Ultraviolet Spectrophotometry of Theophylline in Plasma in the Presence of Barbiturates

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Ultraviolet spectrophotometry is widely used for the analysis of theophylline in blood, for purposes of monitoring therapy, and for pharmacokinetic studies. Phenobarbital, a component of common oral theophylline preparations, interferes with assays now in use. The modification of the method of Schack and Waxler [J. Pharmacol. Exp. Ther. 97, 283 (1949)] presented in this paper eliminates barbiturate interference. After solvent extraction and back extraction into NaOH, the pH of the alkaline solution is adjusted from pH 13 to 10 before spectrophotometric measurement. This shifts the barbiturate absorption maximum from 255 to 240 nm, permitting accurate analysis of theophylline, the spectrum of which is unaffected by the pH change.

Additional Keyphrases: emergency procedure • toxicology • monitoring drug concentrations

Therapeutic monitoring of plasma theophylline (1,3-dimethylxanthine) concentration is of established value in patients being treated for status asthmaticus, as well as in management of patients on long-term therapy with theophylline preparations. Although there is not complete agreement, therapeutic plasma concentrations of theophylline reportedly range from 5 to 20 mg/liter (1–3). Theophylline, because of its strong ultraviolet absorption in alkaline solution (absorptivity, 575), can be measured by ultraviolet spectrophotometry. In most published reports on the clinical significance of plasma theophylline concentrations and pharmacokinetic studies (1–5), the spectrophotometric method of Schack and Waxler (6), or modifications of it, has been used. In all instances, spectrophotometry was at a final pH of 13. Under this condition, barbiturates, including phenobarbital (a component of widely used oral theophylline preparations), interfere with the assay. In our modification, the pH is adjusted to pH 10 before the spectrophotometric measurement. The resulting hypochromic shift in the phenobarbital spectrum is sufficient to permit resolution of the theophylline absorption peak, which is essentially unaffected by this pH adjustment. The described procedure is suitable for emergency as well as routine use.

Materials and Methods

Apparatus

Double-beam recording spectrophotometer. A Model 402 instrument (Perkin-Elmer Corp., Norwalk, Conn. 106852) was used for these studies with quartz cuvettes (2.5 ml capacity, 1 cm pathlength).

Reagents

All reagents were AR grade.

Chloroform/isopropanol (95/5 by vol).

Phosphate buffer: 0.5 mol/liter, pH 7.4.

NaOH, 0.1 mol/liter.

NH₄Cl, 2.0 mol/liter.

Stock standard: Dissolve 10 mg of theophylline (Sigma Chemical Co., St. Louis, Mo. 63178) in 10 ml of methanol. Store at −15 °C. Working plasma standard, 10 mg/liter. Add 1 ml of stock standard to 100 ml of drug-free plasma or serum. Divide it into 4-ml aliquots and store at 15 °C. The working standard is stable for at least six months.

Procedure

Place 3 ml of sample (or plasma standard), 2 ml of buffer, and 30 ml of chloroform/isopropanol mixture into a 60-ml separatory funnel. Extract gently for about 5 min and filter the chloroform through Whatman No. 1 filter paper. Add 3 ml of NaOH to 25 ml of the filtered chloroform and shake gently for 5 min; use 50-ml round-bottomed centrifuge tubes with ground-glass stoppers. After centrifugation, remove 2.0 ml of the aqeous phase, add 0.1 ml of NH₄Cl, mix, and determine the ultraviolet absorption spectrum. Use a solution containing NaOH and NH₄Cl in the same ratio as the reference solution. If theophylline is present, there will be an absorption peak at 275 nm.

Subtract the absorbance (1 cm, 1 g/dl) at 300 nm from that at 280 nm and determine the concentration by comparison with an identically processed plasma standard. Subtracting the absorbance at 300 nm eliminates some baseline errors resulting from traces of endogenous color or turbidity.

Results and Discussion

A standard curve was prepared by extracting plasma samples containing various concentrations of added theophylline and analyzing them as described. Drug concentration and absorbance (A₂₈₀ − A₃₀₀) were linearly related over a range of 2 to 20 mg/liter. The sensitivity (curve b, Figure 1) is sufficient that smaller volumes of serum can be used when necessary. For 10 replicate analyses of a plasma containing 8 mg/liter, the coefficient of variation was 4.1%. Analyses of a pool of plasma (stored frozen) during six months showed a between-day CV of 5.6%. Analytical recovery of theophylline averaged 84.9%, but this need not be considered because in taking plasma standards through the entire extraction procedure the incomplete recovery of theophylline is corrected for. A specimen from a patient receiving a theophylline preparation yielded the spectrum represented by curve a in Figure 1. Because the standard curve is linear, a single standard can validly be used in the analysis of clinical specimens that arrive in the laboratory unpredictably and sporadically.

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Received April 7, 1975; accepted May 27, 1975.
Twelve negative plasmas from fasted patients showed an
average absorbance corresponding to 0.5 mg/liter (range,
0–0.9 mg/liter). An occasional drug-free plasma from a non-
fasting person will show an absorbance corresponding to
between 1 to 2 mg/liter, which possibly reflects other in-
gested xanthine compounds or their metabolites. Koyo-
sooko et al. (5) reported a higher background.

Ultraviolet spectrophotometry cannot, of course, resolve
a mixture of compounds with overlapping spectra. Any
weakly acidic ultraviolet-absorbing drug that reaches a suf-
ficiently high concentration in the blood theoretically will
interfere. Phenylbutazone and sulfonamides are commonly
used drugs that could interfere, but are not likely to be co-
administered with theophylline. Contrary to common be-
ief, caffeine is not extracted from the solvent by the dilute
NaOH; 50 μg of caffeine added to chloroform/isopropanol
was not extracted by NaOH (0.1 mol/liter). Because its
pKa is greater than 14, caffeine has negligible acidic prop-
erties. An hour after a subject ingested two strong cups of
cocoa, a plasma “theobromine” concentration correspond-
ing to 1.5 mg of theophylline per liter was obtained. The-
obromine-containing medications will interfere, but rarely
are used.

Phenobarbital interference cannot be passed over as
lightly as the drugs just mentioned, because it is included
in a number of widely used oral theophylline preparations,
such as Teldral (Warner-Chilcott). Decreasing the pH from
13 to 10 by adding NH₄Cl shifts the phenobarbital peak
from 255 to 240 nm, away from the theophylline maximum.
At 280 nm the interference from phenobarbital is negligi-
ble. Figure 2 shows these relationships. A specimen from a
patient receiving phenobarbital and theophylline yielded
the spectrum shown in Figure 3.

The differential spectrophotometric method of Gupta
and Lundberg (7) can be used if barbiturates are present,
but requires 5 ml of blood, is probably insufficiently sensi-
tive for therapeutic monitoring, and, as the authors indi-
cate, was intended for use in diagnosis of drug overdose.
Schack and Waxler (6) suggested that if the particular bar-
biturate was known, a correction might possibly be made
by using a two-wavelength measurement, simultaneous
equations, and the molar absorption value, but no data
were given to support this suggestion. No foreknowledge
of the use of barbiturates is required in the procedure de-
scribed here.

A potential drawback to spectrophotometric procedures
is that they are subject to interferences from metabolites
that have absorption spectra similar to that of the parent
drug. However, this is a problem only if metabolites reach
sufficiently high concentrations in the blood and if they are
extracted in the same analytical procedure. Cornish and
Christman (8) have shown that theophylline is metabolized
primarily by oxidation to 1,3-dimethyluric acid rather than
by demethylation. Although found in urine, the more polar
uric acid metabolites are not present in blood, nor are they
extracted by the described procedure (1). Thompson et al.
(9) analyzed specimens from two patients receiving theo-
phylline by a specific high-pressure liquid-chromatograph-
ic procedure and by Schack and Waxler’s (6) spectrophoto-
metric method. The latter showed an average positive bias
of 4 to 5%, with a maximal discrepancy of 1.6 mg/liter at a
concentration of 15.6 mg/liter. The discrepancy did not
correlate with concentrations of another metabolite, 3-
methylxanthine, which is extracted with only about 20% the
efficiency of theophylline (1, 9) and is present in plasma
in only much lower concentrations. The discrepancy,
which is insignificant for clinical monitoring, probably cor-
responds to the nonspecific background absorbance dis-
ussed above. Hypoxanthine and xanthine, 10 mg of each
added to plasma per liter, did not interfere.

Procedures in which liquid (9) or gas-liquid chromato-
graphic techniques are used (10, 11) may be more sensitive
than the described procedure, may resolve theophylline

Fig. 1. Spectra obtained on analysis of (a) plasma from pa-
tient receiving theophylline and (b) 10.0 mg/liter standard in
plasma

Fig. 2. Effects of pH on phenobarbital interferences
1, theophylline (10 mg/liter) at pH 10; 2, phenobarbital (20 mg/liter) at pH 13;
3, phenobarbital (20 mg/liter) at pH 10

Fig. 3. Spectrum obtained on analysis of plasma from a pa-
tient receiving theophylline and phenobarbital
from other drugs, and may therefore be preferable for pharmacokinetic studies, but a spectrophotometric procedure is suitable for round-the-clock management of acute asthmatic emergencies, as well as chronic care. The procedure described eliminates the most important and common source of interference, can be completed in 20–30 min, and is technically suited for emergency and routine use.

This work was supported in part by USPHS Research Grant No. 1 R01 MH19017 from the National Institute of Mental Health, NIH. I gratefully acknowledge the technical assistance of Miss Mullin Chiong.

References


Double-Antibody Radioimmunoassay of Serum Insulin: Effect of Use of Hormone-Depleted Human Serum

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Human serum can be depleted of insulin and growth hormone by treatment with dextran-coated charcoal or by dialysis, or both. Inclusion of such hormone-depleted serum in the standard curve of a double-antibody radioimmunoassay for immunoreactive insulin more nearly mimics the conditions under which an unknown human serum sample is assayed. Compared to the usual standard curve in which serum albumin is the only protein, the addition of hormone-depleted serum can cause an increase (by an average 64% under the conditions we used) in the absolute value for apparent insulin in serum. The effect of hormone-depleted serum should be tested in the standard curves of double-antibody radioimmunoassays and included routinely in these standard curves if it changes the results. When used in conjunction with an eventual reference standard for human insulin, this modification of the insulin assay may make the measured values of human serum insulin from different laboratories more comparable.

In radioimmunoassay for immunoreactive insulin in serum, an unknown serum is compared to a standard curve prepared by use of increasing amounts of an insulin preparation that serves as a standard. The insulin standard is usually dissolved in a buffer solution containing bovine (1) or human (2) serum albumin as the only protein. The absence of human serum from the standard is thus an uncontrolled variable and might affect the absolute value of insulin determined in an unknown serum, particularly if it is assayed relatively undiluted. Because the absolute value for serum insulin is often used diagnostically (3), factors affecting its determination can be clinically important. Therefore, we examined the effect of insulin-depleted human serum on the standard curve of a double-antibody radioimmunoassay for immunoreactive insulin.

Methods

Preparation of hormone-depleted human serum. Human serum was treated with dextran-coated charcoal or dialyzed, or both.

Treatment with dextran-coated charcoal: (a) prepare a suspension of dextran-coated charcoal (4); (b) centrifuge at 10 000 × g for 10 min at 4 °C, beginning with a volume of charcoal suspension four times that of the serum to be