

Pharmacokinetics Of Adriamycin After Intravenous Administration In Rat.

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Citation

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Abstract

Pharmacokinetics of adriamycin in the rat testis, heart and plasma was investigated using male Wister rats. The control group received 0.9% saline given I.V. One group of adriamycin treated rats received 20mg/kg adriamycin administered intravenously. The second group received 1mg/kg adriamycin also given I.V. Thereafter, the animals were sacrificed 1, 3, 12 and 24 hours and the testes and hearts were removed. The plasma was recovered from the blood. Using fluorimetric method, the amount of adriamycin in the tissues and plasma was quantitated. The half-life of the drug in the tissues and plasma was calculated from the graph of log adriamycin concentration (ug/g) vs. time. The pattern of distribution in the heart and plasma at the two doses used in the pharmacokinetic studies revealed an exponential fall in drug concentration with time. In the testis on the other hand, after the initial drop on the level of the drug in this organ, the level of the drug was elevated with time. This ability of the testis to accumulate adriamycin renders it highly susceptible to the lethal effects of adriamycin and thus accounts for the observed drug induced testicular toxicity.

INTRODUCTION

Adriamycin (ADR) is currently one of the most useful antineoplastic agents. It is effective and useful in the treatment of malignant lymphomas, acute lymphoblastic leukemia, carcinomas of lung, breast, ovary, endometrium, testis and thyroid (1). The clinical usefulness of ADR is hampered by the occurrence of many adverse effects including congestive heart failure, damage to gastrointestinal mucosa, nausea and vomiting, myelosuppression, alopecia as well as damage to the kidney, liver and nervous system (1,2,3). As and Hsu (3) have shown that ADR is toxic to germinal and somatic cells of the mouse after acute administration.

Further, we have also observed and reported that the drug decreased calmodulin level, cyclic adenosine monophosphate (CAMP) and PDE activity in the testis of rats chronically treated (5). In order to understand fully the mechanism of adriamycin induced effects on the testis, understanding of the pharmacokinetics of the drug in this organ is essential and hence this investigation. This study was extended to the heart and plasma in order to correlate the results obtained in the testis with the previously reported ones.

MATERIALS AND METHODS

Male Wister rats weighing between 250 – 300 g were used in the studies. The rats were obtained from the Department of animal and Environmental Biology of the University of Port Harcourt and kept in the departmental animal house at least 7 days before use. They were fed with food and water ad libitum. The animals were divided into two groups viz the control group consisted of five rats and these were given 0.9% saline solution intravenously. The treated groups were further divided into two sub-groups. One sub-group consisted of twenty (20) rats and these were intravenously administered 20mg/kg adriamycin dissolved in 0.9% saline. The other sub-group consisted of twenty (20) rats and these received 1mg/kg adriamycin which was dissolved in 0.9% saline and given intravenously. Thereafter, five (5) rats in each sub-group were sacrificed at 1, 2, 12 and 24 hours post intravenous injection. The control animals were also sacrificed at 24 hours after injection. The plasma was collected from the blood and the heart and testis were immediately excised from each animal. The heart and testis were homogenized with 10 volumes of 0.3N HCl in 50% ethanol per gram of tissue. The homogenate was centrifuged at 20,000g for 20 minutes at 4°C. Tissue homogenates and extracts were protected from light and kept at 0-4°C during processing.

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The fluorimetric method of bachur et al. (6) was used to quantitate the level of adriamycin in the heart and testis. The amount of drug contained in each tissue was determined by comparing the fluorescence of the standard solutions of adriamycin made in the 0.3N HCL50% ethanol solution. The fluorescence was measured at a wavelength of 585 nm in 5cm quartz cuvettes with Aminco Bowman spectrophotofluorometre using an activation wavelength of 470 nm. The spectrophotofluorometre was standardized with extracts from control animals before it was used.

Plasma concentration of adriamycin was determined by the method of Benjamin et al. (7)

The plasma was sonicated for dispersion of insoluble materials. Two ml. of 0.3N HCl in 50% ethanol was added to 2ml of plasma and allowed to stand at 4C for hours. Thereafter, the gelled mixture was centrifuged at 20,000g for 20 minutes. The fluorescence of the supernatant solution, total drug fluorescence was determined as described above. Adriamycin Hydrochloride was obtained from synthesis and chemistry Branch, National cancer, institute, NLH, Bethesda MD and Farmitalia, Milan, Italy.

RESULTS

Table 1 show the distribution of ADR (20mg/kg) at 1,3, 12 and 24 hours after i.v. administration in the rat heart, testis and plasma. The pattern of distribution of the drug is the same in the heart and plasma but different in the testis. In the heart, the highest concentration of the drug was observed at one hour after administration. The levels of ADR at 20mg/kg and 1mg/kg in the heart after one hour of drug administration were 39.33 1.33 ug/g tissues and 3.63 0.37 ug/g tissues, respectively. The level of the drug thereafter decreased with time and thus by 24 hours after injection, the level of ADR in the heart of rats given 20mg/kg was 7.07 1.17 ug/g tissues whereas the level of the drug in the heart of rats given 1mg/kg was 0.82 0.04 ug /g tissues. The drug plasma levels were much lower than the levels observed in the heart but the pattern of disappearance was the same as that observed in the heart.

In the testis of rats given ADR (20mg/kg), the level of ADR was 3.07 0.47 ug /g tissues after one hour of drug administration, whereas the level of the drug in the rats given ADR (1mg/kg) was 0.78 0.00 ug/g tissues at 3 hours and rose to 2.23 0.23 ug /g tissues at 12 hours after the administration of the drug. The rise in the level of the drug was again observed at 24 hours after drug injection (2.4 0.23 ug/g tissue). This pattern of distribution was also observed in

the testis of rats given 1mg/kg ADR.

The half life of ADR as indicated by fluorescence is shown in table 2. In the heart, the half life of ADR (20 mg/kg) within the first 12 hours of drug injection was 9.35 hours, whereas at 1mg/kg, the half life was 8.88 hours.

Figure 1

TABLE 1: DISTRIBUTION OF ADRIAMYCIN (ADR) FLUORESCENCE IN NORMAL RAT PLASMA, HEART AND TESTIS

DOSE	HOURS AFTER TREATMENT	HEART ^a	TESTIS ^a	PLASMA ^b
20mg/kg	1	39.33±1.3	3.07±0.47	1.20±0.00
	3	26.00±0.5	1.67±0.17	0.55±0.01
	12	14.50±0.5	2.23±0.23	0.30±0.00
	24	7.07±1.17	2.40±0.23	0.13±0.02
1mg/kg	1	3.63±0.37	0.78±0.00	0.21±0.05
	3	NOT DETERMINED	NOT DETERMINE D	NOT DETERMINE D
	12	1.50±0.00	0.39±0.00	0.13±0.03
	24	0.82±0.04	0.52±0.00	0.039±0.00

^a values represent micrograms per gram weight and mean S.E. of five animals.

^b values represent micrograms per milliliter and are mean S.E. of five animals after intravenous injections of ADR (20 mg/kg or 1mg/kg) and 0.9% saline, the animals were sacrificed at 1,3,12 and 24 hours. The heart, testis and plasma were removed from each animal and the distribution of ADR was determined as described in the METHODS.

Figure 2

TABLES 2: THE HALF LIFE OF ADRIAMYCIN (ADR) FLUORESCENCE IN NORMAL RAT PLASMA, HEART AND TESTIS.

ADR HALF LIFE (HRS)				
DOSE	TIME INTERVAL (HRS)	HEART	TESTIS	PLASMA
20mg/kg	1-12	9.65	34.35	5.73
	12-24	(60.34)	(5.34)	(2.32)
		16.25	-79.30	12.55
	1-12	8.88	11.27	16.52
	12-24	(6.72)	(1.43)	(0.75)
		17.01	-23.99	--

The half of ADR fluorescence in the rat plasma and testis was determined from the results of pharmacokinetic studies presented in table 1.

Values in parenthesis represent concentration of adriamycin (ADR) at zero time (Co) expressed in uN. Co was calculated by extrapolation from the graph of log ADR concentration (ug/g) vs. time and then converted to um. Graph was constructed from the data in table 1.

From 12 hours to 24 hours after the injection of the drug, the half life of the drug (20 mg/kg) was 16.25 hours and that of 1 mg/kg was 17.01 hours. The plasma life of ADR at 20 mg/kg in the first 12 hours of drug administration was 5.73 hours whereas that of 1mg/kg was 16.52 hours. The plasma half life of ADR at 20 mg/kg from 12 hours to 24 hours after the injection of the drug was 12.55 hours. The half life of ADR (1mg/kg) in the plasma within the first 12 hours to 24 hours after the injection of the drug was 16.52 hours and that between 12 and 24 hours after administration was not determined. In the testis, the half life of ADR was longer than those obtained in the plasma and heart. In the first 12 hours after drug injection, the half of ADR (20mg/kg) was 79.30 hours. At 1 mg/kg, the half life of ADR in this tissue in the first 12 hours after drug injection was 11.27 hours while at 12 to 24 hours interval the half life was 23.99 hours.

DISCUSSION

The pattern of distribution of adriamycin in the heart and plasma at the two doses used in the pharmacokinetic studies revealed an exponential fall in drug concentration with time. The results of this study are in agreement with the reports of other investigators (7,8,9,10,11) and accordingly authenticate the adriamycin pharmacokinetics in the rat testis which hitherto had not been reported.

The low concentration of the drug in the plasma indicates that the drug is rapidly taken up by body tissues. The testis, on the other hand, had a different pattern of distribution, ie, after the initial drop of the level of the drug in this organ, the level of the drug was elevated with time. Weiss and Manthei (12) have suggested that the differences observed in the tissue distribution of doxorubicin (adriamycin) may be due to differences in the ability of the tissues to excrete the accumulated drug by active transport process. These authors agree that heart cells as well as those of liver and kidney possess this pump and hence the drop in the concentration with time, whereas other tissues as bone marrow, spleen and some tumor cells lack or are deficient in their ability to excrete the drug. Testis may belong to the family of these tissues which lack or are deficient in their ability to excrete the drug.

In conclusion, factors such as (1) the ability of the testis to accumulate adriamycin and probably inability to excrete the drug and (2) our finding that adriamycin binds to calmodulin (a calcium binding protein found in abundance in the mammalian testis) (13) make this testicular organ highly susceptible to the lethal effects of adriamycin and consequently may account for the observed drug induced testicular toxicity

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