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Note

Quantitative determination of 1-(2-chloroethyl)-3-(β-D-glucopyranosyl)-1-nitrosourea in blood and urine of man*

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A newly synthesized antitumor drug, 1-(2-chloroethyl)-3-(β-D-glucopyranosyl)-1-nitrosourea (GANU), is a water-soluble nitrosourea derivative. In animal experiments, GANU exhibited high activity against experimental tumors; its action was mainly associated with inhibition of DNA synthesis [1, 2]. In this communication, a method for the estimation of blood levels as well as of the urinary excretion of GANU has been developed using high-performance liquid chromatography (HPLC).

MATERIALS AND METHODS

GANU was thoroughly dissolved in physiological saline solution just before injection. In the case of drop infusion, 250 ml of saline were used, and 20 ml of saline were used in the case of bolus injection.

Blood samples were collected at 15, 30, 55, 65, 75, 90, 120 and 180 min after injection. A 2-ml volume of the blood sample was heparinized with 0.1 ml of heparin and immediately poured into a tube with 8 ml of a cooled mixture of isotonic citrate buffer (pH 4.0) and 0.9% sodium chloride solution (1:3, v/v). The mixture obtained was carefully mixed to avoid hemolysis. The successive procedures are summarized in Fig. 1. The sample thus obtained was dissolved in 0.1 *M* acetate buffer (pH 4.0) and used for HPLC.

The HPLC apparatus was from Waters Co. Ltd. A stainless-steel column (300 × 3.9 mm) was packed with μBondapak C_{18} (8—10 μm). As the mobile

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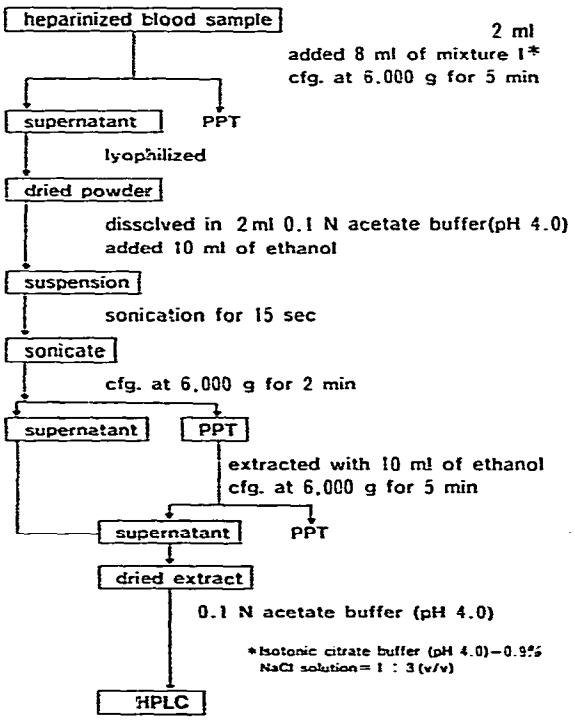

Fig. 1. Procedure for the extraction of GANU from human blood. Whole blood was used for the extraction of GANU. The mixture of isotonic citrate buffer (pH 4.0) and saline solution (1:3, v/v) was used to avoid the decomposition of GANU during the extraction procedures.

phase, a water—methanol mixture (9:1, v/v) was used throughout the experiments. Details of the conditions for the determination of GANU are described in the legends to the figures. The elution pattern was detected at 254 nm using a recorder from National Electric Co. Ltd.

Urine was also collected into bottles containing 0.1 *M* acetate buffer (pH 4.0) at different time intervals, then centrifuged at 6000 *g* for 10 min. The supernatant solution thus obtained was immediately used for HPLC. The HPLC procedure was the same as that for blood samples.

Water used for these experiments was prepared by a water purifier, Milli-R/Q, Millipore Co. Ltd. Methanol was chromatographic grade and was obtained from Wako Chemical Co., Tokyo, Japan. All other chemicals of the highest quality were obtained from commercial sources.

The solution of the authentic sample of GANU was prepared by dissolving 2 mg of GANU in 1 ml of water, and used for HPLC. Also, 2 mg of GANU were dissolved in 10 ml of pooled plasma and extracted by the method described in Fig. 1. Urine samples containing various concentrations of GANU were used for HPLC without further purification.

RESULTS

Separation of authentic samples

When a water—methanol mixture (9:1, v/v) was used as mobile phase, the retention time of GANU was 4.6 min (Fig. 2A), but the peak decreased in size when 0.2 *M* ammonia solution (pH 8.4) was used as mobile phase (Fig. 2B).

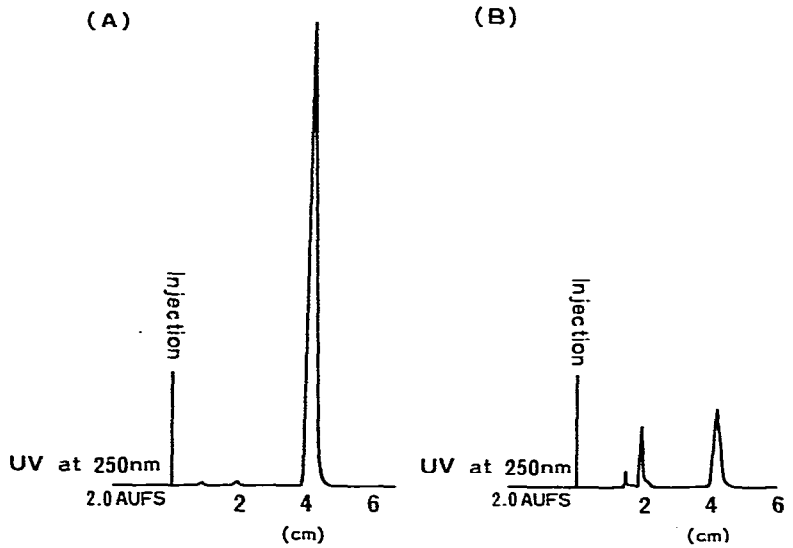

Fig. 2. Elution pattern of GANU and its decomposition product by different eluting solutions. (A) GANU is eluted at 4.6 min without any decomposition product when a water—methanol mixture (9:1, v/v) is used as mobile phase. (B) 0.12 *M* ammonia solution (pH 8.4) has been used as mobile phase. The height of the GANU peak has decreased and a new peak has appeared at 2.1 min.

Recovery of GANU from pooled plasma

The HPLC pattern of GANU extracted from plasma is presented in Fig. 3A. The peak of authentic GANU has a retention time of 4.6 min and no disturbing peak has been observed in the area of the GANU peak (Fig. 3B). The recovery of GANU was calculated to be approximately 68%.

Calibration curve for the estimation of GANU in human blood

The calibration curve is linear until a GANU concentration of 5 μg/ml. The calibration equation has been calculated to be $y = 5.209x - 0.208$ ($r = 0.999$) and the recovery has been estimated as 68.8%. The coefficient of variation has been estimated as 3.8%.

Calibration curve for the estimation of GANU in human urine

The calibration curve is linear until 500 μg/ml GANU. The recovery of GANU from urine is 76.4%, and the *r* value 0.7754.

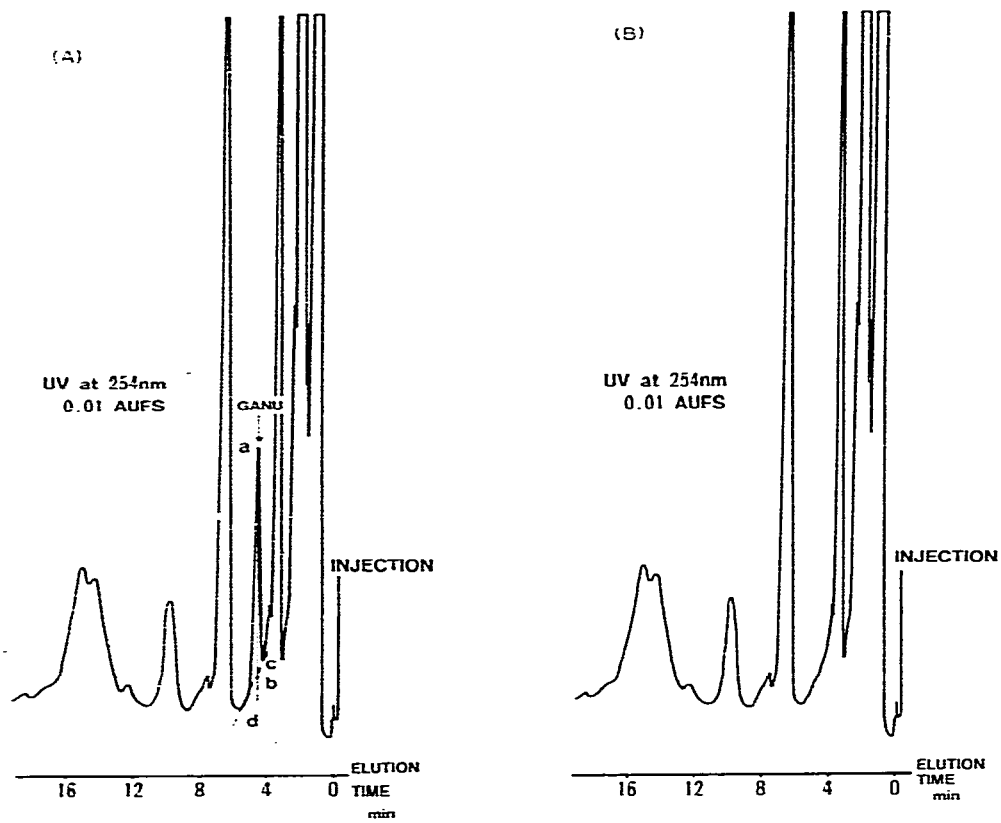

Fig. 3. Chromatograms of GANU extracted from human plasma. HPLC conditions: column 30 × 0.4 cm, μBondapak C_{18}; mobile phase, water—methanol (9:1, v/v); flow-rate 2 ml/min; chart speed 0.5 cm/min. (A) Authentic GANU was mixed with pooled human plasma and extracted by the method described in the text. The concentration was determined using the height between a and b. (B) Blank plasma assayed by the same method. No disturbing peak was observed in the region of the GANU peak.

Concentration of GANU in human blood

The concentration of GANU has been determined in the blood of two patients with cancer for each of the doses 60, 80, 100, 120, 140, and 160 mg/m^2. From the observed concentrations of GANU in the blood the pharmacokinetic data calculated by one-compartment theory have been demonstrated as shown in Table I.

In addition, the same dose of GANU was injected by bolus injection into two patients. The concentration of GANU was assayed, and the pharmacokinetic data are presented as well (Table I).

Excretion of GANU in human urine

As shown in Table II, the excretion of GANU (dose administered 120 mg/m^2) in the urine was 49.5 μg/dl in the initial hour, but after that no GANU was detected in the urine in either case. The excretion rate of GANU was estimated to be 2.11%. Metabolites of GANU such as 1-(2-chloroethyl)-3-(β-D-

TABLE I

COMPARISON OF PHARMACOKINETIC PARAMETERS BETWEEN DROP INFUSION AND INTRAVENOUS BOLUS ADMINISTRATION

Dose administered = 120 mg/m², n = 2.

	Drop infusion	Bolus injection
Elimination constant (min^{-1})	0.0873	0.162
Half-time of blood level (min)	7.98	4.63
Distribution volume (l/m²)	18.0	5.66

TABLE II

URINARY EXCRETION OF GANU IN HUMANS AFTER CONSTANT INTRAVENOUS DROP INFUSION OF GANU

Dose administered = 120 mg/m².

Subject	Time after administration (h)	Urine volume (ml)	Concentration of GANU (μg/ml)	Amount recovered (mg)	Percentage of dose
A	0—1	60	34.8	2.09	1.11
	1—2	180	N.D.*	—	—
	2—6	220	N.D.	—	—
	6—24	630	N.D.	—	—
	0—24	1090	—	2.09	1.11
B	0—1	92	64.2	5.90	3.11
	1—2	63	N.D.	—	—
	2—6	1100	N.D.	—	—
	6—24	1980	N.D.	—	—
	0—24	3235	—	5.90	3.11

*N.D. = not detected.

glucopyranosyl)urea (GAU) and β-D-glucopyranosylamine (1-AG) have never been detected by the method described above.

DISCUSSION

The assay method for GANU has been established by HPLC in acidic solution. GANU is freely soluble in water but is unstable in the light, at high temperatures and at alkaline pH [3]. Therefore, to prevent the degradation of GANU in blood and urine of patients, it was essential that the biological materials are immediately mixed with acid solution after collection, as shown in Fig. 1. By adding the acid solution, the extraction and purification of GANU from biological material have been quantitatively developed. However, it was not necessary to add saline solution in the case of urine. The recovery of GANU from pooled plasma has been estimated as approximately 68%, and the recovery from urine has been estimated to be 76%. The limit of sensitivity was 100 ng/ml in blood and 5 μg/ml in urine.

The concentration of GANU in the blood and urine of the patients who have been administered GANU either by drop infusion or bolus injection has been assayed by the method described above. GANU was detected until 90 min in cases of drop infusion and 30 min with bolus injection. On the other hand, GANU was only detected in the urine in the first hour. GANU was not detected after 1 h of drop infusion at bolus injection because of the dilution of GANU in urine. Metabolites of GANU such as GAU and 1-AG have not been detected in either blood and urine; this is probably due to the low concentration of these compounds in biological materials. The quantitative determination of these metabolites should be investigated by a different method. However, the method described in this communication has been sufficient to assay the concentration of GANU in human blood and urine and to work out details of the pharmacokinetics of this compound.

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