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Note

High-performance liquid chromatographic determination of acetaminophen in plasma: single-dose pharmacokinetic studies

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Acetaminophen (paracetamol) is extensively used as a non-prescription analgesic and antipyretic agent [1, 2]. Recent studies suggest that excessive doses and/or excessively high plasma concentrations of acetaminophen may be associated with hepatotoxicity [1—4]. Thus understanding of its pharmacokinetic properties in humans might be of value in preventing clinical toxicity.

High-performance liquid chromatography (HPLC) is applicable to quantitation of acetaminophen in human plasma [5—14]. However, a sensitivity range of 0.1—0.2 μg per ml of plasma is needed for evaluation of acetaminophen pharmacokinetics following single therapeutic doses. The present paper describes an HPLC method with this degree of sensitivity that is applicable to single-dose pharmacokinetic studies as well as to quantitation of acetaminophen following overdosage.

EXPERIMENTAL

Materials

Acetaminophen and the internal standard 2-acetaminophenol (2-AAP) (Fig. 1) were obtained from Aldrich (Milwaukee, WI, U.S.A.). All other reagents, analytical grade or better, were purchased from commercial sources and were used without further purification.

Apparatus and chromatographic conditions

A Waters Assoc. (Milford, MA, U.S.A.) liquid chromatograph was used. The instrument was equipped with a Model 6000A solvent delivery system,

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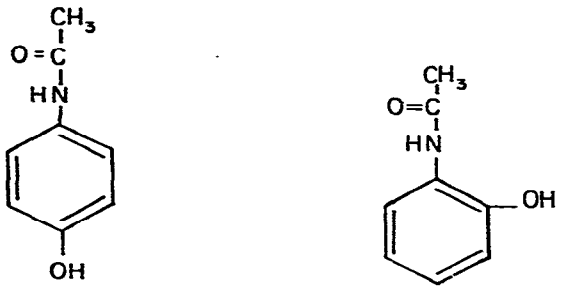

ACETAMINOPHEN 2-ACETAMINOPHENOL

(internal standard)

Fig. 1. Structural formulae of acetaminophen and of 2-acetylaminophenol, the internal standard.

a Model 440 Ultraviolet detector operated at 254 nm, and a 30 cm × 3.9 mm stainless steel μBondapak C_{18} reversed-phase column. A 10 cm × 8 mm Radial-Pak radial compression C_{18} cartridge was used alternatively. The injection system was a Model U6K sampling loop, or a WISP Model 710B automatic sample processor. Detector output was quantitated using either a strip chart recorder or a Model 730 data module.

The mobile phase consisted of either: (a) 3.5% acetonitrile—sodium acetate buffer (pH 4.0), operated at a flow-rate of 2 ml/min; or (b) acetonitrile—methanol—water (6:6:88), operated at a flow-rate of 1.8—2.2 ml/min. All analyses were performed at room temperature.

Stock solutions

Standard solutions of acetaminophen and of 2-AAP were prepared by dissolving 100 mg of each compound in 100 ml of methanol. Working solutions were prepared by appropriate dilution with methanol. Solutions are stable for at least one year when stored at 4°C.

Preparation of samples

Internal standard (15 μg) was added to a series of 13-ml round-bottom culture tubes equipped with PTFE-lined screw-top caps. The organic solvent was evaporated to dryness at 40—50°C under mildly reduced pressure. To a series of calibration tubes were added variable amounts of acetaminophen ranging from 0.1—15 μg. Again, the organic solvent was evaporated to dryness. Drug-free control plasma (0.5—1 ml) was added to each of the calibration tubes; 0.2—1.0 ml of unknown plasma was added to all other tubes.

Ethyl acetate (5 ml) was added to each tube, and the tubes were gently agitated on a Vortex-type mixer for 30 sec. After centrifugation for 10 min at 400 *g*, an aliquot (approximately 4.5 ml) of the organic phase was transferred to a 13-ml tapered glass centrifuge tube. The organic solvent was evaporated to dryness at 40—50°C under mildly reduced pressure. The residue was redissolved in 100 μl of methanol, of which 10—20 μl were injected into the sampling loop.

Clinical pharmacokinetic study

A healthy 26-year-old male volunteer (68 kg) participated after giving written informed consent. He received single 650-mg doses of acetaminophen on two occasions separated by one week. On one trial, a sterile solution of acetaminophen (13 ml of a 50 mg/ml solution [propylene glycol—ethyl alcohol—5% dextrose (40:10:50, v/v)] diluted to 50 ml with 5% dextrose) was infused into an antecubital vein over a period of 5 min by a constant-rate infusion pump. For the other trial, the subject ingested two 325-mg tablets of acetaminophen (Parke-Davis, Ann Arbor, MI, U.S.A.) in the fasting state with 100—200 ml of tap water.

Venous blood samples were drawn into heparinized tubes from an indwelling cannula, or by venipuncture, at multiple points in time during the 12 h after each dose. Plasma concentrations of acetaminophen in all samples were determined using the method described above.

Plasma acetaminophen concentrations were analyzed by weighted iterative non-linear least-squares regression techniques described in detail previously [15]. After intravenous administration, data points were fitted to the following function:

$$C = Ae^{-\alpha t} + Pe^{-\pi t} + Be^{-\beta t} \quad (1)$$

where C is the plasma acetaminophen concentration at time t after dosage. A, P, and B are hybrid intercept terms having units of concentration; they were appropriately corrected for the 5-min infusion period [16]. The exponents α, π, and β are hybrid quantities having units of reciprocal time. Using standard pharmacokinetic methods [17], coefficients and exponents from the fitted function were used to determine the following kinetic variables for acetaminophen: initial distribution half-life, intermediate distribution half-life, elimination half-life, volume of the central compartment, total volume of distribution using the area method, and total clearance. Assuming that all of acetaminophen clearance is accounted for by hepatic biotransformation, the predicted extraction ratio was calculated as the quotient of hepatic clearance and estimated hepatic blood flow (21 ml/min/kg) [18].

After oral acetaminophen administration, plasma concentrations were fitted to the following function:

$$C = -(A + B)e^{-ka(t - t_0)} + Ae^{-\alpha(t - t_0)} + Be^{-\beta(t - t_0)} \quad (2)$$

where C is the plasma concentration at time t after dosage. As in eqn. 1, A and B are hybrid intercept terms, and α and β are hybrid exponents; ka is also a hybrid exponent, representing the apparent phase of drug absorption; t_0 is the lag time elapsing prior to the start of first order absorption. The absolute bioavailability of orally administered acetaminophen was calculated as the area under the plasma concentration curve (extrapolated to infinity) after oral dosage divided by the area under the curve following intravenous administration [17].

RESULTS

Evaluation of the method

Under the described chromatographic conditions, acetaminophen and its internal standard yielded two symmetric, well-resolved peaks (Fig. 2). Endogenous plasma substituents did not interfere with peaks corresponding to acetaminophen or the internal standard.

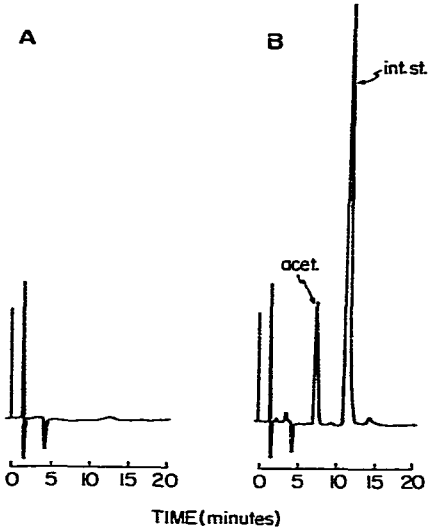

Fig. 2. Chromatograms of plasma extract from a subject (A) prior to administration of acetaminophen; (B) 2.5 h after a single intravenous dose of acetaminophen, showing peaks corresponding to acetaminophen (acet.) and to the internal standard (int. st.).

Either peak area ratio or peak height ratio can be used to quantitate detector response. The relation of detector response ratio to plasma acetaminophen concentration is linear. The day-to-day coefficient of variation in the slope of calibration curves (n = 10) was 6.0%. The sensitivity of the method is approximately 0.1—0.2 μg of acetaminophen per ml of plasma. Coefficients of variation for identical samples ranging in concentration from 0.25—15 μg/ml did not exceed 5% (Table I). The mean deviation between pairs of duplicate samples analyzed during pharmacokinetic studies (n = 45) was 2.4%. Mean recovery of acetaminophen was 90.1% (S.D. = ± 4.5%; range 79—100%). Recovery of the internal standard was 93.8 ± 6.0%.

A series of fourteen samples (concentration range: 0.26—48.8 μg/ml) were analyzed using mobile phase (a) and the stainless-steel column, with quantitation of detector response by peak height ratio. Results were compared with analysis of the same samples using mobile phase (b) and the radial compression cartridge, with detector response quantitated by peak area ratio. The correlation coefficient was 0.999, with a regression line slope of 0.94. The mean deviation between the two methods for identical samples was 3.4%.

TABLE I

REPLICABILITY OF IDENTICAL SAMPLES

Concentration (μg/ml)	Coefficient of variation* (%) (n = 6 at each concentration)
0.25	3.6
0.5	2.0
1.0	1.6
2.5	2.4
5.0	1.8
10.0	4.7
15.0	0.6

*Standard deviation divided by mean, expressed in percent.

TABLE II

KINETICS OF INTRAVENOUS AND ORAL ACETAMINOPHEN

Kinetic variables	Route of administration	
	Intravenous	Oral
Lag time (min)	—	4.7
Absorption half-life (min)	—	11.6
Initial distribution half-life (min)	1.6	—
Intermediate distribution half-life (h)	0.12	—
Elimination half-life (h)	2.6	2.5
Total clearance (ml/min/kg)	4.46	—
Total area under the curve (μg/ml·h)	35.7	26.7
Predicted extraction ratio	0.21	—
Absolute bioavailability	1.00	0.75

Clinical pharmacokinetic study

Table II shows kinetic variables for acetaminophen after intravenous and oral administration to the same volunteer. Values of elimination half-life were 2.6 and 2.5 h, respectively, by the two routes of administration (Fig. 3). After a lag time of 4.7 min, oral acetaminophen was absorbed with an apparent half-life of 11.6 min. Based on comparison of areas under the curve following oral and intravenous administration, absolute bioavailability of oral dosage was 75%. This is very close to the predicted extraction ratio of 79% based on the ratio of hepatic clearance after intravenous dosage to hepatic blood flow.

DISCUSSION

The present paper describes a rapid and sensitive quantitative assay for acetaminophen in plasma. The drug and its internal standard are readily extracted into ethyl acetate at neutral pH with no special sample preparation. Since drug-free plasma samples yield no contaminating peaks, clean-up pro-

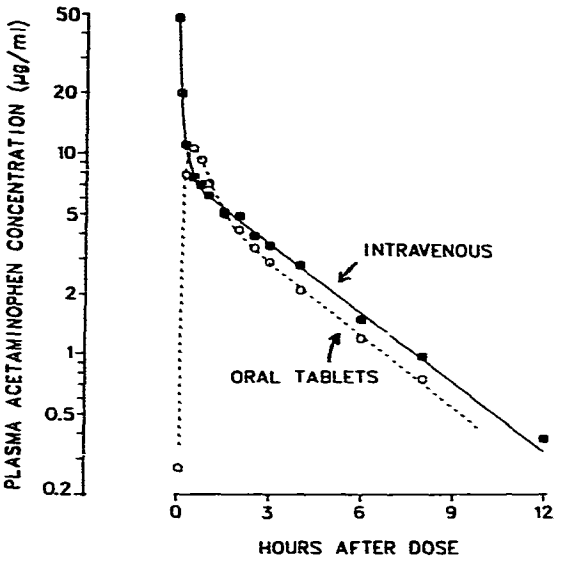

Fig. 3. Plasma acetaminophen concentrations following intravenous and oral administration of 650 mg to a healthy volunteer subject. Solid and dashed lines represent computer-determined pharmacokinetic functions consistent with eqns. 1 and 2, respectively. See Table II for kinetic analysis.

cedures are not required. The organic extract is evaporated to dryness, reconstituted, and chromatographed directly. The method is adaptable to an automatic sampling system, such that up to 100 samples can be analyzed in a 24-h period.

Meaningful data on the pharmacokinetics of acetaminophen following single therapeutic doses in humans require reliable routine quantitation of plasma concentrations as low as 0.1—0.2 μg per ml of plasma. The described method achieves this level of sensitivity, and its application to single-dose pharmacokinetic studies of acetaminophen is illustrated. Disappearance of acetaminophen from plasma following intravenous infusion was consistent with a sum of three exponential terms. The elimination half-life was 2.6 h. Total acetaminophen clearance was 4.5 ml/min/kg, predicting an extraction ratio of 21% assuming hepatic blood flow to be 21 ml/min/kg. The absolute bioavailability of oral acetaminophen in tablet form was 75%. Thus, the systemic availability of oral acetaminophen is less than 100%, and appears to be accounted for by first pass extraction rather than incomplete absorption.

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