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PROTEOLYTIC DEGRADATION OF POLY (ADP-RIBOSE) POLYMERASE IN RATS WITH CARRAGEENAN-INDUCED GASTROENTEROCOLITIS

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The aim of the research was to study the activity of poly (ADP-ribose) polymerase in small intestinal homogenate of rats with chronic carrageenan-induced gastroenterocolitis, as well as mechanisms of regulation of the enzyme in this pathology. Twenty Wistar Albino Glaxo rats were divided into two groups. Animals of group 1 (n = 10) consumed 1 % carrageenan solution for 28 days, which resulted in the development of gastroenterocolitis confirmed morphologically. The control group consisted of intact animals (n = 10). The activity of poly (ADP-ribose) polymerase (PARP) in the homogenate of small intestine, as well as caspase-3, matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9) serum levels were determined. Obtained data were statistically processed using the Mann-Whitney U test and calculating median and interquartile range (Me, 25th–75th percentile) with the help of the GraphPad Prism 5 application. The development of carrageenan-induced gastroenterocolitis was accompanied by an increase in caspase-3, MMP-2, MMP-9 concentrations in blood serum and a decrease in the activity of PARP in small intestinal homogenates. The reduced activity of PARP in chronic carrageenan-induced gastroenterocolitis may be due to the proteolysis of this enzyme under the action of caspase-3, MMP-2, and MMP-9.

KEY WORDS: gastroenterocolitis, carrageenan, rats, poly (ADP-ribose) polymerase, caspase-3, matrix metalloproteinases

ПРОТЕОЛІТИЧНА ДЕГРАДАЦІЯ ПОЛІ (АДФ-РИБОЗА) ПОЛІМЕРАЗИ ЩУРІВ ПРИ КАРАГЕНАНОВОМУ ГАСТРОЕНТЕРОКОЛІТІ

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Метою дослідження стало вивчення активності полі (АДФ-рибоза) полімерази у гомогенаті тонкого кишечника щурів з хронічним карагенан-індукованим гастроентероколітом, а також механізмів регуляції даного ферменту при зазначеній патології. Двадцять щурів популяції Wistar Albino Glaxo були розділені на дві групи по десять тварин у кожній. Тварини дослідної групи вживали 1 % розчин карагенану протягом 28 днів, що призводило до розвитку гастроентероколіту, який було підтверджено морфологічно. Контрольна група складалася з інтактних тварин. Визначали активність полі(АДФ-рибоза) полімерази (ПАРП) у гомогенаті тонкого кишечника та рівні каспази-3, матриксної металопротеїнази-2 (ММР-2) і матриксної металопротеїнази-9 (ММР-9) у сироватці крові. Отримані дані статистично оброблялися з використанням тесту Манна-Уїтні і розрахунку медіани і межквартільного діапазону (Me, 25-й-75-й процентилі) за допомогою програми «GraphPad Prism 5». Розвиток карагенан-індукованого гастроентероколіту супроводжувався підвищенням сироваткових каспази-3, ММР-2, ММР-9 на тлі зниження активності ПАРП у гомогенаті тонкого кишечника тварин. Зниження активності ПАРП при хронічному карагенан-індукованому гастроентероколіті може бути обумовлено протеолізом даного ферменту під дією каспази-3, ММР-2 і ММР-9.

КЛЮЧОВІ СЛОВА: гастроентероколіт, карагенан, щури, полі (АДФ-рибоза) полімераза, каспаза-3, матриксні металопротеїнази

ПРОТЕОЛИТИЧЕСКАЯ ДЕГРАДАЦИЯ ПОЛИ (АДФ-РИБОЗА) ПОЛИМЕРАЗЫ КРЫС ПРИ КАРРАГЕНАНОВОМ ГАСТРОЭНТЕРОКОЛИТЕ

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Целью работы стало изучение активности поли (АДФ-рибоза) – полимеразы в гомогенате тонкого кишечника крыс с хроническим каррагинан-индуцированным гастроэнтероколитом, а также механизмов регуляции данного фермента при указанной патологии. Двадцать крыс популяции Wistar Albino Glaxo были разделены на две группы по десять особей в каждой. Животные опытной группы употребляли 1 % раствор каррагинана в течение 28 дней, что приводило с развитию гастроэнтероколита, подтвержденного морфологически. Контрольная группа состояла из интактных животных. Определяли активность поли (АДФ-рибоза) – полимеразы (ПАРП) в гомогенате тонкого кишечника и уровни каспазы-3, матриксной металлопротеиназы-2 (ММР-2), матриксной металлопротеиназы-9 (ММР-9) в сыворотке крови. Полученные данные статистически обрабатывались с использованием теста Манна-Уитни и расчета медианы и межквартильного диапазона (Me, 25-й-75-й процентили) с помощью приложения «GraphPad Prism 5». Развитие каррагинан-индуцированного гастроэнтероколита сопровождалось повышением каспазы-3, ММР-2, ММР-9 в сыворотке крови и снижением активности ПАРП в гомогенате тонкого кишечника животных. Снижение активности ПАРП при хроническом каррагинан-индуцированном гастроэнтероколите может быть обусловлено протеолизом данного фермента под действием каспазы-3, ММР-2 и ММР-9.

КЛЮЧЕВЫЕ СЛОВА: гастроэнтероколит, каррагинан, крысы, поли (АДФ-рибоза) – полимераза, каспаза-3, матриксные металлопротеиназы

INTRODUCTION

Poly (ADP-ribose)-polymerase (PARP) is a fairly large protein consisting of 1014 amino acid residues, which is involved in the regulation of a number of intracellular functions [1], including DNA repair, cell proliferation and differentiation, apoptosis, necrosis, gene expression [2], and the degradation of PARP occurs under the influence of various intracellular proteases such as caspases, calpaines, granzymes, cathepsins, and matrix metalloproteinases [3].

The role of PARP in the development of inflammatory processes seems to be quite controversial. PARP has been shown to regulate the expression of certain pro-inflammatory proteins, in particular, tumor necrosis factor-α (TNF-α) and inducible NO-synthase (iNOS). It has been known that inflammation results in the development of oxidative stress, which is accompanied by DNA damage. In response to oxidative damage to DNA, PARP activation occurs, which in turn potentiates upregulation of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) whose action mediates the expression of proinflammatory factors [2, 4]. Thus, PARP is considered to exert pro-inflammatory effects, however, taking into account the pleiotropic effects of this enzyme, it is important to note that the impaired delicate balance of PARP inside the cell in the direction of both hyper- and hypoactivation can make the pathological process more severe.

OBJECTIVE

Nowadays the activity and role of PARP in the development of chronic carrageenan-induced inflammation of the gastrointestinal tract have not been studied, therefore the aim of the research was to study the activity of PARP in small intestinal homogenates of rats with chronic carrageenan-induced gastroenterocolitis, as well as the mechanisms of its regulation.

MATERIALS AND METHODS

Twenty female white WAG rats, which were kept in standard conditions of the vivarium, were used in the experiment. The animals were randomly divided into 2 groups. Group 1 consisted of animals exposed to the food additive λ-carrageenan for 4 weeks (n = 10). Group 2 served as a control group and included ten intact animals. Long-term oral administration of λ-carrageenan led to the development of chronic gastroenterocolitis confirmed morphologically [5, 6].

All experimental procedures were performed in accordance with the provisions of the European Convection for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes and Directive 2010/63/EU on the protection of animals used for scientific purposes adopted on September 22, 2010.

Laboratory animals were removed from the experiment by decapitation. Fragments of small intestine were taken immediately after

decapitation. They were perfused with a cooled saline solution. Powdered and shredded small intestine was used to prepare a homogenate in 0.25 M Tris-HCl buffer (pH = 7.4) containing 0.32 M of sucrose. After 15-minute centrifugation at 3,000 rpm. (1,200 g), the supernatant was obtained in which the PARP activity was determined using the method based on the electrophoretic separation of poly-ADP-ribosylated histone proteins from nuclei followed by the quantitative determination of poly-ADP-ribose in them [7]. Caspase-3 blood serum concentrations were measured using ELISA kit manufactured by *eBioscience* (Vienna, Austria). The content of matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9) in blood serum was determined using ELISA kits produced by *Quantikine* (Minneapolis, USA). The Awareness Technology Stat Fax 303 Plus Microstrip Reader was used to register the optical density of solutions.

The statistical processing of the data obtained in our research was carried out using the GraphPad Prism 5 application. Median and interquartile range (Me, 25th–75th percentile) were calculated. To assess the differences in quantitative characteristics between independent groups, the Mann-Whitney U test was used. The results were statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

It was established that the development of chronic carrageenan-induced gastro-enterocolitis was accompanied by a decrease in the activity of PARP in small intestinal homogenates. In animals of group 1 whose representatives had been administered with carrageenan solution, the activity of PARP was 3-fold reduced compared to the control group (Fig. 1).

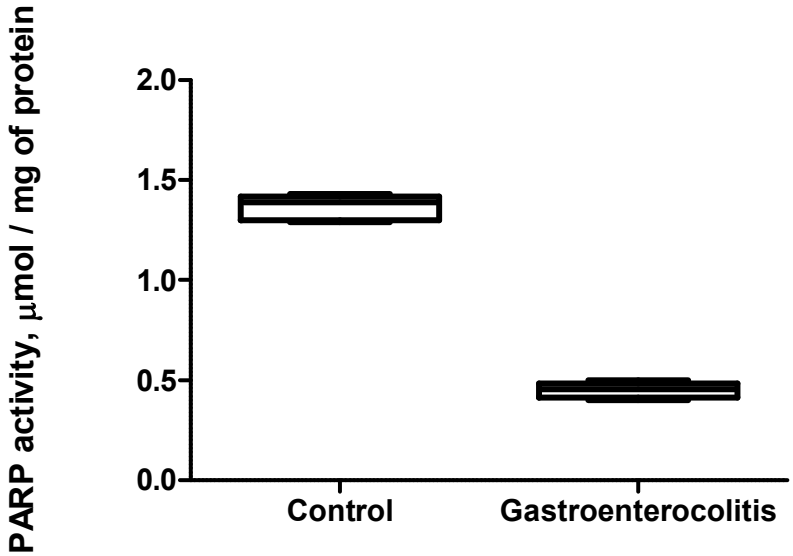

Fig. 1. The activity of poly (ADP-ribose) polymerase in small intestinal homogenates of rats with carrageenan-induced gastroenterocolitis

Among the proteases that are potentially capable of participating in the proteolytic degradation of PARP, we have selected MMP-2, MMP-9, and caspase-3. The four-week oral intake of the food additive λ-carrageenan was found to cause the upregulation of caspase-3 whose level statistically significantly exceeded the same parameter of the control group ($p < 0.001$) more than 38 times (Fig. 2).

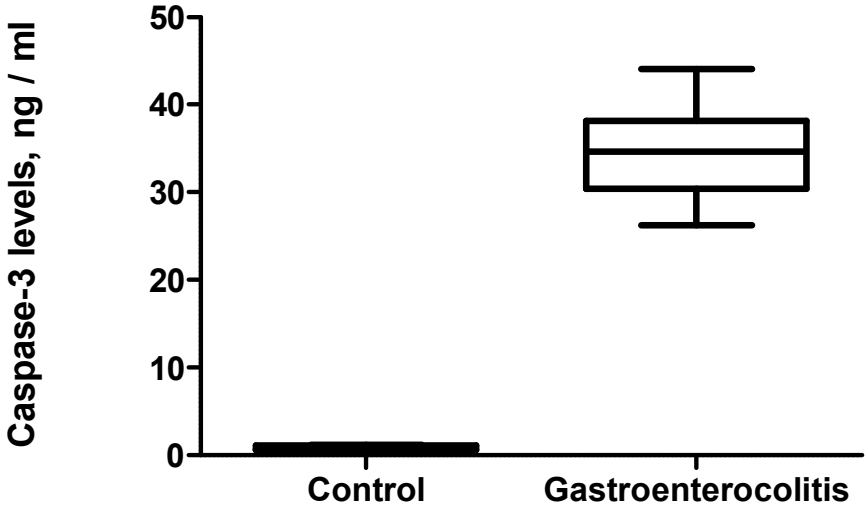

Fig. 2. Caspase-3 blood serum levels in animals with carrageenan-induced gastroenterocolitis

The comparison of the content of metalloproteinases in blood serum of rats with carrageenan-induced gastroenterocolitis and their serum levels in healthy animals allowed us to find out that the disease was accompanied by a significant increase in both MMP-2 and MMP-9 serum levels ($p < 0.01$). For instance, we found a 1.5-fold increase in serum MMP-2 concentrations in animals from group 1 compared to the control animals (Fig. 3).

At the same time, the concentration of MMP-9 was statistically significantly 3.7 times higher ($p < 0.01$) in blood serum of rats with gastroenterocolitis (Fig. 4).

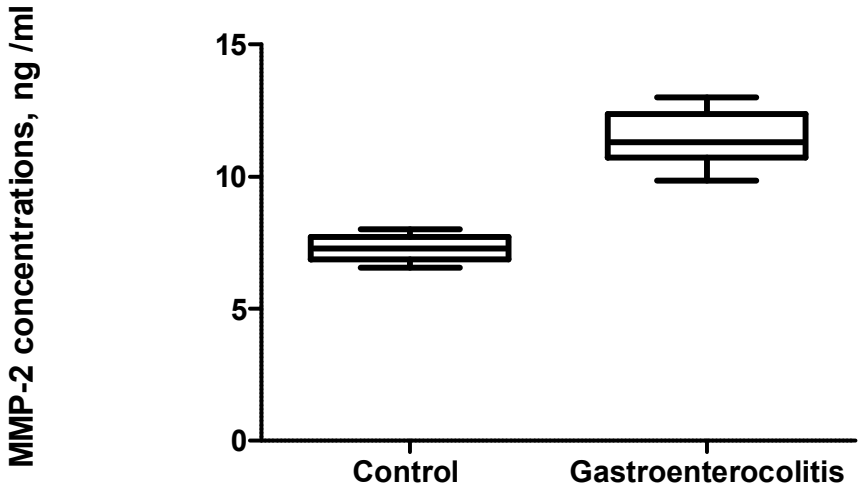

Fig. 3. Matrix metalloproteinase-2 blood serum concentrations in animals with carrageenan-induced gastroenterocolitis

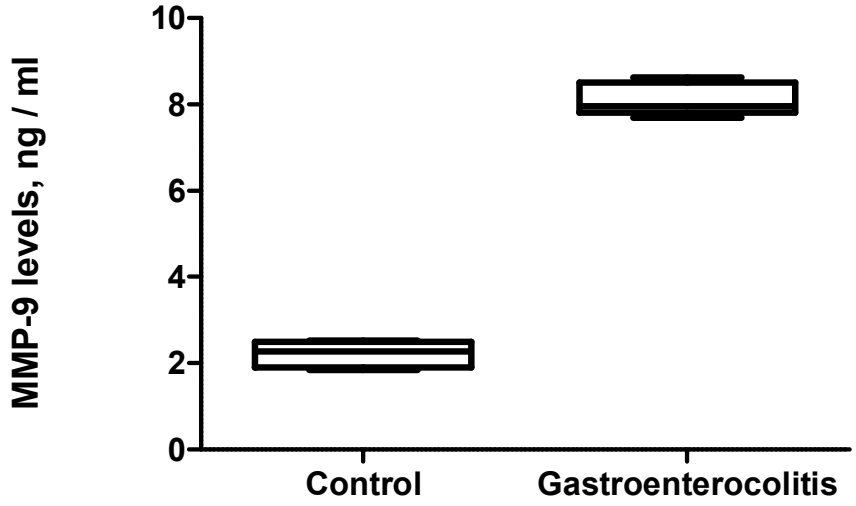

Fig. 4. Matrix metalloproteinase-9 blood serum levels in rats with carrageenan-induced gastroenterocolitis

Taking into account the reported data on the involvement of MMP-2, MMP-9, and caspase-3 in the degradation of PARP [3, 8], the observed decrease in PARP activity might be explained by the cleavage and inactivation of this enzyme by caspase-3 and metalloproteinases. However, we presume that the effect of MMP-2 and MMP-9 on the breakdown of PARP in carrageenan-induced inflammation is less pronounced compared to caspase-3, since only a slight elevation of blood metalloproteinases is observed in the animals from group 1 against the background of considerable activation of caspase-3.

The involvement of PARP in the regulation of the expression of proinflammatory factors makes it possible to explain the early decrease in the activity of inducible NO synthase [9, 10], one of the reasons of which is probably the abnormal PARP-dependent regulation of NF-κB. As a result, NF-κB-mediated expression of inducible NO synthase is affected.

Nowadays, there is no doubt that chronic inflammation is connected with malignant transformation of cells. It has been known that the prolonged oral exposure to carrageenan results in the development of not only chronic inflammation but also gastrointestinal tumors [11]. We assume that the PARP deficiency may serve as one of the factors of tumor transformation, since a decrease in the PARP activity reduces the reparative DNA ability, which is the cause of malignancy.

CONCLUSIONS

1. Chronic gastroenterocolitis developed due to a 4-week consumption of carrageenan is accompanied by a decrease in the activity of PARP and elevation of MMP-2, MMP-9 and caspase-3 in blood serum of animals.

2. Reduction of the PARP activity in chronic carrageenan-induced gastroenterocolitis can be explained by proteolysis of this enzyme under the influence of caspase-3, MMP-2 and MMP-9.

3. Diminished DNA reparative abilities in case of PARP deficiency may serve as one of the factors of malignization in chronic carrageenan-induced intestinal inflammation.

PERSPECTIVES OF FURTHER RESEARCH

It is promising to study other proteolytic enzymes that can participate in the degradation of poly (ADP-ribose) polymerase in chronic carrageenan-induced gastroenterocolitis, affecting the rate of DNA repair, apoptosis, and cell proliferation in this way.

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