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Theophylline Protein Binding in Humans

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Abstract □ Theophylline protein binding was 58–82% in serum from six normal adults and 42 asthmatic patients, 1–25 years old, who were given 5 mg of theophylline/kg. The binding range was greatest in young patients, but the proportion of protein-bound drug did not correlate with age. Theophylline protein binding was higher than previously reported. The effect of binding should be considered in patients who do not have optimal bronchodilation from theophylline despite total serum theophylline concentrations of 10–20 μg/ml.

Keyphrases □ Theophylline—protein binding in humans □ Protein binding—theophylline in humans □ Relaxants, smooth muscle—theophylline, protein binding in humans

Drug-protein interactions may influence drug distribution, metabolism, and excretion (1). The extent of theophylline binding to plasma proteins is said to be negligible (2) and is not usually considered when theophylline clearance rates are calculated or when total serum theophylline concentrations are measured.

In this study, theophylline protein binding was measured in serum samples from normal adults and patients with asthma.

EXPERIMENTAL

After informed consent was obtained, six normal adult volunteers not previously treated with theophylline and 42 patients with asthma, 1–25 years old, who had received long-term treatment with theophylline, were given 5 mg/kg po of the drug[1]. Venipuncture was performed 2 hr later. Serum was separated and frozen at −10° for up to 1 month.

Protein binding studies were carried out at room temperature (22°). Serum, 2 ml, was placed in each membrane cone[2]. The cones were centrifuged three times at 1500 rpm for 8–10 min to produce 100 μl of filtrate each time. Theophylline concentrations in the last two ultrafiltrate samples and in an aliquot of retentate were measured by high-performance liquid chromatography[3] (HPLC) (3).

[1] Quibron Elixir, Mead Johnson Canada, Candiac, Quebec, Canada.
[2] Amicon Centriflo cones (CF50), Amicon Corp., Lexington, MA 02173.
[3] Model 8500, Varian Instrument Division, Palo Alto, CA 94303.

Table I—Serum Theophylline Concentrations and Protein Binding Values

Subjects Age, years	*n*	Serum Theophylline Concentration ± *SD*, μg/ml	Theophylline Protein Binding ± *SD*, %	Range, %
Asthmatics, 1–5	10	9.9 ± 4.4	72.1 ± 7.9	58–82
Asthmatics, 6–11	16	9.0 ± 5.2	70.3 ± 4.0	64–76
Asthmatics, 12–25	16	13.4 ± 3.2	70.3 ± 3.2	65–74
Normal adult volunteers, 26–37	6	13.3 ± 2.2	71.8 ± 5.9	66–79

The extent of theophylline protein binding was calculated using:

$$\beta = \frac{D_b}{D_t} = \frac{D_t - D_f}{D_t} \qquad \text{(Eq. 1)}$$

where β represents the fraction of total drug bound to protein, D_b is the amount of drug bound, D_f is the amount of free drug (protein-free filtrate), and D_t is the total amount of drug present (bound plus free). Drug binding was expressed as β%. No corrections for volume change or membrane adsorption were required.

RESULTS AND DISCUSSION

Serum theophylline concentrations and theophylline protein binding values are summarized in Table I. Theophylline protein binding was similar in patients with asthma and in normal adults. The range of binding was greatest in young children, but the proportion of protein-bound drug did not correlate with age.

Previously reported theophylline protein binding values are summarized in Table II. Theophylline was 42–69% bound in plasma from normal adults and in serum from asthmatic mothers who had ingested the drug (4, 5). A similar range was found when theophylline was added to plasma from normal adults who had not received the drug (6). The extent of binding found in both normal adults and patients in the present study was higher, being 58–82%. This result may be due to the various methods of measuring protein binding and theophylline and because either serum or plasma was used, both from dosed patients and from subjects to whose serum drug was added *in vitro* (4–6).

Protein binding has been assumed to be insignificant as far as theophylline distribution, metabolism, and excretion are concerned. Jenne

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Table II—Summary of Theophylline Protein Binding Studies

Source	n	Procedure	Assay	Percent Bound	Reference
Rabbit plasma	—	Equilibrium dialysis: cellophane membrane	UV spectrophotometry	5–10	11
Bovine serum albumin (2%) solutions	—	Equilibrium dialysis: cellophane membrane	UV spectrophotometry	20	7
Plasma of normal dosed volunteers	7	Equilibrium dialysis: cellophane membrane	UV spectrophotometry	59 ± 3	4
Serum from dosed nursing mothers	4	Ultrafiltration: cellophane membrane	HPLC	42–69	5
"Spiked" normal adult plasma	21	Ultrafiltration: membrane cones[a]	LSC[b]	56.4 ± 3.8	6
"Spiked" cord plasma of full-term infants	21	Ultrafiltration: membrane cones[a]	LSC	36.4 ± 3.8	6
"Spiked" plasma of cirrhotic patients	4	Ultrafiltration: membrane cones[a]	LSC	32.3–40.2	8

[a] Amicon Centriflo cones (CF-50), Amicon Corp., Lexington, MA 02173. [b] LSC = liquid scintillation counting of ^{14}C-theophylline.

et al. (2) concluded that, since only 20% theophylline was protein bound (7), binding need not be considered when the volume of distribution was measured. However, Aranda *et al.* (6) reported decreased binding, which might be important when determining effective serum theophylline concentrations in premature infants with apnea. Piafsky *et al.* (8) found reduced binding in cirrhotic patients but did not determine if greater availability of free drug in the liver increased plasma clearance. In the present study, theophylline protein binding did not increase with age. Therefore, it is unlikely that it contributes to the decreased clearance rates found in older patients (9, 10).

Considerable variation in unbound serum theophylline could be possible in patients, based on the 29% range of protein binding found in this study. The effect of binding should possibly be considered in patients who do not have optimal bronchodilation from theophylline despite total serum theophylline concentrations of 10–20 μg/ml.

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Liquid Chromatography in Pharmaceutical Analysis X: Determination of Chlorzoxazone and Hydroxy Metabolite in Plasma

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Abstract □ A method for the high-pressure liquid chromatographic determination of chlorzoxazone and its hydroxy metabolite in human plasma samples is presented. The separation of the compounds is achieved on an octadecylsilane column with a mobile phase of absolute methanol–distilled water (40:60) at a flow rate of 2.0 ml/min (3100 psig). The chromatographic separation is achieved within 10 min. The overall analysis time is about 45 min, which includes extraction of the drug and metabolite from plasma followed by high-pressure liquid chromatographic separation and quantification. The accuracy of the procedure is in the 1–5% range.

Keyphrases □ Chlorzoxazone and hydroxy metabolite—high-pressure liquid chromatographic analysis in plasma □ High-pressure liquid chromatography—analysis, chlorzoxazone and hydroxy metabolite in plasma □ Relaxants, skeletal muscle—chlorzoxazone and hydroxy metabolite, high-pressure liquid chromatographic analysis in plasma

Chlorzoxazone (I) is one of the most useful agents in the treatment of painful muscle spasms, especially in combination with acetaminophen. It can be used as a potent long-acting central muscle relaxant (1, 2). Clinical studies (3) indicated its therapeutic utility and showed no significant side effects (4). Its metabolism was studied (5, 6), and the major metabolite was 6-hydroxychlorzoxazone (II). Chlorzoxazone is rapidly and completely absorbed from the GI tract. The intact drug essentially disappears from the human body in 7 hr, with less than 1% found in urine. Synthesis of II was reported previously (7).

Analytical methods previously reported for I in biological fluids are scarce. The initial and most commonly used method is a spectrophotometric assay (5), involving extraction of drug from biological fluid and reextraction into basic solution with the absorbance read at 289 nm. Other titrimetric (8), GLC (9, 10), and TLC (11) methods were reported for I but were not adapted to the analysis of the intact drug or metabolites in biological samples.

In continuing efforts to apply high-pressure liquid

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