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Short communication

Detection and quantification of mitoxantrone in human organs

A case report

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Introduction

Mitoxantrone, 1,4-dihydroxy-5,8-bis{[2-[(2-hydroxyethyl) amino]ethyl]amino}-9,10-anthracenedione dihydrochloride (DHAD), CL 232,315, NSC 301739, is a synthetic anthraquinone designed to reduce or eliminate the cardiac toxicity seen with the structurally related doxorubicin [1]. A monograph [6] summarizes current therapeutic and biochemical studies. Our studies of mitoxantrone include evaluation of patients with solid tumors in phase-I clinical trials [2], experimental chemotherapy of patients with acute leukemia in relapse [3], and biochemical studies on the interaction of aminoanthraquinone analogs with DNA [4, 5]. The objective of the present work was to investigate whether DHAD could be detected and quantified in the organs of a patient who died 9 days after the completion of experimental chemotherapy with mitoxantrone. To our knowledge no such studies have been reported.

Case report

The patient (66-year-old man) had diffuse histiocytic lymphoma that recurred after multiple chemotherapy regimens including doxorubicin, the last dose of which had been given > 5 months previously. Treatment with DHAD was elected because of his rapidly deteriorating condition. Treatment consisted of 10 mg/m^2 DHAD (21.8 mg total dose) and 20 mg/m^2 dexamethasone given daily for 4 days, and 2 mg vincristine given on days 1 and 8. Superficially detectable tumor masses decreased by > 50% after day 3, and antitumor response, according to physical findings, was estimated to be 90% on day 8. Recurrent pleural effusions were removed on days 5 and 11. On day 8 the patient developed oral candidiasis and oral nystatin treatment was initiated. The patient became febrile (while pancytopenic) and broad-spectrum antibiotic therapy was instituted. Hepatic chemistry showed a decrease in the transaminases and a progressive increase in bilirubin up to 35 mg/dl and of LDH up to 3,588 units. His general condition deteriorated and he expired from presumed sepsis on day 13, approximately 215 h after the last dose of DHAD.

Postmortem examination 8 h after death revealed a dramatic shrinkage of clinically involved lymph nodes and of the liver. Extensive involvement of pleura, spleen, liver, and bone marrow was found. Hepatic changes consisted mainly of steatosis and cholestasis, without evidence of biliary obstruction or hepatitis, and the presence of scattered microscopic foci of lymphoma. Disseminated candidiasis, involving the stomach, esophagus, liver, kidney, and the right atrial endocardium, was suggested as the immediate cause of death.

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Analytical methodology

Serum was obtained by letting whole blood obtained by venipuncture clot at room temperature for 20 min, followed by centrifugation (500 *g*, 10 min). Pleural fluid aliquots were taken from thoracentesis specimens. Tissue samples from the patient and control were obtained at autopsy. They were hand-homogenized in 0.1 *M* Na_2HPO_4 (pH = 7.4), followed by dilution to yield 50 mg (wet weight) tissue per milliliter Na_2HPO_4 solution.

Sample preparation for blood, pleural fluid, and all tissues was as follows: after addition of 50 ng anthracenedione diacetate (internal standard) to 1-ml samples, they were acidified to pH = 1.5 with 3 *M* HCl, followed by prewashing with 4 ml chloroform (no drug is extracted at pH = 1.5). After centrifugation (50,000 *g*, 4° C, 10 min), the aqueous layer was retained and the pH adjusted to 11 with 30% NH_4OH. The drug was extracted with 4 ml dichloromethane (vortex 2 min, centrifuge 50,000 *g*, 4° C, 10 min). The organic layer was transferred into a prewashed vial. To the remaining aqueous layer 3 *M* NaOH was added and the dichloromethane extraction repeated. The combined organic fractions were evaporated to dryness (N_2 stream, water bath at 35° C). The residues were reconstituted with 125 µl mobile phase (see below) and 100-µl aliquots injected into the chromatograph (Waters Associates, Milford, MA). High-performance liquid chromatography (HPLC) was carried out on a µBondapak C_{18} column (Waters Associates), 30 cm long × 3.9 mm i.d., 10 µm particle size. The mobile phase was a mixture of 53% methanol and 47% of an ion pair reagent (pentanesulfonic acid, PIC B-5, and heptanesulfonic acid, PIC B-7, prepared according to directions from Waters Associates). Flow rate: 1.4 ml/min; detection: UV absorbance at 254 nm (sensitivity setting: 0.005 AUFS); chart speed: 0.2 cm/min.

Quantification was accomplished by establishing an independent calibration curve for serum and each tissue, utilizing normal samples to which successively larger known amounts 0, 50, 100, 250, 500 ng/ml) of pure DHAD (courtesy of Lederle Laboratories, Pearl River, NY) and constant amounts of internal standard (50 ng) were added.

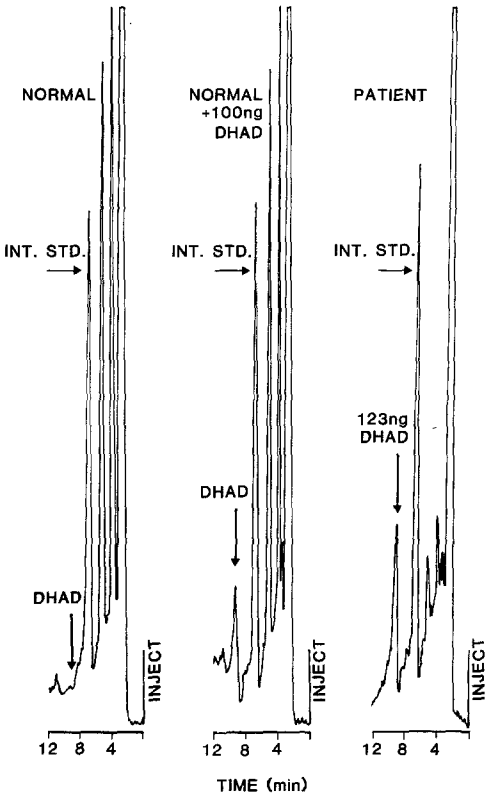

Fig. 1. Detection and quantification of mitoxantrone (dihydroxyanthracedione, DHAD) in heart tissue obtained at autopsy. *Left*: extract from patient who had not been treated with DHAD and did not have cardiac disease. *Middle*: normal heart tissue spiked with 100 ng DHAD. *Right*: extract from patient treated with DHAD. *Arrows* indicate the chromatographic retention time of DHAD in the HPLC profiles

Results and discussion

Retention times of DHAD were determined using pure compounds and confirmed by co-chromatography. It can be seen in Fig. 1 that the profiles of early eluting peaks were different in the patient and the control; this was not related to DHAD and represented repeatedly observed patient-to-patient tissue variability. HPLC profiles of the spleen, liver, and kidney tissues were similar in appearance to Fig. 1, and DHAD was detected in each of the patient samples.

Quantification was accomplished by calibration curves obtained using spiked samples. Plots of DHAD vs observed peak height ratios (DHAD/int.std.) yielded straight lines. Typical regression analysis values were: intercept = −0.008, slope = 0.0025, and $r = 0.996$. The drug was quantified in each tissue using a separate calibration curve prepared from undiseased human tissue of the same type obtained at autopsy. Table 1 summarizes the DHAD content of each tissue.

The DHAD concentrations in the pleural effusion and serum were 23 ng/ml and 44 ng/ml, respectively, 8 h after the last dose of DHAD. On day 11 the DHAD level in the pleural effusion was below the detection limit (< 10 ng/ml) and the serum level was 14 ng/ml (lower limit of quantification).

The following conclusions may be drawn: (a) substantial amounts of DHAD were present in all four organs (heart, liver, spleen, kidney) 9 days after the last day of drug administration; concentrations were approximately 200-fold that in the peripheral blood serum taken 2 days prior to death; (b) the same concentration (approximately 2 μg/g) of DHAD was found in the heart, spleen, and kidney, suggesting that these organs have the same affinity for the drug, thus diminishing the likelihood of tissue-specific binding sites; (c) a higher concentration (4.2 μg/g) of DHAD was found in the liver; since the liver weighed 2,500 g, it contained approximately 12% of the total dose administered; (d) the DHAD concentration in peripheral blood serum had decreased to a very low level (44 ng/ml) 8 h after the last administration of mitoxantrone and was present only at the limit of quantification (14 ng/ml) on day 11. These findings are in agreement with the expected pharmacokinetic behavior of the DHAD in blood [2]; (e) DHAD was distributed in the pleural fluid at a somewhat lower concentration than in serum 8 h after the last dose of DHAD (23 ng/ml vs 44 ng/ml) and was not detected in the pleural fluid 6 days later.

Table 1. DHAD content of organs 9 days after last dose[a]

Organ	Weight (g)	DHAD (μg/g)	Total DHAD (mg/organ)
Heart	440	2.4	1.1
Liver	2,500	4.2	10.5
Spleen	470	2.2	1.0
Kidney	315	2.2	0.7

[a] Dose of DHAD: 21.8 mg (10 mg/m^2) daily on days 1, 2, 3, 4. Patient died on day 13; autopsy 8 h after death

The fact that significant amounts of DHAD were found in several major organs 9 days after the last drug administration and 3 days after serum levels had fallen to the lower limit of quantification implies a reservoir function of the viscera, which may be important in developing therapeutic regimens for mitoxantrone.

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