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USE OF ION-EXCHANGE CHROMATOGRAPHY IN THE SPECTROPHOTOMETRIC ASSAY FOR THE ANTINEOPLASTIC AGENT, 2-DIMETHYLAMINO-3',4'-DIHYDROXYACETOPHENONE HYDROCHLORIDE, IN BIOLOGICAL FLUIDS

GEORGE T. BRYAN AND HITOSHI TAKAHASHI
The Division of Clinical Oncology, University of Wisconsin Medical School, Madison, Wisc. 53706 (U.S.A.)
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SUMMARY

A simple, rapid, and precise method for the determination of 2-dimethylamino-3',4'-dihydroxyacetophenone hydrochloride in whole blood, plasma, tissue homogenates, or urine has been described. The method is based upon stabilizing this compound in borate buffer prior to column chromatography on anion- and cation-exchange resins followed by ultraviolet absorption spectrophotometry. Utilizing this method, it was observed that nearly 50 % of the administered compound is excreted unchanged in the urine of patients with cancer, and that it is slowly, and poorly metabolized by rat liver *in vitro*.

INTRODUCTION

2-Dimethylamino-3',4'-dihydroxyacetophenone hydrochloride (NSC-62512) (AP) has demonstrated moderate but reproducible antineoplastic activity against Leukemia 1210, P288, P1798 and the Dunning Ascites Leukemia[1]. On the basis of this activity, AP was evaluated for human toxicity in clinical Phase I trials[1].

To investigate the mechanism of action of AP, and to facilitate studies of the blood levels and urinary excretion of this drug by patients, a method was developed for the quantitative determination of AP in biological materials. It was observed that AP could be stabilized in alkaline borate buffer[2], and that chromatography on anion- and cation-exchange resins could be employed for the purification of AP in biological samples prior to quantitative estimation by ultraviolet spectrophotometry. The development of this method for the determination of AP in blood and urine forms the basis of this report.

EXPERIMENTAL

Materials

AP was provided by the Clinical Branch, Collaborative Research, National Cancer Institute, U. S. Public Health Service. Dowex 2 (borate$^-$), 8 % cross-linkage,

200 to 400 mesh, and Dowex 50W (H^+), 12 % cross-linkage, 200 to 400 mesh, were prepared as previously described[2]. The chromatography columns were similar to those utilized earlier[3], and were operated in groups of 4 to 20 under air pressure distributed with a manifold. A Beckman model DU spectrophotometer (Beckman Instruments Inc., Fullerton, Calif. 92634, U.S.A.) or a Bausch and Lomb Spectronic 505 recording spectrophotometer (Bausch and Lomb, Rochester, New York 14602) with matched quartz cells were employed for reading the samples.

Ion-exchange chromatography

Aqueous solutions, or whole blood, plasma, tissue homogenates, or urine samples containing known quantities of AP were diluted to 120 ml with 50 ml of 0.1 *M* borate buffer[2] (pH 9.0) and distilled H_2O. The sample was then applied to a 1.0 × 3.0 cm resin bed of Dowex 2 ($borate^-$) and passed through the column by gravity. Then 50–100 ml of 0.05 *M* borate buffer (pH 9.0) were passed through the column followed by 20 ml of 0.5 % boric acid solution to remove catecholamines and similar compounds which might later interfere with the assay of AP. The effluents from the Dowex 2 ($borate^-$) column were discarded. The borate column was then placed above the second column of 0.7 × 1.5 cm resin bed of Dowex 50W (H^+) and the AP eluted from the borate column onto the Dowex 50W (H^+) column with two 25-ml aliquots of 0.2 *N* HCl passed through under gravity. The borate column was then removed and the resin discarded. The column of Dowex 50W (H^+) was washed with 50 ml of 0.2 *N* HCl passed through the column under 0.25–0.5 p.s.i. pressure. Finally, the AP was eluted from the Dowex 50W (H^+) with two 25-ml aliquots of 1.0 *N* HCl which were pooled and an aliquot read spectrophotometrically at 232 mμ, 282 mμ, and 311 mμ against an appropriate blank or 1.0 *N* HCl.

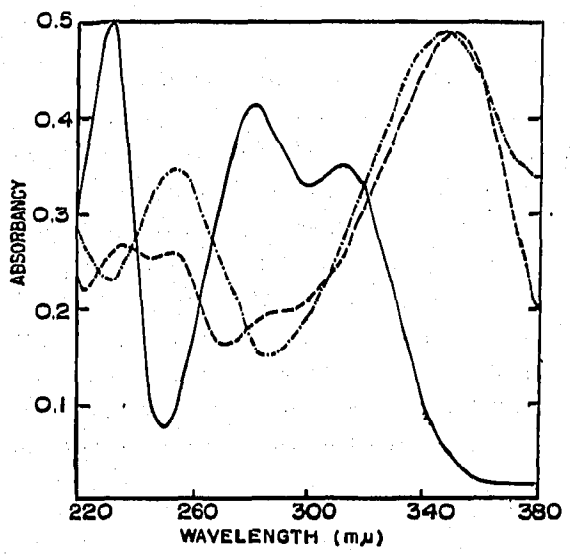

Fig. 1. Ultraviolet absorption spectra of AP in 0.1 *M* phosphate buffer at pH 2.0 (———), 7.4 (– – – –), and 12.0 (–·–·–·–); and in 1.0 *N* HCl. A concentration of 4 × 10^{-5} *M* AP was employed for each solution.

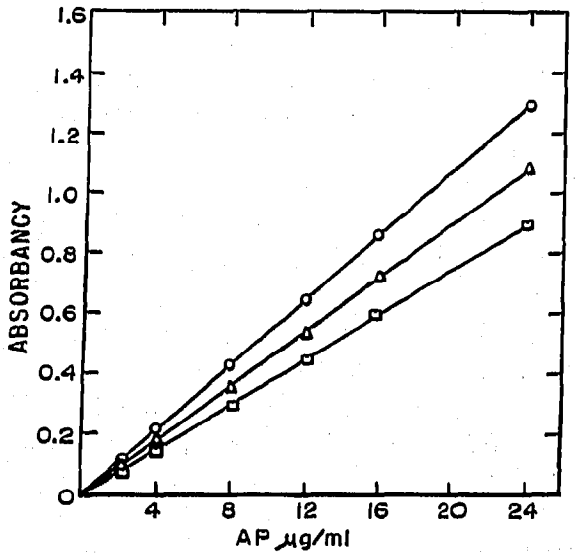

Fig. 2. Standard curves of AP in 1.0 *N* HCl read at 232 mμ (O—O), 282 mμ (△—△), and 311 mμ (□—□).

Spectrophotometric determination

Ultraviolet absorption spectra of standard AP solutions were obtained in 1.0 *N* HCl and in 0.1 *M* phosphate buffer at pH 2.0, 7.4, and 12.0 (Fig. 1). In 1.0 *N* HCl solution or in 0.1 *M* phosphate buffer at pH 2.0 identical spectra were observed with absorption maxima at 232 mμ, 282 mμ, and 311 mμ. Standard curves were constructed for AP in 1.0 *N* HCl at all three wavelengths of maximal absorption (Fig. 2). The absorption curves obtained in 0.1 *M* phosphate buffer at pH 7.4 and 12.0 demonstrated different wavelengths of maximal absorption (Fig. 1). AP was noted to be unstable in 0.1 *M* phosphate buffer (pH 9.0) as evidenced by a shift in spectra with time and the formation of a yellow color after one day. However, AP demonstrated marked stability in 0.1 *M* borate buffer at pH 9.0.

Sample preparation

The stock solution of AP (2×10^{-3} *M*) was prepared by dissolving AP in 0.2 *N* HCl. This was frozen and stored in the dark. Immediately before each experiment, aliquots of this stock solution were diluted as needed, and were analyzed as described, or were added to whole blood, plasma, serum, tissue homogenates, or urine samples prior to analysis. Whole blood, plasma, or serum samples were prepared by adding standard AP solutions to 5 ml of whole blood, plasma, or serum prior to dilution with borate buffer and distilled H_2O. Preliminary deproteinization of these samples was not necessary. The human and rat urine specimens were collected and stored as previously described[2] prior to analysis. Urine samples were prepared by adding standard AP solutions to 0.25–1.0 % of a 24-h human or rat urine sample prior to dilution with borate buffer and distilled H_2O. Blanks for whole blood, plasma, serum, tissue homogenates, or urine were prepared in the above manner except that AP

TABLE I

RECOVERY OF AP FROM VARIOUS BIOLOGICAL SAMPLES FOLLOWING CHROMATOGRAPHY ON DOWEX 2 ($BORATE^-$) AND DOWEX 50W (H^+)

Sample No.	*Biological specimen*	*AP added (μg)*	*AP recovered (μg)*	*% Recovery*
1	—	0	0	0
2	—	232	190	83
3	—	464	385	83
4	—	696	646	92
5	—	928	947	102
6	—	928	835	90
7	—	1392	1141	82
8	Human serum	928	874	94
9	Human serum	464	441	95
10	Human serum	464	436	94
11	Human serum	464	422	91
12	Human serum	464	422	91
13	Human urine	464	455	98
14	Rat liver homogenate	928	879	95
	Average % recovery ± S.D.[a]			92 ± 6

[a] S. D. = $\sqrt{\Sigma(X-\bar{x})^2/(n-1)}$.

TABLE II

PERCENTAGE OF ADMINISTERED AP RECOVERED INTACT IN 24-h URINE OF HUMAN OR RAT

Subject	*Route of AP administration*	*Dose (mg/kg body weight)*	*Quantity present in 24-h urine (mg)*	*% of dose present in 24-h urine*
Human	6-h intravenous infusion[a]	300	9,672	54
Human	6-h intravenous infusion[a]	300	7,500	42
Rat	intraperitoneal[b]	200	10.6	17
Rat	intraperitoneal[b]	200	17.1	27
Rat	intraperitoneal[b]	300	16.0	17

[a] Administered as described in Ref. 1.
[b] Administered as single intraperitoneal dose.

was omitted from the mixture. All analytical samples were compared with standard curves of AP prepared in 1.0 *N* HCl at the three peaks of maximal ultraviolet absorption (Fig. 2).

RESULTS AND DISCUSSION

The ultraviolet absorption spectra of AP in 1.0 *N* HCl and 0.1 *M* phosphate buffer at pH 2.0, 7.4, and 12.0 are presented in Fig. 1. The computed standard curves of AP in 1.0 *N* HCl at 232 mμ, 282 mμ, and 311 mμ are demonstrated in Fig. 2. Prior chromatography of AP-containing solutions on anion- and cation-exchange resins removed many potentially interfering substances. Thus quantities of AP as low as one μg/ml of effluent from Dowex 50 (H^+) could be measured.

The recovery of added quantities of AP in aqueous solutions or from biological materials is presented in Table I. Recoveries averaged 92 ± 6 % (S.D.) over a wide range of concentrations. The recoveries were comparable to those reported for L-dihydroxyphenylalanine in human urine when a similar method of chromatographic isolation was employed[2]. These recoveries could only be achieved because of the surprising stability of AP in borate buffer within the range of pH 7.0–9.0. This stability of AP, structurally similar to dihydroxyphenylalanine, was markedly contrasted with its rapid deterioration in phosphate buffer over the same pH range. The ultraviolet absorption spectral characteristics of recovered AP following chromatography were identical to those demonstrated in Fig. 1 for 1.0 *N* HCl solutions.

Following intravenous infusion of AP over a 6-h period to two patients at a dosage level of 300 mg/kg body weight[1], very low quantities of AP were detected in the serum at the completion of the infusion. The concentrations present in these patients were 31.5 μg/ml and 27.5 μg/ml of serum respectively after 6-h of infusion of about 18.0 gm of AP. Analysis of 24-h urine specimens obtained from these patients for the 6-h period of the infusion and the 18-h period following infusion revealed that nearly 50 % of the administered AP was excreted intact in the urine (Table II). When AP was administered to rats as a single intraperitoneal injection at a dosage level of 200 or 300 mg/kg of body weight, then about 20 % was excreted intact in a 24–h urine specimen (Table II).

When AP was incubated *in vitro* with a rat liver homogenate in 0.1 *M* phosphate buffer (pH 7.4) nearly 70 % of the AP was recovered (Fig. 3). These data suggest that

AP is not metabolized rapidly by liver, and support the observation that large quantities of AP occur in the urine of patients or rats without prior significant metabolic alteration.

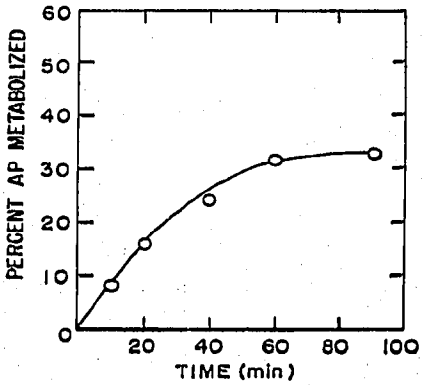

Fig. 3. Percentage of AP metabolized *in vitro* by a rat liver homogenate. 4×10^{-3} moles of AP were incubated at 37° for 90 min in a 10% rat liver homogenate in 0.1 *M* phosphate buffer at pH 7.4. The enzyme reaction was terminated by the addition of 4 ml of 95% ethanol prior to the analysis for AP by the method described in the text.

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