete an assay in duplicate.

The GLC determination of the opium alkaloids has been described (4–9). These methods involve initial extraction of the alkaloids, followed by GLC as either the free bases or derivatives. The direct injection of hydrochlorides of various other classes of drugs, which relies on subsequent quantitative breakdown to the free base, was reported (10, 11).

In the present study, a GLC method was investigated involving derivatization of the alkaloid hydrochlorides in papaveretum without prior extraction, followed by direct injection into the gas chromatograph. The acetate derivatives of the hydrochlorides of morphine and codeine are formed, while the hydrochlorides of noscapine and papaverine are not derivatized due to the absence of any free hydroxyl groups. Since no extractions are required, some error-prone steps are eliminated. This study apparently was the first application of this technique specifically to the hydrochlorides of the opium alkaloids in papaveretum.

EXPERIMENTAL

Materials—Standards of morphine, codeine, papaverine, and noscapine bases[1] were used, together with morphine hydrochloride[2]. These materials were dried at 120° for 2 hr immediately before use. Anhydrous analytical reagent grade pyridine was used, and all other solvents were also analytical reagent grade.

A solution of 100 mg of squalane in 100 ml of 95% ethyl acetate and 5% acetic acid was prepared as the internal standard solution.

Equipment and Operating Conditions—The analysis was performed on a gas chromatograph[3] equipped with dual flame-ionization detectors and a 5-mv recorder with a chart speed of 10 mm/min. The columns used were 1120-mm (4-ft) × 3-mm o.d. glass-lined metal tubing[4] packed with

[1] All alkaloid bases were BP grade material, recrystallized before use until they assayed at >99.5% by both GLC and nonaqueous titration.
[2] Customs Department, Sydney, Australia.
[3] Perkin-Elmer F30.
[4] Scientific Glass Engineering, Melbourne, Australia.