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CONTRIBUTION TO RESEARCH ON THE PROTECTIVE EFFECT OF PROANTHOCYANIDOL-BP1 ON ADRIAMYCIN-INDUCED CARDIOTOXICITY

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The anthracycline antibiotic adriamycin (ADR) is among the most effective drugs used for treatment of hematopoietic malignancies as well as for advanced solid malignant tumours. Unfortunately, ADR-dose-related cardiotoxicit limits the use of this drug in clinical practice. A number of cardioprotectors have been tested so far but only a few of them have shown significant cardioprotection without altering the clinical efficancy of ADR. There have been many demonstrations in animal models that natural antioxidant compounds, including flavanoids, diminsh or prevent both acute and chronic pathologic changes related to ADR treatment. The aim of this study was to evaluate whether the administration of proanthocyanidol - BP1 to ADR -treated mice could diminish or prevent ADR-cardiotoxicity. Mice were pretreated with BP1 (20mg/kg ip) or saline 3 days before ADR treatment. On the fourth day of the experiment ADR was given in a dose of 15 or 20 mg/kg ip 1h after BP1 or saline. Histological changes related to ADR toxicity in the left ventricle were evaluated 4 days after ADR treatment. No morphological changes were found in the hearts of mice receiving either BP1 or saline. In saline pretreated mice receiving 15 mg/kg ADR multiple, single or small groups of cardiac myocytes with myofibrillar lysis were found widely spread throughout the myocardium. In saline pretreated mice receiving 20 mg/kg ADR necrotic areas of myocytes were found in the left ventricle. However, mice pretreated with BP1, regardless of the ADR dose, had less pronounced histological changes. In 7 out of 8 mice in the ADR20BP1 group only a few single cardiac myocytes with myofibillar lysis were found. On the basis of the light microscopy evaluation of the hearts of ADR-treated mice it was noted that myofibrillar lysis was the prevalent type of cardiac damage. In BP1-pretreated mice the degree of cardiac damage related to ADR toxicity was less intensive in comparison with unprotected

mice. The results obtained suggest the need of further evaluation of BP1 as a potentially cardioprotective compound.

Key words: Adriamycin; cardiotoxicity; proanthocyanidols; protection

INTRODUCTION

The anthracycline antibiotic adriamycin (ADR) is among the most effective drugs for treatment of hematopoietic malignancies as well as for advanced solid malignant tumors (Carter, 1975). Unfortunately, ADR-dose-related cardiotoxicity limits the use of this drug in clinical practice (Saltiel and McGuire, 1983; Caulfield and Bittner, 1988; Dardier et al., 1989; Allen, 1992).

Cardiac tissue appears to be particularly sensitive to anthracycline damage in comparison with other tissues. Some features of cardiac tissue might explain this sensitivity. First, flavin-containing enzymes reduce ADR to its semiquinone radical that generates superoxide ($O_2^{\cdot}$) and hydroxil radicals (OH*) in reaction with molecular oxygen (Bachur et al.,1977; Doroshow, 1983). In addition, cardiac tissue contains low levels of antioxidant enzymes (SOD, superoxide dismutase; CAT, catalase; GSH-Px, gluthathione peroxidase) (Doroshow et al, 1980) which protect the myocardium from free-radical-induced injury. Furthermore, ADR itself simultaneously reduces the level of GSH-Px which effectively scavenges $O_2^{\cdot}$ and hydrogen peroxide (Doroshow et al., 1980; Davis, 1991).

Cardioprotection against ADR-induced toxicity is important because it could raise the therapeutic index of the drug. A number of cardioprotectors have been tested so far but only a few of them, particularly ICRF-187, (Spyer et l., 188) have been tested so far but only a few of them, particularly ICRF-187, (Spyer et al, 1988) have shown significant cardioprotection without altering the clinical efficancy of ADR. There have been many demonstrations using animal models, that various natural antioxidant compounds diminish or prevent acute and chronic pathologic changes related to ADR treatment (Doroshow et al, 1981; Lenzhofer et al, 1983; McCay, 1985, Powell and McCay, 1988; Danesi et al, 1990; Ferrari et al, 1991; Griesser-Aleksić et al., 1994). A number of reports on the protective effect of proanthocyanidols on the vascular endothelium (Barbier et al., 1988; Dugen, 1985; Serafini et al., 1994; Leake, 1995) as well as their ability to scavenge free-radicals have been published recently (Laughton et al, 1991; Bogdanović et al, 1995.). Their free-radical scavenging feature is several times stronger in comparison with vitamin E (Masquelier, 1987; Uchida et al, 1987; Kovač and Pekić, 1991). The proanthocyanidols are products of polymerization of flavan-3-ols to oligomers. These compounds belong to the flavanol group of compounds, which are derivates of phenyl-2-chromans (Kovač and Pekić, 1991). In our previous evaluation of the effects of proanthocyanidol-BP1 it was shown, by ESR- spectroscopy, that BP1 was strongly antioxidant. On model of acute drug toxicity involving cell lines BP1 demostrated a significant protective effect in a range of concentrations of 4-40 ug/ml (Naajman, 1994, Bogdanović et al., 1995).

The aim of this study was to evaluate if administration of BP1 to ADR-treated mice could diminish or prevent ADR-cardiotoxicity.

MATERIALS AND METHODS

NMRI mice of both sexesaged 6-8 weeks and weighing 25-30 g were used in the experiment. The mice were divided into 6 experimental groups of 16 mice each. They were housed 8 to a cage and raised in standard environmental conditions.

Proanthocyanidol-BP1 was extraced from the seeds of grapes according to the patented procedure of Pekić and Kovač, (1993). BP-1 was reconstituted in 0.85% sodium chloride (saline) and sterilized by membrane filtration (pore size 0.22μm). It was stored in tightly closed plastic tubes and protected from light until used.

Adriamycin (ADR), doxorubicin hydrochloride for injection of clinical grade quality was supplied by InexHemofarm, Vršac. ADR was reconstituted in sterile saline 1h before administration and was protected from light until used.

BP1, saline, and ADR were administered by intraperitoneal (ip) injection in a constant volume of 10 ml/kg according to the following scheme: 20 mg/kg BP1 or a similar volume of saline alone were administered 3 days before ADR, on the same day but 1 h before ADR, and during the next 5 consecutive days after ADR treatment. ADR was administered at once on the fourth day of the experiment at doses of 15 or 20 mg/kg 1h after BP1 or saline injection. Protected (ADR15 BP1, ADR20BP1) and unprotected (ADR15NaCl, ADR20NaCl) groups were formed, in that way.

Control mice were treated with saline (10ml/kg ip:K) and another group received BP1 only (20 mg/kg ip; BP1).

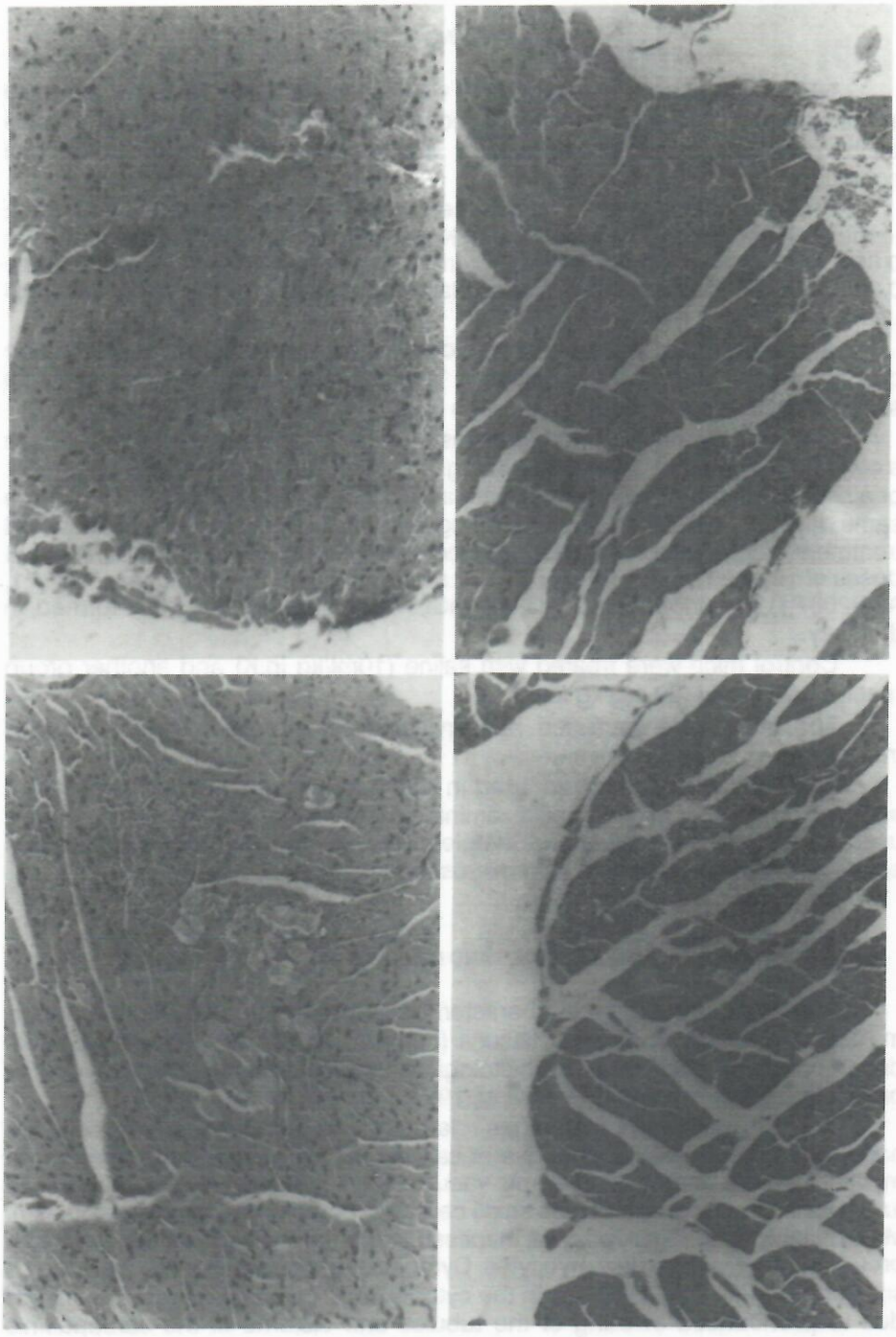
The mice were sacrificed by cervical dislocation four days after ADR treatment.

The hearts were removed, fixed in saline formaline embedad in paraffine and cestions cut for microscopis examination. Areas from the left ventricle were used for histological evaluation. Samples were stained with HE (haematoxylin eosin) and examined undera light microscope.

RESULTS AND DISCUSSION

Adriamycin produces a characteristic pattern of morphological abnormalities in a variety of different tissues (Ferrans, 1978). Mice develop ADR-induced cardiomyopathy that has histological features similar to cardiomyopathy produced by ADR in men (Myers et al, 1977; Ferrans, 1978).

The histopathological changes related to anthracycline cardiotoxicity are nonspecific, degenerative changes of cardiac myocytes usually in the form of myofibrillar lysis and/or cytoplasmic vacuolization. yofibrillar lysis is recognized furing light microscopic study as small cells with homogeneous pale cytoplasm. Myofibrillar lysis is regarded as a manifestation of interference of the drug with protein synthesis in cardiac myocytes. Cytoplasmic vacuolization, recognized as smaller or bigger light vacuoles in the cytoplasm of cardiac myocytes, is mainly due to pronounced swelling of the tubules and cisterns of the sarcopasmic



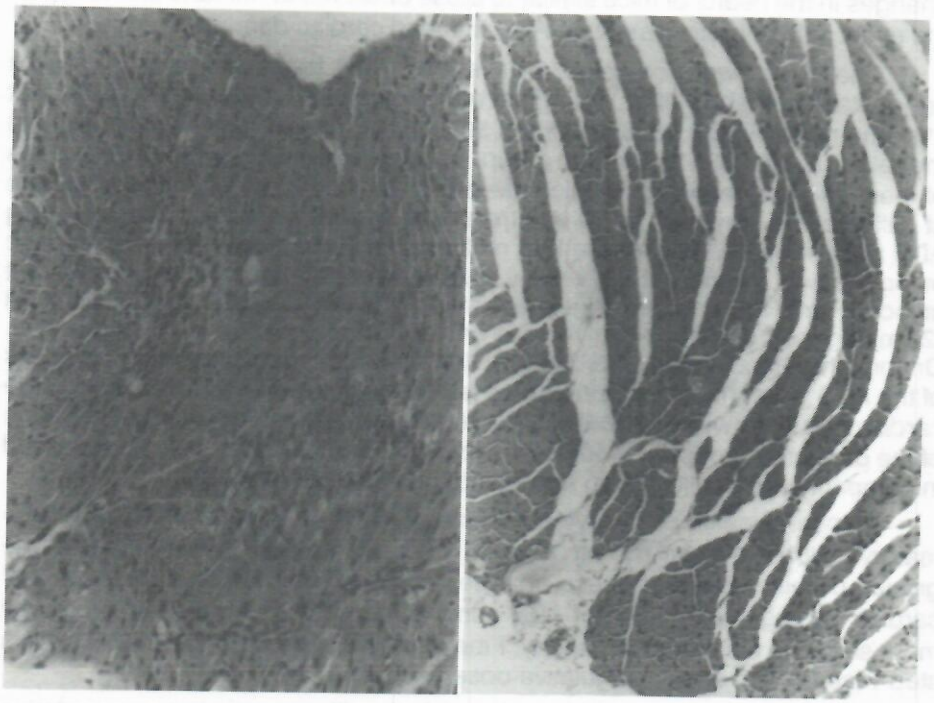


Figure 1. Effect of BP1 on ADR-induced cardiac damage. Histology of the left ventricle of the mouse heart. Light microscopy x 200.
No morphological changes in the hearts of saline (a) BP1 (b.) treated mice were found. Mice receiving 15 mg/kg ADR and saline pretreated, had multiple, single or small groups of cardiac myocytes with myofibrillar lysis wild spread (c.); in saline pretreated mice receiving 20 mg/kg ADR necrotic areas of myocytes were found (e.) Mice pretreated with BP1, regardless of the ADR dose, had a few single cardiac myocytes with myofibrillar lysis (d,f).

reticulum (SR) as well as dilatation of T – system tubules and accumulation of lipid. SR dilatation may be a manifestation of free-radical-induced damage to the membranes of the SR (Dardir et al. 1989). The nuclei of cardiac myocytes in experimental animals and in humans could be unchanged, pyknotic or completely absent depending on the degree of myocyte damage, (Dardir et al., 1989; Allen, 1992). In general, the histopathlogic al changes are present in subendocardial myocytes at earlier stages, but in advanced stages they spread widely throughout the myocardium.

The purpose of this morphological study was to evaluate ADR-induced changes in the hearts of mice similar to those observed in humans and to report the effect of BP1-pretreatment on ADR-induced cardiac damage in mice.

The hearts were sampled 4 days after ADR treatment because it was suggested (Doroshow et al., 1981) that morphological changes were maximal at this time point. Figure 1a. and 1b. are sections of the left ventricle from mice in the saline (a.) and BP1 (b.) control group. No morphological changes in the hearts of these mice were found. All mice given BP1 (20 mg/kg ip) or a similar volume of saline alone survived the 10-day treatment period. Mice receiving 15 mg/kg ADR and pretreated with saline had multiple, single or small groups of cardiac myocytes with myofibrillar lysis, widly spread throughout the entire thickness of myocardium (Figure 1c). In saline pretreated mice receiving a higher dose of ADR (20 mg/kg) necrotic aread of myocytes were found in the left ventricle (Figure 1e). On the other hand, mice treated with BP1 before ADR administration, regardless of the ADR dose, had less pronounced histologic changes, i.e. only a few single cardiac myocytes with myofibrillar lysis were found (Figure 1d,f). In 7 out of 8 mice of the protected group (ADR20BP1) histological changes were less pronounced in comparison with mice given saline and the same dose of ADR.

Hypereosinofilia of myocytes, nuclear pyknosis, polymorphonuclear inflammatory infiltrate around the myocyte or fibrosis were not seen in either of the groups of mice. Some of these changes could appear in the early stages of cardiac damage, within 24h of ADR administration, and might be detected by more sensitive electron microscopy. Fibrosis is a chronic change that occurs in step-wise fashion and is cumulative-dose-related.

The pathogenesis of ADR-cardiotoxicity is still uncertain but accumulation of druginduced reactive oxygen radicals in mitochondria and SR, as well as the proposed role of lipid peroxidation indicate an apparent oxidative nature of this toxicity (Saltiel and McGuire, 1983; Myers et al.,1986; Thomas and Aust, 1986; Sinha et al., 1987; Neri et al., 1993). Antioxidant substances e. g. tocopherol and thiols (Houlihan and Ho, 1985; Powel et al., 1988; Ferrari et al., 1991; Griesser-Aleksić, 1994) can greatly diminish ADR-cardiotoxicity in some animal models (Doroshow et al., 1981; Masquelier, 1987; Caulfield and Bittner, 1988). However, the majority of so far tested free-radical scavengers have received the most preclinical and clinical attention. Various flavanoid compounds, particularly proanthocyanidols, have recently come into focus as potential cardioprotectors (Masquelier, 1987; Barbier et al., 1988; Kovač and Pekić, 1991; Serafini et al., 1994; Leake, 1995; Van Acker et al., 1995; Withehead et al., 1995).

On the basis of the light microscopy evaluation of the hearts of ADR-treated mice it was noted that myofibrillar lysis was the prevalent type of cardiac pathologic damage.In BP1-pretreated mice obviously sparse single cardiac myocytes with myofibrillar lysis were found, i. e. the degree of cardiac damage was less intensive in comparison with unprotected mice. The results obtained suggest the need for further evaluation of BP1 as a potentially cardioprotective compound.

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PRILOG ISPITIVANJU PROTEKTIVNOG DELOVANJA PROANTOCIJANIDOLA-BP1 NA KARDIOTOKSIČNOST IZAZIVANU ADRIAMICINOM

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SADRŽAJ

Antraciklinski antibiotik adriamicin (ADR) jedan je od najefikasnijih lekova u lečenju hematoloških i uznapredovalih solidnih malignih tumora. Nažalost, klinička upotreba ovog leka značajno je ograničena činjenicom da ADR izaziva oštećenje miokarda zavisno od doze.Do sada je ispitan veliki broj kardioprotektivnih jedinjenja ali je samo nekoliko pokazalo značajnu kardioprotekciju a da pri tom ne umanjuju kliničku efikasnost ADR. U brojnim eksperimentima na životin-

jama pokazano je da prirodna antioksidativna jedinjenja, uključujući i flavanoide, smanjuju ili sprečavaju i akutne i hronične patološke promene izazvane davanjem ADR. Cilj ovoga rada bio je da se ispita da li davanje proantocijanidola - BP1 miševima tretiranim ADR može smanjiti ili sprečiti ADR-izazvanu kardiotoksičnost. Miševima je 3 dana pre ADR davan BP1 (20 mg/kg ip) ili sterilan fiziološki rastvor. Četvrtog dana eksperimenta miševima je jednokratno dat ADR u dozi od 15 ili 20 mg/kg ip 1h posle BP1 ili fiziološkog rastvora. Četiri dana nakon davanja ADR ispitane su histološke promene vezane za toksičnost ADR u levoj srčanoj komori. U srcu miševa koji su primili samo BP1 ili fiziološki rastvor nisu nađene morfološke promene. U srcu miševa koji su pre tretirani fiziološkim rastvorom a kasnije primili 15 mg/kg ADR nađene su mnogobrojne, pojedinačne ili manje grupe mišićnih ćelija sa lizom miofibrila difuzno raspoređene po celom miokardu. U srcu miševa koji su pored fiziološkog rastvora primili 20 mg/kg ADR nađena su polja nekrotičnih mišićnih ćelija. Miševi pre tretirani sa BPI, bez obzira na dozu ADR, imali su manje izražene histološke promene na srcu. Kod 7 do 8 miševa iz ADR20 BP1 grupe nađene su samo pojedinačne srčane ćelije sa liziranim miofibrilama. Na osnovu pregleda svetlosnom mikroskopijom zapaženo je da je najčešći oblik srčanog oštećenja ADR-tretiranih miševa bila liza miofibrila. Kod miševa štićenih sa BP1 stepen srčanog oštećenja izazvan ADR je manji u poređenju sa neštićenim miševima. Dobijeni rezultati ukazuju da je potrebno nastaviti ispitivanje sa BP1 kao potencijalno kardioprotektivnim jedinjenjem.