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Protective Effect of Allopurinol in Intestinal Ischemia-Reperfusion Injury in Rats

Ochronny wpływ allopurynolu w urazie niedokrwiennym jelita cienkiego u szczurów

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Abstract

Background. Acute intestinal ischemia of various origin may lead to tissue anoxia followed by morphological changes (even necrosis) and disorders of cellular metabolism. These changes may be reversible if duration of ischemia is not too long. Restoration of blood circulation is accompanied by synthesis and release of reactive forms of oxygen in significant amounts. Reaction of xanthine oxidase is one of the main sources of this phenomenon. Allopurinol – a competitive inhibitor of xanthine oxidase may show a protective effect on cellular oxidative stress.
Objectives. Evaluation of the influence of intestinal ischemia-reperfusion injury on intensity of histopathological changes and some biochemical parameters in rats on allopurinol therapy vs the control group.
Material and Methods. All experiments were performed on inbred male Buffalo rats, 14 individuals in each studied group. Intensity of mucosal ischaemic changes in small intestine was diagnosed by 6-degree point scale according to Chiu et al. [12]. Activity of the xanthine oxidase, superoxide dismutase, β-glucuronidase and malonyldialdehyde concentration were determined in supernatants of small intestine homogenates.
Results. Deep mucosal changes were observed in small intestine wall of the rats after 50-minute ischemia. However, reperfusion caused partial withdrawal of the changes. Small intestines of rats treated with allopurinol were damaged to a considerably lesser extent. Xanthine oxidase activity was not influenced by 50-minute ischemia and 60-minute reperfusion, although it was strongly inhibited by allopurinol. Superoxide dismutase activity was increased in ischemia and reperfusion and presence of allopurinol amplified this rise. β-glucuronidase activity and malondialdehyde concentration were unchanged in comparison with the control group (p-value > 0.05).
Conclusions. It was found that allopurinol had protective role in intestinal ischemia-reperfusion injury in rats. In response to oxidative stress, caused by temporary ischemia and reperfusion, activity of antioxidative enzyme – superoxide dismutase rose and allopurinol amplified this increase (**Adv Clin Exp Med 2006, 15, 2, 233–240**).

Key words: ischemia-reperfusion intestinal injury, xanthine oxidase, superoxide dismutase.

Streszczenie

Wprowadzenie. Ostre niedokrwienie jelita cienkiego, wywołane różnymi przyczynami, powoduje niedotlenienie tkanki, w następstwie czego są m.in. zmiany martwicze oraz zaburzenia metabolizmu komórkowego. Odwracalność tych zmian jest uzależniona od czasu trwania niedokrwienia, a przywróceniu krążenia naczyniowego towarzyszy synteza i uwalnianie znacznych ilości reaktywnych form tlenu (RFT). Za jedną z głównych przyczyn wydzielania RFT uważa się reakcję oksydazy ksantynowej. Allopurynol – inhibitor kompetycyjny oksydazy – może więc wykazywać ochronny wpływ na komórkę w warunkach stresu oksydacyjnego.
Cel pracy. Ocena wpływu niedotlenienia i reperfuzji na głębokość zmian histologicznych w śluzówce jelita oraz na niektóre wskaźniki biochemiczne komórek jelitowych szczurów leczonych i nieleczonych allopurynolem.
Materiał i metody. Do badań przeznaczono wsobny szczep samców szczurów rasy Buffalo w liczbie 14 osobników w każdej z badanych grup. Ostre niedokrwienie jelita cienkiego wywoływano zaciśnięciem pnia tętnicy krezkowej. Stopień zmian niedokrwiennych śluzówki jelita cienkiego oznaczano sześciostopniową skalą punktową.

W supernatantach homogenatów jelitowych oznaczano aktywności oksydazy ksantynowej, dysmutazy ponadtlenkowej, β-glukuronidazy oraz ilości dialdehydu malonowego.
Wyniki. Po zaciśnięciu tętnicy krezkowej stwierdzono istotne niedokrwienne zmiany patologiczne w śluzówce jelit, a reperfuzja powodowała częściowe wycofywanie się tych zmian. Jelita szczurów leczonych allopurynolem w znacznie mniejszym stopniu ulegały uszkodzeniu. 50-minutowe niedokrwienie i późniejsza reperfuzja nie wpływały na aktywność oksydazy ksantynowej, allopurynol natomiast silnie ją hamował. Aktywność dysmutazy ponadtlenkowej zwiększała się w wyniku niedotlenienia i reperfuzji. Wzrost ten potęgowała obecność allopurynolu. Aktywność β-glukuronidazy oraz zawartość dialdehydu malonowego nie wykazywały zmian istotnych statystycznie w porównaniu z wynikami uzyskanymi w grupie kontrolnej.
Wnioski. Stwierdzono ochronny wpływ allopurynolu na stopień zaawansowania zmian histologicznych śluzówki jelit. W odpowiedzi na stres oksydacyjny, wywołany przejściowym niedokrwieniem i reperfuzją, aktywność enzymu antyoksydacyjnego – dysmutazy ponadtlenkowej – zwiększała się, a allopurynol ją wzmagał (**Adv Clin Exp Med 2006, 15, 2, 233–240**).

Słowa kluczowe: uraz niedokrwienny jelita, oksydaza ksantynowa, dysmutaza ponadtlenkowa.

Acute intestinal ischemia is a serious disease, whose prolongation may lead to irreversible local necrotizing changes of mucosa at first and of whole intestine wall, finally. Sometimes serious total and organ changes lead to the death of the patient [1, 2]. Acute ischemia may be a consequence of arterial embolism or acute thrombosis of abdominal cavity vessels, especially of mesenteric artery superior, including whole small intestine segment and right part of the colon. Limited intestinal ischemia takes place in case of strangulation ileus in consequence of getting stuck of intestinal loop in abdominal hernia, its intussusception and block of vascular pedicle. Sometimes acute intestinal ischemia may be caused by injuries of abdominal cavity or by iatrogenic damage of vascular trunks which occurs during surgical treatment. Etiopathogenesis of the development of total and organ alteration in acute intestinal ischemia is complicated and is not clear until now. Growing ischemia is the primary cause of this phenomenon and its direct consequences are different disorders in cellular metabolism. Their reversibility is dependent on the time of ischemia lasting. Acute intestinal ischemia is caused also by decrease of the vascular perfusion during prolongating shock. In consequence of reperfusion and restoration of blood circulation significant amounts of oxygen reactive forms are generated and tissue cells are intensively damaged by them. OFR may be generated in a few ways. One of the main sources is reaction of xanthine oxidase. Also electron transport in respiratory chain in mitochondria, granulocyte migration to tissues touched by ischemia and activation of these granulocyties are very important causes of OFR synthesis. Oxidative stress may be neutralized by intensification of antioxidant defense system and thus a chance for new therapeutic methods may be given [3–7]. Allopurinol, a known competetive inhibitor of xanthine oxidase, may have a significant protective effect on the quantity of generated OFR and on the development of morphological damage caused by temporary ischemia and reperfusion [8–11].

The aim of the study was to evaluate effects of acute intestinal ischemia caused by block and reperfusion of mesenteric artery superior on intensity of histological changes in ischemic intestine mucosa and on some biochemical parameters in intestine homogenates. The role of allopurinol in protecting intestines from damage caused by OFR was also studied.

Material and Methods

All investigations were performed on male inbred Buffalo rats with average weight ranging between 180–220 grams. Comission of Bioethics at Wrocław Medical University has given permission No 8/03 on February 19, 2003 for realization of the project. The animals were maintained in stable conditions, fed standard diet (LABOFED – H produced by Kcynia – Poland, permission No 38/2003) and had free access to water and food. All surgical procedures were performed in clean but not sterile conditions under general anesthesia, which was produced by intramuscular administration of Bioketan R (Biowet – Poland) at a dose of 60 milligrams per one kilogram of body weight. For experiments 70 rats were used, divided into 5 groups (14 animals in each one). In order to induce acute intestinal ischemia the abdominal cavity was opened using epi- and mesogastric midline incision. After exposition of branching-off of mesenteric artery superior the trunk of mesenteric artery was prepared from intestine mesentery. Above this branching-off a delicate arterial clamp type of “buldock”was tightened on the vascular pedicle for the 50-minut period. Soon after the clamping, almost entire small intestine together with typhlon (blind gut) and ascending colon started to be visibly livid. A pulse on vascular arcades

was disappearing, what was manifested by the lack of light reflections on vessels. Gradually peristaltic movements were disappearing also. After 50 minutes the entire end segment of ileum (about 6 cm) was resected, washed out with physiological saline and divided into four fragments. The first one was fixed in 5% buffered formaldehyde solution and was used for preparing of hematoxyline and eosine (H-E) stained paraffin preparations. Remaining fragments, designated for biochemical analysis were temporarily frozen and stored at –80°C. In the second group of the animals, after 50-minute intestinal ischemia the arterial clamp was removed and blood circulation was restored. After 60-minute reperfusion the end part of ileum was resected and its appropriate fragments were prepared for histological and biochemical studies as described above. For estimation of the allopurinol influence the substance was administrated in drinking water to the next two groups of rats at the mean dose of 100 milligrams per kilogram of body weight/day during three-day period. Next acute ischemia was induced and intestinal specimens were taken as described above. In the control group of rats (shame operated) after dissection of proper trunk of mesenteric artery superior the arterial clamp was not applied. Abdominal cavity was closed and after 110 minutes opened again for dissection of appropriate parts of ileum as in the groups of rats with acute intestinal ischemia. Biochemical analysis of supernatants obtained after centrifugation of small intestine homogenates in 0.05M phosphate buffer pH 7.5 consisted in determination of activities of following enzymes: superoxide dismutase (SOD) (Randox-Ransod Cat. No SD 125), β-glucuronidase (Sigma Diagnostic Inc. Cat. No 325 B), xanthine oxidase (XOD) (in agreement with the protocol of Boehringer Mannheim, Biochemica Information, 1975) and also determination of malondildehyde (MDA) (OXIS Biotech. LPO-586 TM Cat. No 21012) concentration. Enzymatic activity was expressed in activity units (U) per one gram of tissue and MDA contents in nanomoles per gram tissue. Results were expressed as mean values with standard deviation (SD). Stastical analysis of results was performed using one-way analysis of variance (ANOVA) and least significant difference test (LSD). In the cases for which Gaussian decomposition cannot be obtained for studied features, the results were verified with non-parametric Mann-Whitney test. When p-values were found below 0.05 the differences were accepted as statistically significant. Abbreviation ND means: no difference; abbreviation NSD means: near statistical difference. In microscopic evaluation of the degree of intensity of ischemic mucosa changes in a intestine wall on the basis of histological specimens the 6-degree point scale according to Chiu et al. [12] was used although intermediate values were also permissible. Results of histopathological investigations were shown as mean values (MVs). Statistical analysis was performed with non-parametric Mann-Whitney test due to the use of semi-quantitative scale for evaluation of results. When p-values were found below 0.05 the differences were accepted as a statistically significant.

Results

The results shown in Table 1 illustrate the intensity of mucosal ischemic changes in a small intestine wall of rats in the 6-grades scale (0–5) according to Chiu at al. [12] in acute ischemia caused by constriction of trunk of mesenteric artery superior and in ischemia with subsequent 60-minute reperfusion. Results obtained for animals untreated with allopurinol and treated with it for three days prior to induction of ischemia-reperfusion processes are compared to results obtained for the control group of rats. Presented data show that 50-minute ischemia is the factor intensifying pathological changes formation in the intestinal mucosa. The changes in the rats not treated with allopurinol are much more advanced and statistically significant ($p < 0.001$). Obtained results show also that the changes formed during 50-minute ischemia are partially reversible. It was observed after 60-minute reperfusion. Withdrawal of the changes is more effective in the rats treated with allopurinol. Animals treated with allopurinol during three days before experiment (group II and III) had considerably decreased XOD activity in the intestinal wall homogenates as compared to the control group and to the animals untreated with allopurinol (group IV and V) (Table 2). The found differences are statistically significant ($p < 0.001$). Ischemia (group IV) and subsequent reperfusion (group V) do not have influence on the enzyme activity. Statistically significant differences are not observed also in the activity of XOD in these groups of animals in comparison with the control group (I). Table 3 presents the SOD activity. Presented studies show that in the rats with 50-minute ischemia (group IV) and with 60-minute reperfusion following ischemia (group V) the SOD activity in intestine homogenates is significantly higher in comparison with the results obtained in the control group (group I). The differences are statistically significant with p-values from < 0.001 to < 0.003. In the group of rats treated with allopurinol (groups II, III) the SOD activity is distinctly higher than in the control group as well as in the

Table 1. Intensity of mucosal ischemic changes in small intestine wall of rats in 6 grade scale (0–5) according to CJ Chiu et al. (Arch Surg 1970, 101, 478–483)

Tabela 1. Zaawansowanie niedokrwiennych zmian śluzówkowych w ścianie jelita cienkiego u szczura w skali 6-punktowej (0–5) według C.J. Chiu et al. (Arch Surg 1970, 101, 478–483)

	I	II	III	IV	V
	Control group (Grupa kontrolna)	Ischemia 50 min (Niedokrwienie 50 min)	Ischemia 50 min Reperrfusion 60 min (Niedokrwienie 50 min Reperfuzja 60 min)	Ischemia 50 min (Niedokrwienie 50 min)	Ischemia 50 min Reperrfusion 60 min (Niedokrwienie 50 min Reperfuzja 60 min)
		allopurinol (allopurynol) (+)	allopurinol (allopurynol) (+)	allopurinol (allopurynol) (–)	allopurinol (allopurynol) (–)
	zaawansowanie histologicznych zmian śluzówkowych w skali 0–5 (intensity of histological mucosal changes in scale 0–5)				
Number of rats (Liczba szczurów)	14	14	14	14	14
Mean value (Wartość średnia)	0	2.25	1.43	4.21	2.25
Statistics (Statystyka)	(II : I) p < 0.001	(III : I) p < 0.001	(IV : I) p < 0.001	(V : I) p < 0.001	
	(III : II) p < 0.002	(V : IV) p < 0.001	(II : IV) p < 0.001	(III : V) p < 0.001	

The differences in 5 group system were estimated in non parametric Mann-Whitney test.
1) grade 0 – lack of changes, 2) grade 1, 3) grade 2, 4) grade 3, 5) grade 4, 6) grade 5.

Różnice w układzie 5 grup oceniano za pomocą nieparametrycznego testu Manna-Whitneya.
1) stopień 0 – brak zmian, 2) stopień 1, 3) stopień 2, 4) stopień 3, 5) stopień 4, 6) stopień 5.

Table 2. Activity of xanthine oxidase in homogenate of rat intestinal wall in acute ischemia-reperfusion injury expressed in mU/g tissue

Tabela 2. Aktywność oksydazy ksantynowej w homogenacie ściany jelita cienkiego u szczura w przebiegu ostrego niedokrwienia i następowej reperfuzji wyrażona w mU/gram tkanki

	I	II	III	IV	V
	Control group (Grupa kontrolna)	Ischemia 50 min (Niedokrwienie 50 min)	Ischemia 50 min Reperrfusion 60 min (Niedokrwienie 50 min Reperfuzja 60 min)	Ischemia 50 min (Niedokrwienie 50 min)	Ischemia 50 min Reperrfusion 60 min (Niedokrwienie 50 min Reperfuzja 60 min)
		allopurinol (allopurynol) (+)	allopurinol (allopurynol) (+)	allopurinol (allopurynol) (–)	allopurinol (allopurynol) (–)
	activity of xanthine oxidase of intestinal wall in mU/g tissue (aktywność oksydazy ksantynowej w ścianie jelita w mU/g tkanki)				
Number of rats (Liczba szczurów)	11	12	13	13	12
Mean value (Wartość średnia)	82.4	7.7	3.1	70.8	75.0
Standard deviation (Odchylenie standardowe) SD	33.4	6.6	3.8	35.1	41.8
Statistics (Statystyka)	(IV : I) ND ND	(V : I) ND ND	(II : I) p < 0.001 p < 0.001	(III : I) p < 0.001 p < 0.001	
	(II : IV) p < 0.001 p <0.001	(III : V) p < 0.001 p < 0.001	(IV : V) ND ND	(II : III) ND p = 0.56 NSD*	

The differences were estimated with LSD test and were verified with non parametric Mann-Whitney test.
ND – non statistical difference; NSD* – near statistical difference.

Różnice w układzie 5 grup oceniano za pomocą testu LSD i dodatkowo weryfikowano za pomocą nieparametrycznego testu Manna-Whitneya.

ND – brak różnic istotnych statystycznie; NSD* – blisko różnicy statystycznie istotnej.

Table 3. Activity of superoxide dismutase in homogenate of intestinal wall in acute ischemia-reperfusion injury expressed in mU/g tissue

Tabela 3. Aktywność dysmutazy ponadtlenkowej w homogenacie ściany jelita cienkiego u szczura w przebiegu ostrego niedokrwienia i następowej reperfuzji wyrażona w mU/gram tkanki

	I	II	III	IV	V
	Control group (Grupa kontrolna)	Ischemia 50 min (Niedokrwienie 50 min)	Ischemia 50 min Reperrfusion 60 min (Niedokrwienie 50 min Reperfuzja 60 min)	Ischemia 50 min (Niedokrwienie 50 min)	Ischemia 50 min Reperrfusion 60 min (Niedokrwienie 50 min Reperfuzja 60 min)
		allopurinol (allopurynol) (+)	allopurinol (allopurynol) (+)	allopurinol (allopurynol) (–)	allopurinol (allopurynol) (–)
	activity of superoxide dismutase of intestinal wall in mU/g tissue (aktywność dysmutazy ponadtlenkowej w ścianie jelita w mU/g tkanki)				
Number of rats (Liczba szczurów)	13	9	12	13	13
Mean value (Wartość średnia)	1.132	5.787	5.397	2.942	3.129
Standard deviation (Odchylenie standardowe) SD	515	2.203	1.929	1.912	814
Statistics (Statystyka)	(IV : I) $p < 0.002$ $p < 0.003$	(V : I) $p < 0.001$ $p < 0.001$	(II : I) $p < 0.001$ $p < 0.001$	(III : I) $p < 0.001$ $p < 0.001$	
	(II : IV) $p < 0.001$ $p < 0.006$	(III : V) $p < 0.001$ $p < 0.001$	(IV : V) ND ND	(II : III) ND ND	

The differences were estimated with LSD test and were verified with non parametric Mann-Whitney test.
ND – non statistical difference.

Różnice w układzie 5 grup oceniano za pomocą testu LSD i dodatkowo weryfikowano za pomocą nieparametrycznego testu Manna-Whitneya.
ND – brak różnic istotnych statystycznie.

animals untreated with allopurinol (groups IV, V). The statistic significance (p) of the differences has values < 0.001. The comparison with the control group shows that ischemia has generated changes in SOD activity but reperfusion has not had any significant influence on the enzyme. Table 4 presents MDA content in homogenates of intestinal wall in acute ischemic-reperfusion injury expressed in nanomoles per gram of tissue. Between the studied groups statistically significant differences are not found. However, a tendency is observed to a delicate decrease of MDA content in intestinal cells of rats treated with allopurinol in comparison with the rats fed without allopurinol and with the control group also. Investigation of β-glucuronidase activity in intestinal wall homogenates does not show statistically significant differences between the studied groups of rats. Neither ischemia nor reperfusion influence the enzyme activity (Table 5).

Discussion

Cells damage caused by ischemia and reperfusion is very important medical problem. Mechanism of the phenomenon is still unexplained. When oxygen influx to the cell is stopped, very important metabolic changes occur as a result of enzyme activity disturbance and changes in functioning of the cell organelles (mitochondria) and membrane transport [13, 14]. Intensity and reversibility of the changes depend on the time of oxygen deficiency in the cell. Restoration of blood circulation and oxygen transportation to the cell are accompanied by production of large amount of oxygen free radicals (OFR). Therefore damage caused by ischemia can increase. It is very important to protect a patient against these dangerous consequences of ischemia and reperfusion after some surgical treatments. This is the reason why substances which are antioxidants or are able to initiate an action of antioxidative protection mechanisms [3–7, 15–17] are searched for. Allopurinol is one of such substances and it is an inhibitor of

Table 4. Malondialdehyde content in homogenate of intestinal wall in acute ischemia-reperfusion injury expressed in nanomoles/gram tissue

Tabela 4. Zawartość dialdehydu malonowego w homogenacie ściany jelita cienkiego u szczura w przebiegu ostrego niedokrwienia i następowej reperfuzji wyrażona w nanomolach/gram tkanki

	I	II	III	IV	V
	Control group (Grupa kontrolna)	Ischemia 50 min (Niedokrwienie 50 min)	Ischemia 50 min Reperrfusion 60 min (Niedokrwienie 50 min Reperfuzja 60 min)	Ischemia 50 min (Niedokrwienie 50 min)	Ischemia 50 min Reperrfusion 60 min (Niedokrwienie 50 min Reperfuzja 60 min)
		allopurinol (allopurynol) (+)	allopurinol (allopurynol) (+)	allopurinol (allopurynol) (–)	allopurinol (allopurynol) (–)
	malondialdehyde content – nmoles/gram tissue (zawartość dialdehydu malonowego – nmol/gram tkanki)				
Number of rats (Liczba szczurów)	14	13	12	13	12
Mean value (Wartość średnia)	25.9	23.1	23.9	27.6	27.4
Standard deviation (Odchylenie standardowe) SD	5.2	7.9	6.5	6.2	10.5
Statistics (Statystyka)	(IV : I) ND ND	(V : I) ND ND	(II : I) ND ND	(III : I) ND ND	
	(II : IV) ND ND	(III : V) ND ND	(IV : V) ND ND	(II : III) ND ND	

The differences were estimated with LSD test and were verified with non parametric Mann-Whitney test.
ND – non statistical difference.

Różnice w układzie 5 grup oceniano za pomocą testu LSD i dodatkowo weryfikowano za pomocą nieparametrycznego testu Manna-Whitneya.
ND – brak różnic istotnych statystycznie.

XOD. By inhibition of the enzyme activity the substance reduces the amount of OFR generated by XOD reaction after reperfusion. As the authors showed in described studies the 50-minute ischemia causes distinct histological changes in small intestine mucosa of rats. The changes are partially reversible what is observed after 60-minute reperfusion. Allopurinol significantly reduces the changes. It can suggest a participation of ORF in their generation. It is confirmed by the fact that the damage can be diminished by earlier application such antioxidants as vitamin C, mannitol [18], allopurinol coupled with vitamin C [8] or melatonine [19].

The reaction catalysed by XOD was considered the main source of OFR. The common opinion that during ischemia xanthine dehydrogenase present in a tissue is transformed into XOD is controversial because of many contradictions in literature. Vastatis et al. [20] did not find any evidence that such a conversion takes place in a horse organism after ischemia and reperfusion of small intestine. Biancardi et al. [21] suggest that XOD is a more productive source of OFR in rat intestine than in human. In presented studies the authors did not observe an increase in XOD activity during ischemia and after reperfusion. Numerous studies show that XOD reaction is not the only source of OFR. Ischemia activates neutrophils and stimulates them to produce OFR. It is well known that the response on a tissue damage is an inflammatory reaction which leads to accumulation of leucocytes producing a large amount of OFR which subsequently induce the synthesis of chemotactic factor causing intensification of leucocytes accumulation and their interaction with endothelium. Allopurinol and SOD reduce these processes [22]. In a response to oxidative stress the cell switches on enzymatic mechanisms neutralizing of OFR, defending in this way important structures and molecules against the radicals. One of the most important elements of the defense system is SOD dismutase which catalyses the dismutation of superoxide anionic radical. Investigations performed in animals showed that SOD activity in homogenates of ischemic intestinal wall and ischemic intestinal wall after reperfusion was significantly higher than in the control group. In the

Table 5. Activity of β-glucuronidase in homogenate of intestinal wall in acute ischemia-reperfusion injury expressed in U/gram tissue

Tabela 5. Aktywność β-glukuronidazy w homogenacie ściany jelita cienkiego u szczura w przebiegu ostrego niedokrwienia i następowej reperfuzji wyrażona w U/gram tkanki

	I	II	III	IV	V
	Control group (Grupa kontrolna)	Ischemia 50 min (Niedokrwienie 50 min)	Ischemia 50 min Reperrfusion 60 min (Niedokrwienie 50 min Reperfuzja 60 min)	Ischemia 50 min (Niedokrwienie 50 min)	Ischemia 50 min Reperrfusion 60 min (Niedokrwienie 50 min Reperfuzja 60 min)
		allopurinol (allopurynol) (+)	allopurinol (allopurynol) (+)	allopurinol (allopurynol) (–)	allopurinol (allopurynol) (–)
	activity of β-glucuronidase of intestinal wall in U/gram tissue (aktywność β-glukuronidazy w ścianie jelita w U/gram tkanki)				
Number of rats (Liczba szczurów)	12	13	12	13	12
Mean value (Wartość średnia)	6.436	6.572	5.388	6.796	5.587
Standard deviation (Odchylenie standardowe) SD	1.239	1.226	1.169	2.190	1.538
Statistics (Statystyka)	(IV : I) ND ND	(V : I) ND ND	(II : I) ND ND	(III : I) ND p = 0.036	
	(II : IV) ND ND	(III : V) ND ND	(IV : V) p = 0.053 ND NSD*	(II : III) p = 0.058 ND NSD*	

The differences were estimated with LSD test and were verified with non parametric Mann-Whitney test.
ND – non statistical difference.
NSD* – near statistical difference.

Różnice w układzie 5 grup oceniano za pomocą testu LSD i dodatkowo weryfikowano za pomocą nieparametrycznego testu Manna-Whitneya.
ND – brak różnic istotnych statystycznie.
NSD* – blisko różnicy statystycznie istotnej.

groups of animals treated with allopurinol for three days prior to induction of ischemia or ischemia and reperfusion the SOD activity was higher than in the control group and higher also than in animals untreated with allopurinol. Gained so for knowledge about SOD activity in intestinal ischemia and reperfusion is not sufficient. An increase of the enzyme activity was observed when melatonine was administrated to the animals before ischemia and reperfusion were generated. However, other investigators observed decrease in SOD activity [8]. It is possible that the differences resulted from incomparable conditions of experiments. During oxidative stress unsaturated fatty acids of cellular lipids are peroxidised by OFR to aldehydes and hydroxyaldehydes. MDA is the most often assayed representative of these substances. Results of presented experiments did not reveal statistically significant differences in concentrations of MDA in studied tissue between the control group and animals with induced ischemia and ischemia-reperfusion, and also between the control group and animals treated with allopurinol. However some regularities can be observed. A little higher MDA concentrations were found in the groups of animals with induced ischemia and ischemia-reperfusion in comparison with the control group. Lower MDA concentrations were found in the animals treated with allopurinol. The facts can suggest protective function of allopurinol. The authors did not observe statistically significant changes in β-glucuronidase activity because cell organella probably were not yet damaged in spite of oxidative stress. The results of carried out experiments show that ischemia and reperfusion of small intestine lead to intestinal mucosa damage which was shown by means of histopathological methods. Biochemical studies confirm, suggested in literature, participation of OFR in the investigated processes and protective influence of allopurinol.

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