STUDIES ON ADRIAMYCIN SIDE EFFECTS ON THE RENAL FUNCTION AND MORPHOLOGY IN RATS

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INTRODUCTION

Adriamycin (ADR) is an anthracyclic antibiotic (Di Marco et al., 1969). It is considered now to be a very effective and useful chemotherapeutic agent in the treatment of many human solid tumors and malignant heamatological processes (Carter et al., 1972). However, its extensive use at doses adequate for effective antitumor therapy is restricted by the appearance of a severe cardiotoxicity (Adamson, 1974; Bristow et al., 1978; and Nagineni, 1985). Little is known about the effect of ADR on the kidney function and its relation to any pathological changes. Our study aims to reveal this relation after 4 and 8 weeks of ADR treatment.

Materials and methods

Animals: Experiments were carried out on white male rats weighing between 200-300 grams. They were allowed acess to food and water ad. libitum. Five rats were injected i.v. with normal saline twice/week (served as control), and twinty rats arranged in two groups underwent treatment with adriamycin (I mg/kg b.w. twice/week i.v.) for four weeks and eight weeks respectively. Urine samples were collected over 24 hours period from control and adriamycin treated groups by using metabolic cages. Then the animals were sacrified for sampling analysis.

Biochemical and histochemical investigations :

Serum and urinary creatinine were determined by the method of Whiting et al., 1982. Creatinine clearance was calculated applying the following formula : Clearance = (P x V / u) m/min.

where P is the plasma concentration (mg/100 ml)

V is the urine volume (ml/min)

u is the urinary concentration (mg/ 100 ml)

Serum and urinary proteins were measured by Lowry et al., 1951, method. One kidney from each animal was taken for histopathological examination; fixed in 10% formalin solution and embedded in paraffin. Sections from 3-7 u thick were stained by haematoxylin and eosin and examined by light microscopy: A piece of the other kidney was dried; lipid extracted and prepared for sodium and potassium determination by the Unicam SP 90 A Series 2 Atomic Absorption Spectrophotometer apparatus following the method of Zettner and Seligson, 1964.

RESULTS

General toxicity and survival :

Toxicity was expressed as severe weakness, weight loss, progressive loss of appetite and middorsal alopecia in the adriamycin treated rats. The mean survival percent was recorded in Fig. 1 where it shows successive decrease in the number of rats in adriamycin treated group over 8 weeks reaching 50% of the treated group.

Biochemical Study :

Table (I) illustrates significant increases in the plasma creatinine of the adriamycin treated rats for 4 week or 8 weeks, while significant decreases were observed in both the urinary creatinine and creatinine clearance in these rats. The effect was more severe after the last period (8 weeks).

In table (2) plasma proteins show significant decreases specially after 8 weeks of adriamycin treatment where the per cent of change was (-27.36). Protein excretions in adriamycin treated rats (Fig. 2) were very high where their means are 30.54 and 40.78 mg/ hour after 4 and 8 weeks respectively.

Table (3) shows the sodium (Na) and potassium (K) contents of the kidney in control and treated rats. Comparing with the control, adriamycin treatment caused significant decreases in the renal contents of potassium while the change in sodium content was not significant.

Histopathological findings : The light microscopic findings in the kidneys of adriamycin treated rats show focal and segmental glomerular sclerosis with thickening of basement membrane of Bowman's Capsule (Fig. I. A)The proximal convoluted tubules

show cloudy swelling (Fig.I.c). 40% of the treated animals show dilated collecting tubules with eosinophilic red casts in their lumens (Fig.I.D). 60% show, focal interstitial inflammation and fibrosis (Fig.1.A). The mentioned pathological findings are seen in both treated groups, but more marked in 8 weeks treated one.

DISCUSSION

The fact that the antitumor antibiotic adriamycin has a glycosidic structure which resembles some glycosides with cardiotoxic effect, and the fact that cardiomyopathy induced by chronic intoxication with adriamycin present very marked alterations in Na and K contents (Olson, 1974) promoted us to study the effect of this drug on the kidney function and morphology and their relation to the Na and K contents of the kidney.

Biochemical studies :

In the present; work the results showed significant decrease in K content, but not any significant change in Na content in the kidney in all adriamycin treated rats. These results can be interpreted by the preliminary studies of Gosalvez et al., 1979 who showed that adriamycin is a potent inhibitor of Na-K ATP ase of native heart microsomes and inhibits K-transport (although not Na-transport) in slices of kidney cortex. In contrast; Richard et al., 1987 found that, the reduction in renal blood flow in adriamycin treated rats contributes to sodium and water retention. An intriguing result is the finding that adriamycin causes a nearly complete inhibition of K-reaccumulation while failing to affect Naextrusion. The explanation may be that the drug has uncoupled the sodium transport aspects of the system from its dependency on K. This would represent a novel inhibitory effect in ion transport (Gosalvez et al., 1979).

Creatinine is a substance that has a maintained plasma level by its continuous endogenous production due to muscle catabolism and is indirectly affected by diet. Given a constant rate of production, and for negligible tubular reabsorption and extra renal losses, the plasma level of creatinine depends directly on the GFR and considered to be a good index of the degree of impairment of GFR. All adriamycin treated rats has developed nephrotoxicity by the time of study as judged by an increased plasma creatinine and decreased its concentration in the urine (Table 1), thus leading to

decreased creatinine clearance. The present results also revealed high concentration of proteins in the urine of adriamycin treated rats. This established proteinuria (Fig. 2) may explain the significant decrease in plasma proteins (Table 2) which was also recorded by Johan et al., 1980. Our results are consistant with the findings of Bertani and Remuzzi, 1983; and Bertani et al., 1986, whome reported that a single injection of 5 mg/kg of adriamycin promotes in rats a state of heavy and persistent proteinurea. Conversly Bertani et al., 1986 showed that the GFR measured as clearance of endogenous creatinine at the end of the experimental period was not different from the values found in control rats although the plasma creatinine was declined. This difference in the results may be related to the mode and time of the drug treatment.

The effect of adriamycin was more severe after 8 weeks treatment.

Pathological findings :

The present study is a trial to correlate proteinuria with changes in kidney function and structure in rats. The detected proteinuria and decreased creatinine clearance in ADR treated rats can be explained by the pathological findings of focal glomerulosclerosis, tubular cloudy swelling and interstitial inflammation with fibrosis. These findings are in agreement with Johan et al., 1980 and Bertani et al.. 1986.

The renal changes appear to be due to toxic effects of the drug on the glomerular structure resulting in an increased permeability with protein casts and proteinuria. This concept is suggested previously by Bertani and Remuzzi, 1983.

The interstitial reaction may be evoked by leakage of protein from the distended tubules and not related to direct effect of the drug (Bertoni et al., 1986). The aggrevation of the lesions; 8 weeks treated rats is due to the cumulative effect of the drug.

Conclusion :

Adriamycin has a toxic effect on the kidney and this effect is related to the period of treatment. The authors advise interrupted courses of treatment if long duration treatment is needed to avoid the comulative effect of the drug, with investigation of kidney function.

SUMMARY

Adriamycin (ADR) is an antitumor antibiotic, which is widly used in can-

cer chemotherapy. Previous studies revealed cases of heavy proteinuria following single injection of 5 mg/kg ADR. Marked alteration in sodium and potassium contents were seen in cases of cardiomyopathy induced by chronic intoxication with ADR. We are interesting to study the relationship between renal sodium and potassium contents and proteinuria, in adriamycin treated rats (1 mg/kg b.w. twice a week for 4 and 8 weeks), and the renal function and morphology. The results showed impaired renal function as revealed by the significant increase in plasma creatinine and decreased urinary creatinine and creatinine clearance in ADR treated rats. ADR treatment also produced significant decrease in plasma proteins and proteinuria . Sodium content of the kidney was not significantly changed, while potassium content was significantly decrease in ADR treated rats. These finding are consistant with the pathological examination which show glomerular sclerosis, cloudy swelling of the proximal convoluted tubules, dilated collecting tubules with protein casts and inflammation of the interstitial tissue. These changes are more marked in 8 weeks ADR treated rats. The study advise interrupted courses of ADR in the long treatment periods, with investigations for the kidney function.

Table (I) : Plasma (P) and urinary (U) creatinine in mg/l , and creatinine clearance (C) in ml/minute, in control and adriamycin treated rats.

	Control			Adriamycin (1mg/kg)					
				4 weeks			8 weeks		
	P	U	C	P	U	C	P	U	C
Mean	7.26	304.6	0.51	7.80	246.80	0.43	9.60	182.60	0.35
± S. E	0.08	1.66	0.006	0.06*	1.78*	0.008*	0.11*	1.76*	0.008*
% of change				+7.44	-18.98	-15.69	+32.23	-40.05	-31.37

n = 5

* = Significantly different from control ($P < 0.005$).

Table (2) : Total plasma proteins (ing / 100 ml) in control and adriamycin treated rats.

	Control	Adriamycin (1mg/kg)	
		4 weeks	8 weeks
Mean ± S. E	6.58 + 0.05	5.86 ± 0.05*	4.78 + 0.05*
% of change		-10.94	-27.36

n = 5

* = Significantly different from control (P < 0.005).

Table (3) : Renal sodium (Na) and potassium (K) contents in control and adriamycin treated rats.

	Control		Adriamycin (1mg/kg)			
			4 weeks		8 weeks	
	Na	K	Na	K	Na	K
Mean	20.56	38.72	20.68	33.16	20.22	29.44
± S. E	±0.24	±0.24	±0.27	±0.34*	±0.28	±0.27*
% of change			+0.58	-14.36	-1.65	-23.97

n = 5

* = Significantly different from control (P < 0.005).

Na and K were expressed as meq/100 g dry fat free tissue.

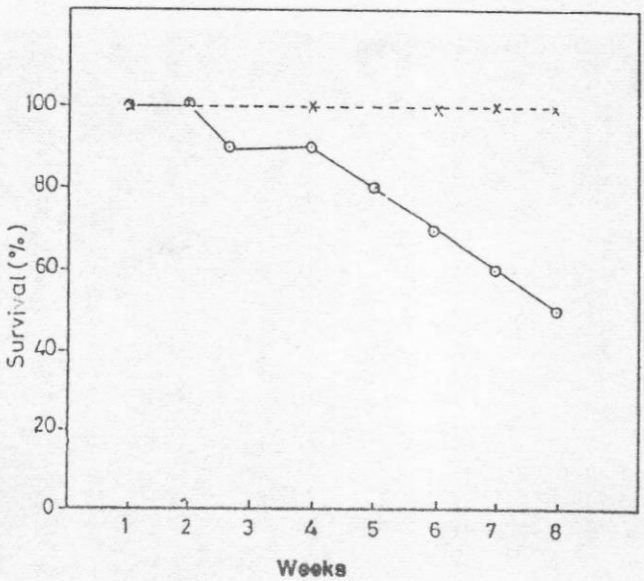


Fig. 1 : Effect of adriamycin on the survival percent in white rats.

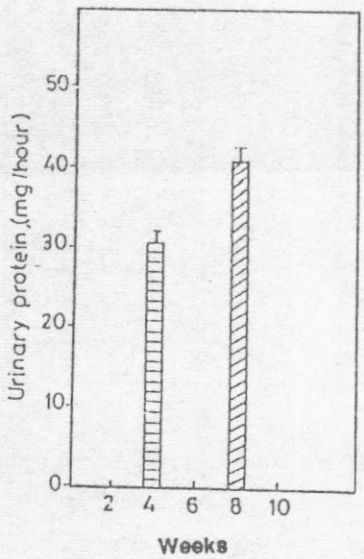
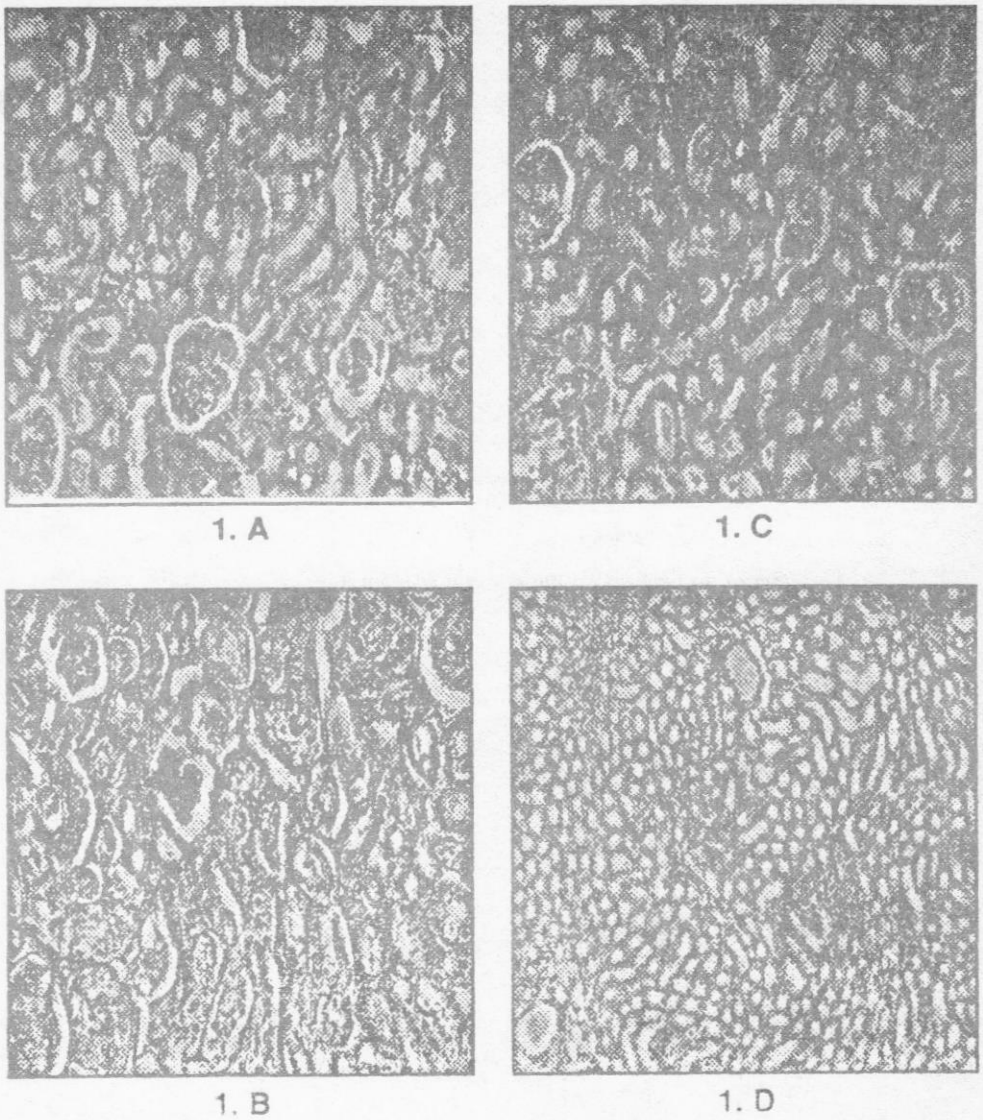


Fig. 2 : Protein excretion in adriamycin treated rats results are expressed as mean ± SD.



1. A

1. C

1. B

1. D

Fig. 1. Sections in the kidneys of adriamycin treated rats. HX E (x400) showing :

- Focal and segmental glumerulo-schrosis and interstitial inflammation. After 4 weeks (A) and 8 weeks (B) treatment respectively.
- Cluody swelling of the proximal convoluted tubules of 8 weeks treatment (C)
- Dilated collecting tubules distended with eoslinophilic protein casts (D) 8 wekks treatment.

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الملخص العربى

دراسات على الآثار الجانبية للادرياميسين على وظيفة وشكل الكلية فى الفئران

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يعتبر عقار الادرياميسين من أهم المضادات الحيوية المستخدمة فى علاج الأورام السرطانية بكافة أنواعها. وفى هذا البحث، حقنت ذكور الفئران البيضاء التى تزن حوالى ٢٥٠حم فى الوريد الذيلى بجرعة قدرها ١محم/كجم من الادرياميسين مرتين أسبوعياً لمدة ٤ أسابيع و٨ أسابيع لمعرفة تأثير هذا العقار على وظيفة وشكل الكلى وأوضحت الدراسة مايلى :-

١- زيادة معنوية فى كرياتينين البلازما الخاص بالفئران المعاملة بهذا العقار بعد المدد المذكورة بينما قل تركيزة فى البول وقلت درجة تنقيته، معنوياً.

٢- ظهور البروتين فى بول الفئران المعاملة بدرجة كبيرة، مع نقص بروتينات البلازما معنوياً.

٣- نقص محتوى أنسجة الكلية من البوتاسيوم وعدم تغير محتواها من الصوديوم معنوياً فى هذه الفئران.

٤- أوضح الفحص الباثولوچى لكلى الفئران المحقونة بالادرياميسين تليف بؤرى فى الكبد، وانتفاخ فى خلايا الأنابيب البولية الملتفة القريبة. وكذلك تمدد الأنابيب الجامعة مع وجود تجمعات بروتينية بداخلها. كما وجدت خلايا التهابية فى النسيج البينى.