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Note

Plasma and tissue concentrations of hycanthone in the mouse determined by reversed-phase high-performance liquid chromatography

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Hycanthone, 1-{[2-(diethylamino)ethyl]amino}-4-(hydroxymethyl)-thioxanthen-9-one (Fig. 1) is widely used in the treatment of schistosomiasis [1]. Hycanthone has activity against murine leukemias L-1210 and P-388 and rat Walker 256 carcinosarcoma [2, 3] and has been studied as a potential anticancer drug in man [4, 5]. Hycanthone is a major metabolite of lucanthone, 1-{[2-(diethylamino)ethyl]amino}-4-(methyl)-thioxanthen-9-one [1, 6], a drug with radiosensitizing properties in vitro [7, 8] and in vivo [9]. The radiosensitizing effects of lucanthone are rapidly reversed when it is removed and exposure to the drug for several hours is necessary to produce the optimal effect [8]. Hycanthone has been reported to enhance the effects of radiation on mammary tumors in mice [10] and it is currently being evaluated as a radiosensitizing agent in man. Metabolic studies have been conducted using radioactively labelled hycanthone [11] and plasma and tissue levels of solvent extractable radioactivity reported following the administration of radioactively

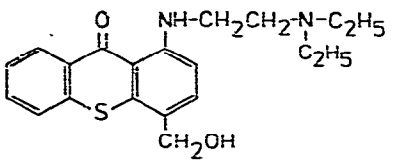

Fig. 1. Hycanthone structure.

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labelled hycanthone to monkeys and rats [12]. Plasma levels of non-labelled hycanthone determined by a normal-phase high-performance liquid chromatographic (HPLC) procedure have been reported in man [5] but the method was not adapted for use with an internal standard and does not resolve hycanthone metabolites. We report a simple reversed-phase HPLC assay for hycanthone and its lipophilic metabolites. This assay has been used to measure the time course of the disappearance of hycanthone from plasma and tissues in the mouse.

EXPERIMENTAL

Animal studies

Male BDF_1 mice weighing between 25 and 30 g were injected intraperitoneally (i.p.) with an aqueous solution of hycanthone methanesulfonate at a dose of 80 mg hycanthone base per kg body weight (343 mg/m^2). Groups of six mice were killed at different times by decapitation and blood collected into heparinized tubes. The different organs were removed and immediately frozen with crushed dry ice. Tissues were homogenized in 4 volumes water at 4°C using a Polytron homogenizer (Brinkman Instruments, Westbury, NY, U.S.A.).

Preparation of samples

Portions (0.5 ml) of plasma or of tissue homogenate (4 ml) were extracted with 8 ml chloroform. The efficiency of extraction of hycanthone was 93%. The chloroform extract was dried with sodium sulfate and taken to dryness under nitrogen. The residue was dissolved in 100 μl acetonitrile and 50 μl injected into the HPLC system. With plasma and most tissues an internal standard of 10 μg hycanthone acid was employed. Analysis was, however, always conducted with and without an internal standard. Standard curves were prepared for each tissue.

High-performance liquid chromatography

Hycanthone and non-polar metabolites were separated by reversed-phase HPLC on a 25-cm C_2-bonded LiChrosorb RP-2 column, 10-μm particle size (Merck, Darmstadt, G.F.R.), under isocratic conditions with 50% 0.01 *N* sodium acetate buffer (pH 4.0) in acetonitrile (Burdick and Jackson Labs., Muskegon, MI, U.S.A.) as the mobile phase at a flow-rate of 2 ml/min. More reproducible results were obtained if, after each separation, the column was flushed with 100% acetonitrile for 10 min. The eluting compounds were detected at 257 nm with a reference wavelength of 520 nm. A Hewlett-Packard 1084B liquid chromatograph and variable-wavelength detector were employed. The output from the detector was fed into a Hewlett-Packard 79850B liquid chromatograph terminal, and peak areas integrated.

Drugs

Hycanthone monomethanesulfonate, lucanthone, hycanthone acid (1-{[2-(diethylamino)ethyl]amino}-4-carboxy-thioxanthen-9-one) and hycanthone N-oxide (1-{[2-(diethylamino-N-oxide)ethyl]amino}-4-hydroxymethyl-thioxanthen-9-one) were supplied by Sterling Winthrop Research Institute (Rensselaer, NY, U.S.A.).

RESULTS AND DISCUSSION

The normal-phase HPLC separation of hycanthone previously reported from this laboratory [5] could not be adapted to an assay with the internal standards available since it failed to resolve hycanthone and lucanthone while the more polar hycanthone N-oxide and hycanthone acid gave broad unresolved peaks. All of these compounds were well separated by reversed-phase HPLC on a C_2-bonded support (Fig. 2). Hycanthone bound so tightly to a C_{18}-bonded support that it could not be eluted with 100% acetonitrile. The procedure for the extraction of hycanthone from plasma is simpler than that previously reported [5]. All of the compounds had an absorption maximum at 257 nm which was chosen as the wavelength for detection. Hycanthone acid was chosen, for convenience, as the internal standard rather than the slowly eluting lucanthone. Although hycanthone acid was a possible metabolite it was not present in the plasma or in tissues other than the liver and gastrointestinal tract.

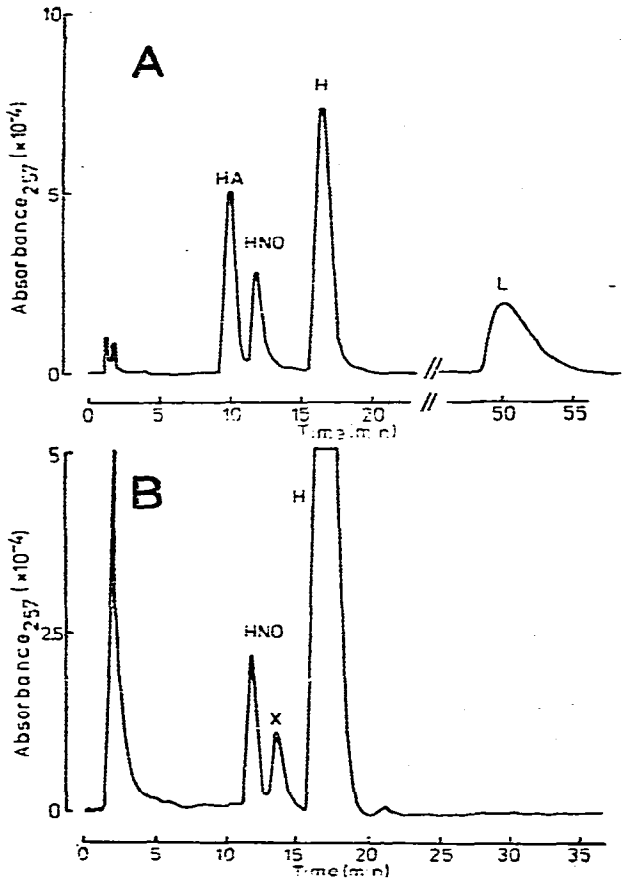

Fig. 2. Chromatograms of A, mouse plasma to which had been added 5 μg/ml each of hycanthone (H); hycanthone acid (HA); hycanthone N-oxide (HNO); and lucanthone (L); B, mouse kidney homogenate 100 min after the i.p. administration of hycanthone 80 mg/kg; X, unidentified metabolite.

All samples were routinely assayed with and without added hycanthone acid. The acid was used as an internal standard only when it was not detected in the tissue under study. Using hycanthone acid as an internal standard in plasma, the assay was linear up to 100 μg hycanthone per ml and had a lower limit of detectability of 50 ng/ml. The coefficient of variation of the assay was 6.4%.

Following i.p. administration, hycanthone was rapidly absorbed and disappeared from the plasma in a biphasic manner (Fig. 3) with a distributive half-life ($t\frac{1}{2}^{\alpha}$) of 19 min, a postdistributive half-life ($t\frac{1}{2}^{\beta}$) of 56 min, and a steady state volume of distribution (Vss) of 43,888 mg per kg body weight. The concentrations of hycanthone in the tissues studied were always higher than in plasma but fell with about the same half-life as the plasma hycanthone (Table I). This suggests a reversible binding of hycanthone to sites within the tissues.

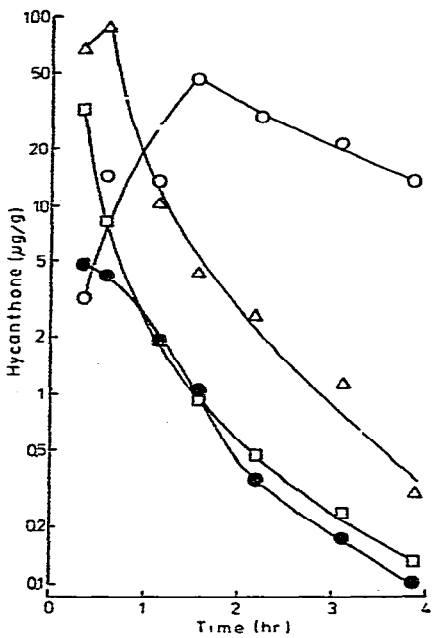

Fig. 3. Plasma and tissue levels of hycanthone •, plasma; □, liver; △, lung; and ○, gastrointestinal tract. Each point represents a pooled sample from 6 mice administered hycanthone i.p. at a dose of 80 mg per kg body weight.

Hycanthone binds reversibly with high affinity to double stranded DNA by intercalation [13] but it may also bind to other macromolecules. Hycanthone has been reported to be an alkylating agent and to bind covalently to tissue macromolecules [14], although this has not been found by all workers [15]. Hycanthone bound covalently to tissue marcromolecules would not be detected by solvent extraction.

Tissues can be divided into three types depending on the time course of the appearance and disappearance of hycanthone. Examples are shown in Fig. 3. In liver, epididimal fat pad and brain the peak levels of hycanthone occur within 15 min of administration. In lung, kidney and heart muscle the peak levels of hycanthone are not reached until 30 min. The ratio of peak tissue concentra-

TABLE I

TISSUE HYCANTHONE CONCENTRATIONS

Tissue	Peak concentration ($\mu g/g$)*	Tissue: plasma concentration ratio**	Biologic half-life (min)***
Plasma	4.9	–	56
Kidney	89.4	21.1	42
Lung	89.2	21.0	38
Fat pad	47.1	9.7	34
Brain	42.8	8.8	35
Liver	32.4	6.7	47
Heart	11.2	2.6	51
Gastrointestinal tract§	47.0	44.3	80

*Values are the mean of 3 determinations on pooled samples from 6 mice.

**The plasma concentration employed was that at the time of the peak tissue concentration.

***This represents the postdistributive half-life ($t_{1/2}^{\beta}$).

§ Including contents. The peak concentration is much delayed (see Fig. 3).

tions of hycanthone to the plasma hycanthone concentrations are shown in Table I. In the gastrointestinal tract, which includes the intestines, caecum and colon together with their contents, there was a delayed rise in the level of hycanthone. This can be attributed to a biliary excretion, which in most species is the major route of elimination for hycanthone and its metabolites [11].

Hycanthone metabolites were present in several tissues. This is in contrast to previous animal studies conducted with radioactively labelled hycanthone in which only liver and bile were found to contain metabolites [9]. Hycanthone N-oxide was detected in kidney (Fig. 2), liver, lung, and gastrointestinal tract, and at low levels in plasma. Hycanthone acid was present in liver and gastrointestinal tract. An unidentified metabolite with a retention time of 14 min was present in liver and in smaller quantities in kidney (Fig. 2), lung, and heart. A second unidentified metabolite with a retention time of 20 min was found only in the gastrointestinal tract. These unidentified peaks may represent either the sulfoxide or deethylated metabolites which have been found in rat bile and liver [11, 12] or the sulfone which has been found in mouse bile [16].

Clinical studies with lucanthone suggest that the gastrointestinal tract is particularly sensitive to the enhancing effects of the thioxanthenones on radiation [17]. This could be due to high levels of hycanthone or its metabolites in the gastrointestinal tract. It could also reflect a dependence of intestinal stem cells on an ability to repair sublethal radiation damage [18]. A major effect of hycanthone is to prevent the accumulation and repair of sublethal radiation damage [8]. The effect of different concentrations of hycanthone and its metabolites in various tissues on the radiosensitizing properties of hycanthone remain to be determined.

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