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Letter to the Editor

Protective Influence of Natural Anthocyanins of *Aronia Melanocarpa* on Selected Parameters of Antioxidative Status in Experimental Intoxication with Sulphide-2-Chloroethyl-3-Chloropropyl

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Abstract

Sulphide-2-chloroethyl-3-chloropropyl is an alkylating agent. It posssesses mutagenic and carcinogenic properties, participates in oxidative processes and can induce lipid peroxidation.The aim of our investigation was to define antioxidative activity of natural anthocyanins after single experimental intoxication with sulphide-2-chloroethyl-3-chloropropyl in mice. Catalase activity in hemolysate, thiobarbituric acid reacting substances (TBARS) concentration in hemolysate and selected organs were determined. The study confirms increased lipid peroxidation as a result of sulphide-2-chloroethyl-3-chloropropyl intoxication, but natural anthocyanines derived from *Aronia Melanocarpa* also seem to play a protective role as an antioxidative agent.

Keywords: Sulphide-2-chloroethyl-3-chloropropyl, anthocyanines, free radicals, catalase

Introduction

Sulphide-2-chloroethyl-3-chloropropyl is an alkylating agent and has not been used as a chemical warfare agent. As a derivative of sulphur mustard it has a similar mechanism of activity and it possesses mutagenic and carcinogenic properties and alkylate DNA leading to a series of reactions and finally cell death [1, 2]. It not only binds to DNA, but also to membranes, RNA and proteins inducing lipid peroxidation [3]. As a lipophilic compound sulphide-2-chloroethyl-3-chloropropyl rapidly penetrates tissues, especially skin and respiratory tract [4]. Besides these local effects, sulphur mustard has also been reported to cause severe systemic injuries of the gastrointestinal tract with general dysregulation and of the hematopoietic system leading to anemia [5, 6].

The ability to generate of oxygen reactive forms seems to play an essential role in toxic activity of sulphide-2-chloroethyl-3-chloropropyl [7].

The aim of the study was to investigate the protective influence of anthocyanine isolated from Aronia Melanocarpa on antioxidative balance disturbed by lipid peroxidation made by sulphide-2-chloroethyl-3-chloropropyl.

Experimental Procedures

Sulphide-2-chloroethyl-3-chloropropyl was synthetised in the Department of Biochemistry, Military Medical Academy.

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Table 1. Catalase activity in red blood cells in rats 24 hours after intoxication (U/g%Hb).

I group	II group	III group	IV group
20.1 ± 1.8	12.5 ± 1.3 P < 0.05	20.8 ± 1.4 P < 0.05	20.9 ± 1.3

Table 2. TBARS concentration in red blood cells in rats 24 hours after intoxication (mmol/g%Hb).

I group	II group	III group	IV group
36.9 ± 5.0	36.7 ± 1.7	36.7 ± 2.1	32.1 ± 1.8 P < 0.05

The investigations comprised 60 Balb·c mice (both sexes) with mean mass 21.0±3.5g, kept in animal quarters with stable temperature and humidity. The animals were divided into four groups. They received once during 24 hours of observation with stomach tube : 1) group I(control)- 0.1 ml of 0.9% NaCl solution; 2) group II – 54.0 mg/kg of body mass of sulphide-2-chloroethyl-3-chloropropyl, which was indicated as DL50 by Strzelczyk et al.[8]; 3) group III - in the form of aqueous solution – 1.0 mg/kg of body mass – anthocyanins from *Aronia melanocarpa* (Agropharm)[16] at the same time with administration of sulphide-2-chloroethyl-3-chloropropyl; 4) group IV- in the form of aqueous solution – 1.0 mg/kg of body mass – anthocyanins from *Aronia melanocarpa* (Agropharm)[16].

During the investigation the animals were fed with standard feeding stuff for small laboratory animals, Murigran, and they had free access to drinking water. After 24 hours the animals were terminated in general narcosis and blood, lungs and small intestine were collected for the determination of catalase activity in hemolysate according to Beers and Sizer [9], TBARS concentration in hemolysate according to Buege [10], whereas TBARS concentration in organ homogenates were determined according to Kennedy [11].

The results were analyzed statistically with U Mann-Whitney non-parametric test, using Statistica program, licence number SP 1054888009G51.

The investigations were carried out with the approval of the Local Ethics Committee No. 11/00.

Results

The effect of sulphide-2-chloroethyl-3-chloropropyl intoxication and anthocyanins on the selected biomarkers of oxidative stress in rats are presented in Tables 1, 2, 3.

Intoxication with sulphide-2-chloroethyl-3-chloropropyl resulted in a statistically significant decrease of catalase activity in red blood cells and TBARS concentration in lungs and small intestine in rats. No statistically significant changes were observed in TBARS concentration in haemolysate of erythrocytes. After administration of anthocyanins the values of catalase activity increased, while TBARS concentration in lungs and small intestine decreased.

Discussion of Results

The results of the present study demonstrate that intragastric administration of a single DL50 dose of sulphide-2-chloroethyl-3-chloropropyl can cause inhibition of catalase activity within 24 hours in red blood cells, indicating that this substance quickly reached the systemic circulation and bound this enzyme. It catalyzes the breakdown of H_2O_2 into H_2O and O_2 [12, 13]. The inhibition of catalase activity results in the accumulation of H_2O_2 in tissues that can undergo iron-catalyzed decomposition, leading to the production of extremely reactive hydroxyl radicals. Hydroxyl radicals have been shown to increase lipid peroxidation, inactivate enzymes and finally cause cell death [13,14].

Gastrointestinal and respiratory systems are often damaged during sulfur mustard intoxication [15]. TBARS concentration in lungs and small intestine tissue homogenates in our investigations significantly increased 24 hours after sulphide-2-chloroethyl-3-chloropropyl administration while in red blood cells they did not change. The results indicate that increased lipid peroxidation due to intoxication as observed by us and also by other investigators could be a consequence of antioxidant enzyme inhibition [4].

Anthocyanins seem to play a protective role after sulphide-2-chloroethyl-3-chloropropyl intoxication. It is possible that application of anthocyanins inhibits lipid peroxidation processes or increases antioxidant enzyme activity.

Obtained data show that catalase activity in hemolysate was significantly increased, while TBARS concentration, both in lungs and small intestine, was decreased. Vijayaraghavan et al. proved that flavonoids protected tissue damage caused by sulphur mustard in animals [16]. It has also been shown that suplementation by thiol-containing compounds like cisteine and glutathione to animals exposed to nitrogen mustard provided significant protection against tissue injury of spleen caused by free radicals [17, 18].

Up to now, the mechanisms of anthocyanins activity have not been fully explained. It is accepted that anthocyanins inhibit lipids oxidation, acting as donors of hydrogen atom. They are radical scavengers reacting with another radical. Anthocyanin radical is more stable than other radicals generated in organism, in comparison to which

Table 3. TBARS concentration in lungs and small intestine in rats 24 hours after intoxication (mmol/g).

	I group	II group	III group	IV group
lungs	7.5 ± 1.1	10.5 ± 2.0 P < 0.05	8.9 ± 1.7 P < 0.05	7.6 ± 1.0
Small intensine	6.8 ± 1.4	20.6 ± 5.4 P < 0.05	14.4 ± 2.7 P < 0.05	7.6 ± 0.7

its half-life is longer. That is why it may "wait" for other, living shorter, radicals, e.g. peroxide ones [19, 20, 21].

Antioxidative properties of anthocyanins seem to play a pivotal role against results of sulphide-2-chloroethyl-3-chloropropyl intoxication. Similar observations were done during the comparative study of the effectiveness of several drugs on the degree of protection against sulfur or nitrogen mustards. The most effective protection was achieved by dexamethasone, sodium thiosulfate and vitamin E, which is one of antioxidative agents [3, 22].

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