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Short Communication

The influence of sufan on myocardial energetic metabolism in the case of adriamycin-induced heart failure

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ABSTRACT

The results of influence of the new non-glycoside structure metabolic cardiotonic drug sufan on the energetic metabolism and oxidative homeostasis indicators in myocardium, brain and spleen of intact rats and rats with adriamycin-induced heart failure are presented. This study shows that sufan increases the coefficient of oxidative/reductive forms of nicotinamide coenzymes and reduces the adriamycin caused deterioration of the energetic metabolism and the pro-oxidative-anti-oxidative homeostasis. It is suggested that the use of the non-glycoside structure cardiotonic drug sufan can be recommended for the prevention of cardiotoxic effects of the anthracycline antibiotics.

KEYWORDS: sufan, adriamycin hydrochloride, heart failure, energetic metabolism, oxidative homeostasis

INTRODUCTION

Adverse drug reactions are a problem in modern pharmacotherapy. The side effects associated with many medications narrow the field of its use. Special medical care and careful control are required during the administration of these drugs. One such drug is an antitumor antibiotic, adriamycin which causes the development of cardiomyopathy that manifests itself first of all with the occurrence of heart failure in patients [1].

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The adriamycin-induced intoxication is also accompanied by severe disturbances of many cardiomyocyte enzyme systems [2]. The administration of cardiac glycosides in the case of adriamycin-induced intoxication does not reduce its severity, but rather enhances the structural abnormalities in the myocardium [3]. Data exist in literature concerning the attempts to reduce the adriamycin cardiotoxicity by the combined use of adriamycin with cardioprotective medications. One of those medications is sufan - a new non-glycoside cardiotonic drug, which acts as a cardioprotector as it relates to the succinic acid derivatives and may have certain effect on the energy metabolism of the heart muscles [4]. Sufan effectively prevents the development of morphological disorders in the myocardium during the adriamycin-induced intoxication [3].

The aim of this investigation is to explore the possibilities of correcting energy metabolism and oxidative homeostasis disorders in the myocardium of rats, for which sufan was administered during the adriamycin-induced intoxication.

MATERIALS AND METHODS

The investigations were conducted on 120 Wistar male-rats, weighing 150-200 g. These animals were divided into 4 groups: 1st group - the control group; 2nd group - animals that have been injected only with sufan (35 mg/kg); 3rd group - animals that have been injected only anthracycline antibiotic; 4th group - animals that have been injected adriamycin in combination with sufan.

Adriamycin was administered intramuscularly once a week (5 mg/kg) during 5 weeks; sufan was administered intramuscularly (i.m.) daily during 5 weeks. The myocardial tissue, brain and spleen of the rats were studied. 10% homogenates were prepared in 0.05 M Tris buffer (pH 7.4). All manipulations were carried out at temperature +4 ºC.

In myocardial tissues the content of nicotinamide coenzymes (nicotinamide adenine dinucleotide oxidized form (NAD^+) and the reduced form (NADH), nicotinamide adenine dinucleotide phosphate oxidized form ($NADP^+$) and the reduced form (NADPH)) was determined using fluorometry; the activity of NAD-hydrolase was determined by the enzymatic method; the content of creatine phosphate (CP) in the myocardial homogenate was determined as the difference between total and free creatine via spectrophotometry; the activity of the creatine phosphokinase (CPK) was assessed using the photo-colorimetric method; the adenine system components were determined with the help of spectrophotometry. In the heptane-isopropanol extracts of tissue homogenates of myocardium, brain and spleen, the content of primary products of lipid peroxidation (LPO), the diene conjugates (DC) (E_{232}/E_{220}) in the heptane and isopropanol phases, was determined as discussed in [5]. In the tissue homogenates the content of secondary LPO products that react with 2-thiobarbituric acid (TBA-active products), mainly the malondialdehyde (MDA), was measured as discussed in [6]. In the tissues of myocardium, brain and spleen, the content of glutathione in the reduced state was determined as per [7]. In the post-mitochondrial fractions of the same tissue, the activity of the glutathione cycle enzymes, glutathione reductase and glutathione peroxidase was determined following [6, 8]. The obtained data were statistically processed using the Student's criterion.

RESULTS AND DISCUSSION

It was observed that the i.m. administration of sufan in intact rats at a dose of 35 mg/kg daily during 5 weeks leads to the reduction of the nicotinamide coenzymes in the reduced form in the myocardium by 10.5%, that had certain effect on the increase of oxidized/reduced forms coefficient (+14.3%). This fact indicates a decrease in the coenzymes' reduction degree, which can be regarded as a positive effect on the functioning of various chains of cell metabolism. In the same experimental conditions sufan showed a small effect on the number of adenine system components and on the content of inorganic phosphate; it increased slightly the level of CP and glycogen in the myocardium (Tables 1-4).

The adriamycin-induced intoxication was accompanied by distinct changes in almost all studied parameters of myocardial energy metabolism. In the nicotinamide coenzyme system the level of oxidized forms decreased by 26% and the level of total number of nicotinamide coenzymes decreased by 9% with some increase in the content of reduced forms. Consequently the ratio of oxidized/reduced forms was reduced by 36.6% and thus the NAD-hydrolase activity increased by 37.5% (see Table 2). Under the influence of adriamycin hydrochloride that was administered i.m. during 5 weeks, the amount and composition of adenine nucleotides were significantly changed, the level of adenosine triphosphate (ATP) decreased by 29%, the level of adenosine diphosphate (ADP) decreased by 14% while the level of adenosine monophosphate increased by 55% and thus the myocardial energy potential was reduced by 20% and the ratio of ADP/ATP increased 1.5 times (see Table 3). The increased level of inorganic phosphate by 31% and the decreased level of CP by more than 2 times and the CPK activity by 1.7 times show the deterioration of the energy metabolism in myocardial tissue (see Table 1). The reduce in the level of glycogen in myocardium to 95.2 ± 8 mg/kg should also be noted (see Table 1). The use of adriamycin leads to the deterioration of the pro-oxidative-anti-oxidative homeostasis in myocardium of rats (see Table 4). Thus, the amount of TBA-active products was increased by 3.6 times, the reduced form of glutathione was reduced by 60%, the activity of glutathione reductase was increased by 28.9% and the activity of glutathione peroxidase was decreased by 25.7%. When comparing the indices of pro-oxidative-anti-oxidative homeostasis in myocardium and in tissues with high sensitivity to toxins and hypoxia

Table 1. The sufan effect on the CP and glycogen content and the CPK activity in rats with adriamycin-induced heart failure.

Animal group	CP, mmol/g	CPK, mcmol CP per 1 g tissue in 1 min	Glycogen, mg/kg
1st (n = 10)	3.40 ± 0.88	5.75 ± 0.22	218.2 ± 13.1
2nd (n = 10)	4.04 ± 0.36	5.45 ± 0.17	276.3 ± 17.9
3rd (n = 10)	1.40 ± 0.20*	3.28 ± 0.24*	95.20 ± 8.0*
4th (n = 10)	3.20 ± 0.44**	4.46 ± 0.18	184.5 ± 11.8**

*p < 0.05 concerning the 1st group (control group).
**p < 0.05 concerning the 3rd group.

Table 2. The sufan effect on the level of nicotinamide coenzymes and the NAD-hydrolase activity in myocardium of rats with adriamycin-induced intoxication.

Animal group	NAD^+, $NADP^+$, mcmol/kg	NADH, NADPH, mcmol/kg	Total number of nicotinamide coenzymes, mcmol/kg	Ratio of oxidized/reduced forms	NAD-hydrolase, mcmol/kg
1st (n = 10)	493 ± 8	345 ± 80	838 ± 13	1.43 ± 0.04	2609 ± 27
2nd (n = 10)	502 ± 11	297 ± 10*	799 ± 11	1.69 ± 0.03*	3015 ± 42*
3rd (n = 10)	365 ± 7*	396 ± 13	764 ± 7*	0.92 ± 0.02*	3589 ± 86*
4th (n = 10)	4430 ± 5**	370 ± 12	813 ± 32	1.2 ± 0.04**	3088 ± 39**

*p < 0.05 concerning the 1st group (control group).
**p < 0.05 concerning the 3rd group.

Table 3. The sufan effect on the adenine nucleotide content and the inorganic phosphate (Pi) in myocardium of rats with adriamycin-induced intoxication.

Animal group	ATP, mcmol/g	ADP, mcmol/g	AMP, mcmol/g	ATP + ADP + AMP, mcmol/g	Pi, mmol/g
1st (n = 10)	2.04 ± 0.07	1.38 ± 0.08	0.89 ± 0.06	4.31 ± 0.2	85.6 ± 6.2
2nd (n = 10)	2.26 ± 0.02	1.42 ± 0.13	0.81 ± 0.05	4.49 ± 0.3	84.2 ± 6.0
3rd (n = 10)	1.44 ± 0.02*	1.17 ± 0.03*	1.36 ± 0.08*	3.97 ± 0.3	112.0 ± 9.0*
4th (n = 10)	1.68 ± 0.13*	1.64 ± 0.12**	1.02 ± 0.05**	4.34 ± 0.4	92.4 ± 7.4

*p < 0.05 concerning the 1st group (control group).
**p < 0.05 concerning the 3rd group.

(brain and spleen) we have observed the same changes, but they were more considerable. Thus, in brain tissue the content of TBA-active products was increased by 16.3 times, the reduced form of glutathione was decreased by 79.5%, the activity of glutathione reductase was not changed and the activity of glutathione peroxidase was decreased by 23.9%. In spleen the content of TBA-active products was increased by 5.1 times, the reduced form of glutathione was increased by 20.7%, the activity of glutathione reductase was increased by 45.3% and the activity of glutathione peroxidase

Table 4. The sufan effect on the pro-oxidative-anti-oxidative homeostasis indices in myocardium, brain and spleen of rats with adriamycin-induced intoxication.

Animal group	TBA-active products, nmol/mg of protein	Glutathione, the reduced form, mg/g	Glutathione reductase, nmol NADPH*/mg protein	Glutathione peroxidase, mcmol glutathione/mg protein per 1 h
1	**2**	**3**	**4**	**5**
Myocardium				
1st (n = 10)	7.23 ± 1.17	0.638 ± 0.062	14.21 ± 0.90	69.12 ± 1.83
2nd (n = 10)	7.25 ± 2.07	0.725 ± 0.092	13.08 ± 1.02	70.52 ± 2.94
3rd (n = 10)	26.19 ± 2.56*	0.253 ± 0.031*	18.32 ± 0.61*	51.36 ± 1.72*
4th (n = 10)	18.36 ± 1.14**	0.348 ± 0.036*	18.71 ± 0.53*	50.19 ± 1.68*
Brain				
1st (n = 10)	8.53 ± 1.72	0.352 ± 0.040	27.35 ± 1.45	68.54 ± 1.92
2nd (n = 10)	8.48 ± 1.82	0.361 ± 0.050	28.03 ± 1.68	67.38 ± 1.36
3rd (n = 10)	138.92 ± 17.8*	0.072 ± 0.012*	25.38 ± 1.52	52.17 ± 1.06*
4th (n = 10)	82.19 ± 12.2*	0.157 ± 0.038*	28.17 ± 1.74	66.15 ± 1.38
Spleen				
1st (n = 10)	8.21 ± 1.54	0.458 ± 0.007	15.18 ± 0.98	17.53 ± 1.91
2nd (n = 10)	8.27 ± 1.75	0.453 ± 0.006	14.01 ± 0.67	17.72 ± 1.67
3rd (n = 10)	42.03 ± 5.06*	0.553 ± 0.007*	22.06 ± 1.12*	16.07 ± 1.38
4th (n = 10)	35.62 ± 3.10*	0.658 ± 0.006	24.31 ± 1.35*	37.12 ± 0.53

*$p < 0.05$ concerning the 1st group (control group).
**$p < 0.05$ concerning the 3rd group.

was not changed (see Table 4). Attention must be paid to the primary products of LPO, such as DC, the amount of which was not changed neither in heptane phase, nor in isopropanol phase in any of the studied organs. This is obviously due to the fact that the unstable DC does not accumulate in tissues, but rather converts to the end products of LPO or it can be reduced with the help of antioxidant defense systems. Under the influence of sufan, in animals that had been injected adriamycin hydrochloride during 5 weeks, the level of CP was increased by 2.2 times and the activity of CPK was increased by 35% in comparison with animals that had been administered adriamycin only (see Table 1). In those animals that had been administered adriamycin in combination with sufan the level of glycogen in myocardium was increased 2.1 times, the level of oxidized forms of nicotinamide coenzymes was increased by 21% and the ratio of oxidized/reduced forms was increased by 30.3% in comparison with rats that had been administered anthracycline antibiotic only (see Table 1). The adenine nucleotides' system also has less changes in animals that had been administered adriamycin in combination with sufan. Thus, the level of ATP was increased by 54%, the level of ADP by 40.5% and the amount of AMP was decreased by 25% (see Table 3). Sufan, during the adriamycin-induced intoxication decreases the amount of TBA-active products and increases slightly the level of the reduced form of glutathione in myocardium and brain; the influence of sufan on the activity of the glutathione cycle enzymes is not considerable (see Table 4). Thus, under the influence of sufan in

myocardium, the content of CP, reduced forms of nicotinamide coenzymes and the general amount of adenines' system compounds were normalized. Other parameters of energy metabolism and oxidative homeostasis were not completely normalized, but they were much closer to control values.

Consequently, it was observed that in case of adriamycin-induced intoxication the energy metabolism was disturbed (the level of oxidized forms and the total amount of nicotinamide coenzymes, CP, glycogen, the ratio of oxidized/reduced forms, the amount of ATP and ADP were decreased and at the same time the level of NAD-hydrolase, the ADP/ATP ratio and the amount of inorganic phosphate were increased). The obtained results are consistent with the literature data [2]. The activation of LPO in myocardium, brain and spleen was also determined. As for the glutathione system, due to the anthracyclin LPO-activation, there is decrease in the level of glutathione in myocardium and spleen, decreased level of glutathione peroxidase activity in myocardium and brain, and the compensatory increase of glutathione reductase in myocardium and spleen. Hence, glutathione system is a sensitive link of the xenobiotic metabolism, which is confirmed by literature data [9]. The pro-oxidative anthracyclin properties play a certain role in the realization of its pharmacological action [10]. Thus, the mechanism of the antibiotic antitumor action is associated with the induction of the single-strand breaks in DNA stimulated by free radicals that are formed during the adriamycin biotransformation in the hepatic microsomes in the presence of NADPH [11]. Free radicals activate the process of LPO. It should be noted that the oxidative homeostasis changes in case of adriamycin-induced intoxication are very similar to those that can be obtained during the radiation damage as per [12]. The use of sufan in combination with the i.m. antibiotic administration showed certain protective effects. These effects concern the vast majority of energy metabolism indicators and the content of TBA-active products of glutathione system. The cardio protective effect of sufan may be due to the fact that this substance is a derivative of succinic acid; it can be included in the Krebs cycle and thus it increases the heart energy potential [13]. Besides the ability of succinic acid to create the high-level energy-rich compounds, it also has a property to improve the reducibility of the pyridine nucleotides and thus it stimulates the cell regenerative processes. According to M. N. Kondrashova [13], succinic acid plays a specific role in restoring the functional tissues activity, which is intensively used during a period of the enhanced re-synthesis of the main sources of energy - CP and glycogen. It is important that, unlike many antioxidants, the sufan activity occurs only under the oxidative stress.

So, the adriamycin-induced intoxication causes significant disturbance in the energy metabolism and the pro-oxidative-anti-oxidative homeostasis in myocardium. The deterioration of the oxidative homeostasis in brain and spleen has the same tendency, but it is more considerable. The use of sufan decreases these metabolic manifestations of the toxic tissue damage. We consider that the use of sufan is promising as a substance that corrects not only the hemodynamic parameters, but also the structural and metabolic deterioration as a result of anthracycline-induced intoxication in myocardium and other organs.

CONCLUSION

The anthracycline-induced intoxication in rats was experimentally created (by the i.m. adriamycin administration at a dose of 5 mg/kg per week during 5 weeks). This intoxication was characterized by the energy metabolism deterioration in myocardial tissues (the decrease of oxidized forms and the total amount of nicotinamide coenzymes, CP, glycogen, ratio of oxidized/reduced forms, the amount of ATP and ADP and at the same time the increase of NAD-hydrolase activity and the ADP/ATP ratio and the amount of inorganic phosphate). It was also characterized by the LPO activation in tissues of myocardium, brain and spleen.

The i.m. use of sufan at a dose of 35 mg/kg during the adriamycin-induced intoxication reduces the severity of energy metabolism and oxidative homeostasis disorders in myocardium, brain and spleen.

CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest with respect to this article.

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