Fluorometric Assay for Measuring Biological Half-Life of Coralyne Sulfoacetate in Dogs and Monkeys

JOE M. FINKEL ˣ **and DONALD L. HILL**

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Abstract □ A spectrophotofluorometric assay for coralyne sulfoacetate was developed. Coralyne was extracted from serum samples with *n*-butyl alcohol, and the drug concentrations were determined fluorometrically at 475 nm when the extract was excited at 325 nm. A biphasic serum decay curve for coralyne was observed for both dogs and monkeys. The biological half-lives for the two phases were 20 and 196 min in dogs and 15 and 142 min in monkeys.

Keyphrases □ Coralyne sulfoacetate—fluorometric analysis in serum □ Fluorometry—analysis, coralyne sulfoacetate in serum □ Antineoplastic agents, potential—coralyne sulfoacetate, fluorometric analysis in serum

Coralyne and some of its derivatives exhibit activity against leukemia L-1210 and P-388 in mice (1, 2). The basis for the biological activity of coralyne may be formation of intercalated complexes with cellular DNA (3, 4). The physiological distribution and metabolic fate of ^{14}C-coralyne in CDF_1 mice and Sprague–Dawley rats were reported (5), as was a preclinical toxicological evaluation of coralyne in dogs and monkeys (6).

The present paper reports serum levels and biological half-lives for coralyne in dogs and monkeys, as determined by a new fluorometric assay.

EXPERIMENTAL

Instrumentation—Fluorescence measurements were made with a spectrophotofluorometer[1] and an *x–y* recorder. The following instrument settings were used for all measurements: slit arrangement, No. 4; photomultiplier shutter, 4 mm; and sensitivity control, 40. Stainless-steel mirrors were inserted in the cell compartment to increase the amount of radiation reaching the photomultiplier tube.

Reagents—Various concentrations of coralyne sulfoacetate[2] in water or *n*-butyl alcohol saturated with water were prepared daily from a stock solution of 100 μg/ml.

Phosphate-buffered saline, 0.05 *M*, pH 7.4, was prepared by the addition of 2.6 g of monobasic sodium phosphate monohydrate and 11.5 g of dibasic sodium phosphate anhydrous to 9.0 g of sodium chloride. The mixture was dissolved and diluted to 1000 ml with water.

Coralyne extractions were made with *n*-butyl alcohol[3] saturated with water.

Analytical Procedures—Serum (0.2 ml) was diluted with an equal volume of phosphate-buffered saline and extracted with 2 ml of *n*-butyl alcohol. The fluorescence of the organic extract was measured at 475 nm with an excitation wavelength of 325 nm. Coralyne concentrations in serum were determined by comparison of the relative fluorescence intensity of extracts of serum to which 1–100 ng/ml of coralyne in *n*-butyl alcohol had been added. The fluorescence observed was proportional to concentration in the range of 10–1000 ng/ml of serum. Pretreatment serum from each animal served as blanks.

Recovery Studies—A 2-ml aliquot of coralyne standard in *n*-butyl alcohol (1–100 ng/ml) was manually shaken with 0.2 ml of serum plus 0.2 ml of pH 7.4 buffer or with 0.4 ml of pH 7.4 buffer in a 12-ml screw-capped centrifuge tube for 1 min and centrifuged at 1640×*g* for 10 min. The organic phase was removed, and its fluorescence intensity was determined as previously described. The fluorescence intensities of these extracts were compared with the intensities of corresponding coralyne standards in *n*-butyl alcohol.

Table I—Values for Selected Pharmacokinetic Parameters for Dogs and Monkeys Receiving a Single Intravenous Dose of Coralyne Sulfoacetate

Animal Model	Decay Phase, min	Pharmacokinetic Parameters[a] y Intercept, μg/ml	Rate Constant (Slope)	$t_{0.5}$, min
Dog	0–60 (α)	3.5, 2.4	−0.0328, −0.0365	21, 19
	90–300 (β)	0.29, 0.25	−0.0044, −0.0030	159, 232
Monkey	0–60 (α)	5.9, 3.3	−0.0483, −0.0439	14, 16
	90–300 (β)	0.22, 0.24	−0.0048, −0.0050	144, 140

[a] Values for individual dogs and monkeys are presented.

Animal Studies—Two female beagle dogs, 10 and 12 kg, and two female rhesus monkeys, 4 and 5 kg, each received a single dose (10 mg/kg iv) of coralyne sulfoacetate. The injection solution (10 mg/ml) was prepared in the phosphate-buffered saline immediately prior to administration. Blood samples were collected at 11 time intervals up through 6 hr after treatment and placed in an ice bath. Each animal served as its own control.

The serum samples were assayed within 3–5 hr after collection. Although the drug remained stable during its storage in an ice bath, longer storage periods required prior extraction into *n*-butyl alcohol.

TLC—Coralyne metabolites soluble in *n*-butyl alcohol might interfere with the fluorometric assay. To determine if such metabolites appeared in the serum, 100- or 500-μl aliquots of the alcohol extract from each sample of serum were spotted on TLC sheets[4]. The sheets were developed in a solvent of chloroform–methanol–acetic acid (80:19:1 v/v/v). Coralyne was located at R_f 0.45 on the sheets under UV light (366 nm).

RESULTS AND DISCUSSION

Fluorescence Studies—Coralyne sulfoacetate showed fluorescence in a neutral or acidic aqueous solution. A twofold increase of fluorescence intensity was observed when coralyne was dissolved in *n*-butyl alcohol. The maximum excitation occurred at 325 nm; at that wavelength, the maximum fluorescence was 475 nm. Preliminary studies indicated that the drug was unstable in 0.1 *N* NaOH but stable in 0.1 *N* HCl, ethanol, and *n*-butyl alcohol at room temperature. The relative fluorescence intensity was linear with the concentration of the drug in *n*-butyl alcohol in the range of 1–100 ng/ml.

The relative fluorescence intensities of a coralyne standard (1 μg/ml) as extracted from pH 7.4 phosphate-buffered saline and from serum were 44.8 ± 0.64 and 33.5 ± 1.0, respectively. These values, the averages of three determinations, were corrected for background interferences of 0.25 ± 0.02 for extracts from the buffer and 1.47 ± 0.03 for extracts from serum. The saturated *n*-butyl alcohol gave a fluorescence background of 0.24 ± 0.02. Concentrations of coralyne in serum as low as 10 ng/ml were detected. The extraction efficiency of coralyne from serum was 67% when compared with a coralyne standard in *n*-butyl alcohol.

Applications—Serum levels of coralyne were analyzed by computer programs. The exponential parameters were estimated by curve stripping of the data as described by Sedman and Wagner (7). After preliminary resolution into individual exponential components, the data were analyzed by a weighted, nonlinear, least-squares program and were fitted to the biexponential equation $C = Ae^{-\alpha t} + Be^{-\beta t}$, where C was the drug concentration in serum, A and B were coefficients, and α and β were rate constants representing the slopes of the two phases.

[1] Aminco-Bowman, American Instrument Co.

[2] Supplied by Drug Research and Development, Division of Cancer Treatment, National Cancer Institute.

[3] Analyzed reagent, J. T. Baker Chemical Co.

[4] Gelman Type SA ITLC.

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The serum decay curves for coralyne in dogs and monkeys exhibited a rapid distribution phase followed by a much slower second phase. Pharmacokinetic values derived by computer analysis are summarized in Table I. Values for the first phase were similar to those derived for rats given an intravenous dose of coralyne (5). As observed for rats, however, a distribution phase may be missing, because distribution of the dose in the total body water of dogs and monkeys would produce a larger serum concentration (15 μg/ml) at t_0 (y intercept).

In dog serum, but not monkey serum, a faintly fluorescent metabolite of coralyne appeared after 45 min at R_f 0.80. This nonpolar metabolite may be the same as that observed in the bile of rats (5). Based on TLC using 10–50-fold more extract than was used to detect coralyne, the contribution of fluorescence of this metabolite to total fluorescence is small.

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Rapid Determination of Gold in Whole Blood of Arthritis Patients Using Flameless Atomic Absorption Spectrophotometry

MICHAEL J. BARRETT, ROY DeFRIES *, and WILLIAM M. HENDERSON ˣ

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*Present address: School of Medicine, Southern Illinois University, Carbondale, IL 62901.

Abstract □ An assay for gold in whole blood of arthritis patients was developed using the graphite furnace atomic absorption spectrophotometer. This method involves no pretreatment of the whole blood except for simple dilution, thereby eliminating some variables and saving laboratory time and expense.

Keyphrases □ Gold—atomic absorption spectrophotometric analysis in whole blood □ Atomic absorption spectrophotometry—analysis, gold in whole blood □ Metals—gold, atomic absorption spectrophotometric analysis in whole blood

Gold salts have been used in rheumatoid arthritis for over 40 years, but few concise and accurate assays for the determination of gold in biological fluids have been developed. Since varying amounts of gold are found in the red blood cells of some patients receiving chrysotherapy (1, 2), an analysis of whole blood rather than blood fractions should be used to determine optimally the total gold content in the circulation.

Most methods of gold analysis (*i.e.*, chemical, UV emission, spectrography, polarography, and neutron activation) require extensive laboratory work to remove interfering fractions and repeated extractions (3, 4). Neutron activation analysis for the estimation of gold is the most sensitive method available (5); but since samples must be dried, sealed, irradiated for several hours, and cooled for several days, there is a possibly prohibitive time delay in reporting results. Furthermore, the assay requires expensive neutron sources with licensed operators and multichannel analyzers, both of which are relatively unavailable (5). This assay is beyond the capabilities of most clinical laboratories, thus rendering it rather impractical (4).

The lack of simple and accurate assay techniques for gold in biological fluids made it difficult to determine absorption, distribution, deposition, and excretion throughout dosing intervals and inhibited evaluation of the pharmacology and the pharmacodynamics of gold (4, 6–8). Atomic absorption spectrophotometric results compare well with those of neutron activation analysis (5), and its simplicity, speed, and range make it ideal for clinical work.

EXPERIMENTAL

Apparatus—The analysis was done using an atomic absorption spectrophotometer[1] with a graphite furnace accessory[2]. Power for the furnace was supplied by a controller[2] with a temperature ramp accessory[2] (Table I) to minimize elemental loss due to rapid gross temperature changes. All samples were injected *via* an automatic sampling system[3]. Since the instrument digital readout did not respond rapidly enough to the atomization signal, a strip-chart recorder[4] was implemented. The chosen emission source was a hollow cathode lamp[5].

Reagents and Standards—The water used in all dilutions was previously deionized by passage through a mixed-bed, ion-exchange column[6] with subsequent filtration through a 5-μm membrane filter[7] to remove any dislodged resin and other particulates.

[1] Perkin-Elmer model 403.
[2] Perkin-Elmer model HGA-2100.
[3] Perkin-Elmer model AS-1.
[4] Perkin-Elmer model 36.
[5] Perkin-Elmer Intensitron (No. 303-6031).
[6] Barnstead Ultrapure (No. D0809).
[7] Metricel.

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