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The Influence of Inosine on Adriamycin-Induced Cardiomyopathy in Rats

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Abstract—*The effect of inosine on adriamycin-induced cardiomyopathy was studied. Total adriamycin (ADR) dose of* 25 mg/kg *i.p. injected in* 15 *equal partial doses* 3 *times a week for five following weeks evoked fully developed cardiomyopathy in rats. Inosine* 200 mg/kg *i.p. injected* 5 *times a week parallel to ADR diminished ADR cardiotoxicity evaluated by electrocardiographic recordings and histopathological examination. Moreover lower cytostatic toxicity was observed as judged by less-expressed leucopenia and lower SGOT activities in inosine treated animals*

INTRODUCTION

THE ANTHRACYCLINE antibiotic adriamycin is a broad spectrum and potent chemiotherapeutic agent [1, 2]. Clinical usefulness of ADR is limited by late, sometimes life-threatening cardiomyopathy which reveals itself in patients administered a cumulative dose exceeding 550 mg/m^2 [3].

Many efforts were undertaken to diminish ADR toxicity while not changing its antitumor activity [4–6]. Several analogs and new anthracycline derivatives were synthetized without satisfying results. Different treatment regimens [7, 8] and some pharmacologic agents like vitamin E [4], coenzyme Q_{10} [9], razoxone [10] and ascorbic acid [11] had also only limited beneficial effect. The mechanism of ADR cardiotoxicity is not clearly established. Some authors suggest that it is due to lipoperoxidation [4], others that generation of free radicals is the most deleterious [5]. Lately it was suggested that it is caused by primary mitochondrial damage with subsequent decrease of high energy phosphates in the cell [12].

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Recently there has been a growing interest in inosine (INO), an ATP metabolite which was proved to increase coronary blood flow [13–15] and myocardial contractility in experimental animals [13–16] and in man [17]. It was also shown that INO protects ischemic myocardium [18] and stimulates the synthesis of high energy phosphates enhancing their content in the heart cell [19–21]. Although Hacker and Newman [22] presented the reduction of acute ADR toxicity by adenosine, mentioning that this effect may not concern all purines, this study was undertaken to determine if inosine influence on myocrdial cells and vasculature can diminish late ADR cardiotoxicity.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats, initial weight 240–250 g, housed in cages receiving normal rat cubes and water *ad libitum*. Animals were divided into three groups of 10. One was treated with 0.05% solution of adriamycin hydrochloride (Adriblastina–Farmitalia) diluted in 0.9% NaCl injected i.p. 3 times a week in 15 equal partial doses for 5 following weeks to the total dose of 25 mg/kg according to the previously elaborated model [23]. The second group received 2% solution of inosine in 0.9% NaCl (Inosine substance—Boehringer Mannheim) in the dose 200 mg/kg i.p. injected 5 times a week parallel to ADR admin-

istered as above. Controls were injected with adequate volumes of 0.9% NaCl. Evaluation of parameters of general ADR toxicity (leucocytosis, hematocrite, SGOT, body weight) and ECG changes (II lead, paper speed 100 mm/sec) was carried out before the onset of the treatment and once a week thereafter. Blood for evaluation was taken from sinus venosus of the eye in ether anesthesia, serum glutamic oxalecetic transaminase (SGOT) was evaluated according to Reitman and Frankel [24].

Main evaluation of cardiotoxicity consisted of histopathological and histochemical examination. Rats were sacrificed 25 days after the last dose of ADR. Slices of myocardium were excised in a routine manner according to the transversal line running through both ventricles in their middle length. The samples were fixed in buffered formalin, then frozen or embedded in paraffin. The paraffin sections were stained with hematoxilin and eosin, with methyl green pyronin or according to the van Gieson method. On the frozen sections the histochemical reactions for acid phosphatase, ATP-ase, TPP-ase, succinic dehydrogenase and sudan black staining were performed. The pathologic grades of myocardial toxicity due to ADR administration were estimated in a blind manner as suggested before [23], according to the scale proposed by Billingham *et al.* [25]. To reveal the percentage of the altered cardiocytes the quantitative analise of 100 microscopic fields in each experimental group was additionally processed.

The data was analysed using an unpaired Student's *t*-test with statistical significance defined for probability values less than 5%. Presented data are mean ± S.D. values.

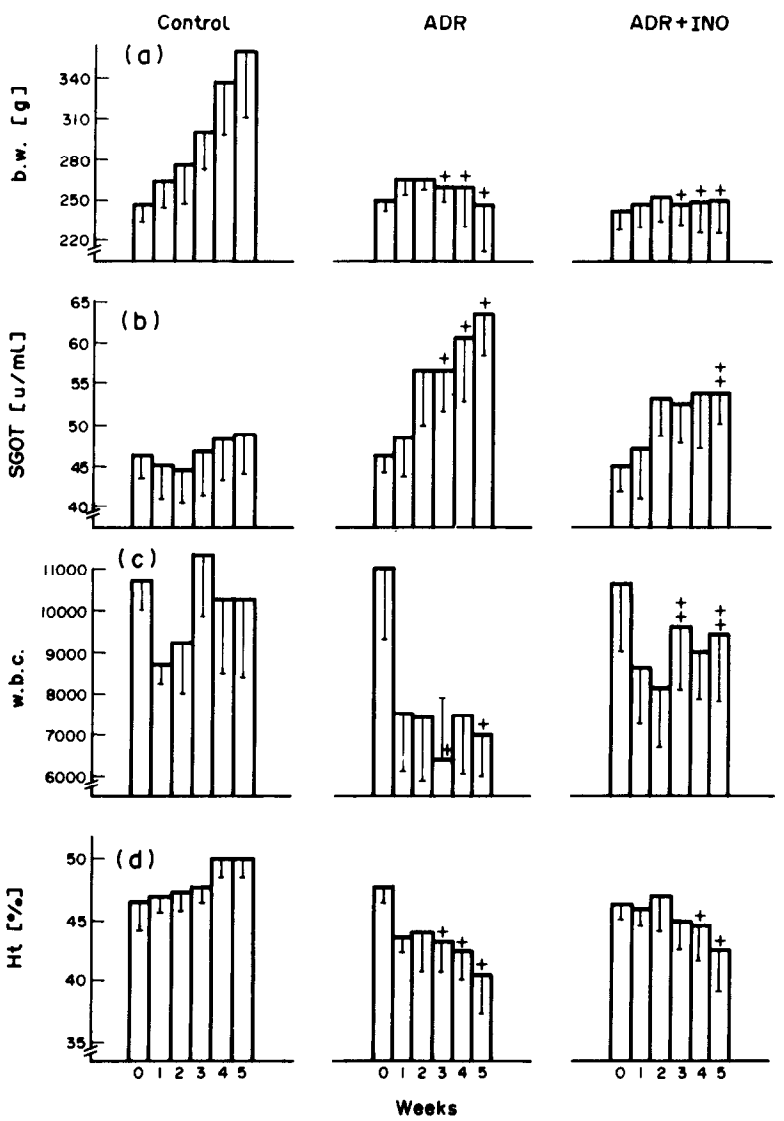

Fig. 1. Changes in general toxicity parameters in rats treated with adriamycin (ADR) or adriamycin plus inosine (ADR+INO) and in control animals. (a) *Body weight* (b.w.); (b) *SGOT activities*; (c) *white blood cells* (wbc) *count*; *Hematocrite* (Ht). †*Significantly different from control.* ‡*Significantly different from adriamycin treatment.*

Fig. 3. Left ventricle of the rat heart 25 days after adriamycin in the total dose of 25 mg/kg i.p. in 15 equal partial doses 3 times a week for 5 following weeks was administered. Marked loss of myofibrills, cytoplasmic vacuolisation (hematoxilin and eosin staining × 200).

Fig. 4. Left ventricle of the rat heart 25 days after adriamycin in the total dose of 25 mg/kg i.p. in 15 equal partial doses, 3 times a week for 5 following weeks was administered parallel with inosine 200 mg/kg i.p. 5 times a week. Loss of fibres, some cells unchanged (hematoxilin and eosin staining, × 200).

RESULTS

Body weight gain was reduced in both experimental groups [Fig. 1a]. Marked ascites has been observed in 50% of rats treated with ADR but not in rats treated also with INO. SGOT activities were higher in the group subjected to ADR treatment with statistical significance to control ($P < 0.01$) after 4 weeks of experiment and to INO treated group ($P < 0.05$) at the end of experiment [Fig. 1b]. Bone marrow supression was less expressed in rats treated with INO. White blood cells count was significantly lower ($P < 0.01$) in ADR treated group after 3 weeks of experiment as compared to control and to the group with INO protection [Fig. 1c]. Hematocrite values were significantly lower after 3 weeks of treatment in both experimental groups [Fig. 1d]. Our previous experiments with INO (dose range 200–400 mg/kg) showed no influence on general toxicity parameters, ECG recordings and morphological changes in the heart muscle of the rat [26]. In electrocardiographic recordings, rats treated with ADR plus INO showed reduction of QRS voltage and prolongation of QRS time which revealed later than in animals treated by ADR alone. These changes developed after the cumulative dose 17 mg/kg of ADR in INO protected group and were more or less comparable to that of 13 mg/kg after ADR administration (Fig. 2).

Histopathology

Heart samples taken 25 days after the total ADR dose was administered showed developed cardiomyopathy with no qualitative but marked quantitative differences betwen both experimental groups. Cells involved were characterized by marked loss of myofibrills, cytoplasmic vacuolisation and nuclear pycnosis (Fig. 3). Focal cell distention and interstitial oedema was also observed. Morphological changes, described above, were less expressed in the INO treated group (Fig. 4). In rats treated with INO they were on average 1 degree smaller in the scale proposed by Billingham *et al.* [25] (Table 1). Quantitative analysis showed significant difference ($P < 0.001$) between the number of involved cardiocytes in both experimental groups (Fig. 5) with about 30% smaller number of altered cardiocytes in INO treated animals. Histochemical reactions confirmed the inosine protection of the myocardial cells. In this group reaction for acid phosphatase was less intensive, while for ATP-ase was more intensive.

DISCUSSION

Development of cardiomyopathy is the main limitation in the anticancer treatment with anthracycline antibiotics. Numerous studies were done to establish its mechanism [4–6, 12, 27, 28]. Some authors suggested that it is due to lipoperoxidation [4], others that free radicals play the most important role [5]. It seems at the moment that mitochondrial damage [12], impairment of mitochondrial enzyme activity [27] and subsequent lack of high energy phosphates [28] in the cell may be responsible for observed changes [5, 6, 12, 29]. The results of the above-mentioned studies may indicate that ADR cardiotoxicity is probably a multifactorial damage. So much so that some of the already used protecting compounds acting on different sites such as vitamin E [4], coenzyme Q_{10} [9], rozoxone [10] or ICRF-159 [30] have had limited success in preventing ADR toxicity. Also different treatment regimens diminished it only to some extent [7, 8].

Our experimental model with ADR treated rats showed marked general ADR toxicity expressed by changes in SGOT activities, leuocytosis, hematocrite and body weight. These changes and the one observed in ECG recordings were similar to that in other studies concerning ADR treatment [29–32].

Inosine, a purine nucleotide which is formed in

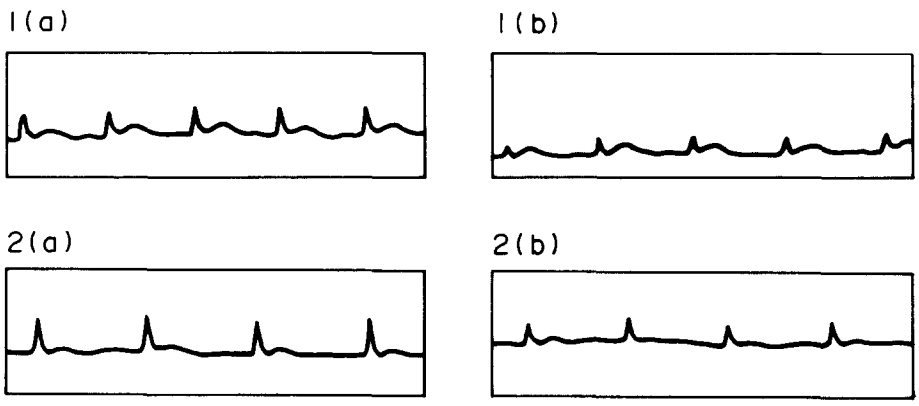

Fig. 2. ECG recordings in rats treated with adriamycin (ADR) and adriamycin plus inosine (ADR+INO); II lead, paper speed 100 mm/sec. (1) *ADR treatment*; (2) *ADR+INO treatment*; (a) *total ADR dose of* 13 mg/kg; (b) *total ADR dose of* 17 mg/kg.

Table 1. *Pathologic grades of myocardial toxicity evaluated according to the* 4 *grade scale proposed by Billingham* et al. [25] *in the rat hearts examined* 25 *days after adriamycin (ADR) and adriamycin plus inosine (ADR + INO) administration (for experimental procedure see Figs.* 3 *and* 4)

	ADR	ADR+INO
Left ventricle	3	2
Septum	3	2
Right ventricle	1	0–0/1

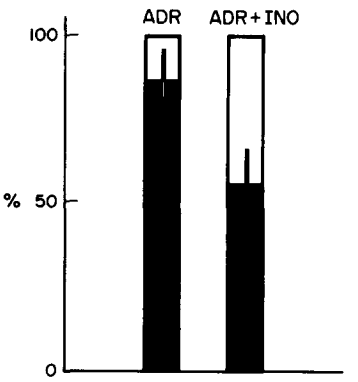

Fig. 5. *Altered and unaltered cardiocytes in* 100 *microscopic fields of the heart samples of rats subjected to adriamycin (ADR) and adriamycin plus inosine (ADR+INO) treatment. Black bars—altered cardiocytes; open bars—unaltered cardiocytes.*

the heart cell from adenosine or inosine monophosphate, was used as a protective agent against late ADR cardiotoxicity. INO increases coronary blood flow [14,15] and myocardial contractility [15,17], it also stimulates the synthesis of high energy phosphates in the heart cell [19–21]. It seems that INO administration slightly diminished the intensification of general ADR toxicity. It also delayed ECG changes to a similar extent as has been noted by others [30,32].

This experiment was designed to study cardiotoxicity and it does not attempt to solve the mechanisms of general toxicity, but the influence of INO on some parameters raises several questions. In ADR plus INO treated rats no ascites were seen. It seems that INO improving heart metabolism [18–21] may prevent, in some way, the features of congestive heart failure. Moreover, as in that case, ascites may be due to an inflammatory response to peritoneal damage, the anti-inflammatory effects of INO in the heart muscle and liver was observed by Kipshidze *et al.* [20]. INO dimished ADR evoked bone marrow toxicity in healthy animals. On the base of this study it cannot be said if this effect would also concern cancer cells. Nevertheless if INO influences ADR antitumor activity in bone marrow it could still be used in other kinds of tumors.

Described morphological changes of the heart muscle were characteristic for ADR toxicity in experimental animals [23, 29, 31] and in man [25]. Inosine protected to some extent myocardium against late ADR cardiotoxicity. Morphological changes were 1 degree lower in the used scale and about 30% lower in quantitative analysis. Histochemical reactions confirmed also less cellular damage.

The mechanism of inosine protection remains to be elucidated. It seems that the increased synthesis of high energy phosphates in the heart cell plays the most important role [19–21]. Moreover inosine-evoked increase in coronary blood flow counteracts the effects of ADR on coronary vasculature. Both these factors may influence the rate of cardiotoxicity and diminish general ADR toxicity. Only partial INO protection of myocardial cells could be explained by the multifactorial cause of cardiotoxicity [4, 5, 12, 28].

Summing up the described results it seems that INO may be a promising agent in limitation of ADR cardiotoxicity especially that it does not reveal toxic effects in humans and has been proved to be effective in some clinical trials [17, 33].

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