PharmacologyOnLine

Archives • 2021 • vol.3 • 405-412

ACTIVITY OF THE INFLAMMATORY PROCESSES IN RATS DURING EXPERIMENTAL CARCINOGENESIS AND THE INFLUENCE OF DRY EXTRACT FROM REISHI MUSHROOMS ON THEM

Herasymets Iryna[1]*, Fira Liudmila[2], Mykhalkiv Mariya[3], Ivanusa Iryna[3]
1Department of Department of Pharmacology with Clinical Pharmacology, I. Horbachevsky Ternopil National Medical University, Maidan Voli 1, 46001 Ternopil, Ukraine
2Department of Pharmacy, faculty of postgraduate education, I. Horbachevsky Ternopil National Medical University, Maidan Voli 1, 46001 Ternopil, Ukraine
3Department of Pharmaceutical chemistry, I. Horbachevsky Ternopil National Medical University, Maidan Voli 1, 46001 Ternopil, Ukraine

* irunaherasymets@gmail.com

Abstract

Changes in the cytokine profile, the content of C-reactive protein in experimentally induced carcinogenesis in rats and the effect of dry extract of Reishi mushrooms on this pathology were studied.

The study was performed on 120 white rats, in which oncopathology was simulated by long-term administration of 1,2-dimethylhydrazine hydrochloride. The level of cytokines, in particular, IL-4, IL-6 and TNF was determined by enzyme-linked immunosorbent assay. The content of C-reactive protein was determined by immunoturbidimetric method. There was a significant increase in the content of pro-inflammatory cytokines IL-6, TNF, C-reactive protein and a decrease in the content of anti-inflammatory cytokine IL-4 in the serum of rats with induced carcinogenesis after 3 months of administration of the toxicant compared to control. The obtained results confirm the significant role of cytokines and C-reactive protein in the pathogenesis of colon cancer. Our studies revealed the effectiveness of the Reishi mushrooms dry extract's effect on the normalization of the cytokine profile and the content of C-reactive protein in rats with simulated oncopathology.

Keywords: *Reishi mushrooms, experimental chronic endotoxicosis, inflammatory processes, dry extract, oncoprotective action*

Introduction

Ukraine is the leader on the European continent in terms of cancer incidence. As of January 1, 2021, more than a million citizens were registered in specialized medical institutions. Cancer mortality in Ukraine is in the second place after cardiovascular diseases [1].

The human body has its own system of protection against oncopathology - leukocytes, which have the ability to recognize and destroy modified and damaged cells, including cancer. But a number of factors, both external and internal, weaken this protection and the system can fail [2]. Thus, the analysis of morbidity and mortality from oncopathology, conducted by the WHO, shows that more than 45% of deaths are associated with the negative impact of the environment, smoking. alcohol abuse, as well as exposure to substances such as asbestos, benzene, aniline dyes, herbicides, pesticides. Sun exposure and harmful eating habits also have a negative effect, in particular, the consumption of large amounts of animal fats, salt, simple carbohydrates, preservatives and chemical dyes, smoked, canned and fried foods [1, 3].

To help the weakened body fight pre-existing cancers or prevent their occurrence in modern traditional and alternative medicine, many drugs have been created that have an oncoprotective effect on the body and help to cope with pre-existing diseases. However, most drugs used in the treatment of oncopathology act indiscriminately, affecting not only cancer but also healthy cells of the body, thereby causing the development of serious adverse reactions [4, 5].

That is why it is important to help the natural mechanisms of the human body protection in performing their oncoprotective functions. This care can be effectively implemented with a number of natural drugs-oncoprotectors [6, 7].

According to modern literature, Reishi mushrooms (Ganoderma Lucidum) have a strong immunomodulatory, hepatoprotective, oncoprotective, anti-allergic (inhibit the release of histamine), anti-inflammatory effect, are an effective radioprotector and have a desensitizing effect on the body [8, 9, 10 11]. That is why we have chosen them for our research.

The aim of the experimental work was to study the anti-inflammatory properties of dry extract of Reishi mushrooms in an experiment on rats with a chemically induced cancer process.

Methods

The experiment was performed on 120 white outbred male rats weighing 190 - 210 g. The animals were kept on a balanced standard diet of the vivarium of Ternopil National Medical University named after I. Horbachevsky of the Ministry of Health of Ukraine in compliance with the rules of good laboratory practice (GLP) and bioethics in accordance to "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" [12].

Chronic carcinogenesis was simulated by the introduction of asymmetric 1,2-dimethylhydrazine hydrochloride (DMH) ("SIGMA-AL DRICH CHE MIE", made in Japan, series D161802), which was previously diluted with isotonic sodium chloride solution. The toxicant was injected subcutaneously into the interscapular area at a dose of 7,2 mg/kg (based on the active substance) once a week for 30 weeks, according to the rat's body weight [4].

Animals were divided into the following groups: 1st – intact control (IK); 2nd - animals affected by 1,2-dimethylhydrazine hydrochloride; 3rd - rats affected by 1,2-dimethylhydrazine hydrochloride, which were corrected with dry extract of Reishi mushrooms (DERM). Intragastric administration of DERM was performed daily for 30 weeks of the experiment against the background of increasing pathological changes at a dose of 100 mg/kg of the animal's body weight, which in our previous studies was established as conditionally therapeutic for this extract [13]. The intact control for the experimental group of animals injected with 1,2-DMH were 8 white rats, which were weekly injected subcutaneously with 0,1 ml of saline per 10 g of body weight.

At the 1st, 2nd, 3rd, 4th, 5th, 6th and 7th month from the onset of the lesion, the animals were removed from the experiment under thiopental anesthesia. The study was subjected to blood serum. Blood was taken from the heart of the animals and was centrifuged at a speed of 1100 g for 30 minutes. The concentration of pro- and anti-inflammatory interleukins in the serum of rats was

determined by enzyme-linked immunosorbent assay using commercial kits (("GE Health care: Amersham", UK) [14]. Quantitative level of serum tumor necrosis factor (TNF-α) was determined by competitive enzyme-linked immunosorbent assay (ELISA) in vitro using the enzyme-linked immunosorbent assay Rat Tumor necrosis factor α (TNF-α) ELISA Kit MBS (USA) according to the manufacturer's method, which was described in the technical documentation [15]. The content of C-reactive protein (CRP) was determined by immunoturbidimetric method [16, 17].

The obtained data were statistically processed in the STATISTICA 12 program. The probability of intergroup differences was determined using the Wilcoxon rank sum test and the Mann-Whitney test. The results of the study were considered plausible at $p < 0.05$ [19, 20].

Results

The development of nonspecific inflammatory response was observed in rats under the conditions of simulated experimental carcinogenesis, including increased levels of CRP, proinflammatory cytokines IL-6, TNF- α and decreased level of anti-inflammatory cytokine IL-4 in the serum of animals with DMH pathology.

The study of CRP content has some clinical significance. Determination of this indicator is carried out in almost all pathological conditions and diseases. Even a slight increase in CRP levels, which can only be detected by a highly sensitive method, increases the risk of developing hypertension, atherosclerosis, diabetes, heart attack or stroke [17, 18].

Thus, under the conditions of induced carcinogenesis a statistically significant increase in the concentration of CRP in the serum of rats was found in the 3rd month from the onset of the lesion by 33% compared with the same indicator of the control group of animals. When DERM was used as a corrective agent the concentration of CRP in serum was lower by 17% ($p < 0,001$) relative to the group of animals with 1,2-DMH toxic lesions in the 3rd month of pathology (Table 1).

We found that at the 5th and 7th month from the onset of DMH lesions, the CRP concentration probably increased by 91% and 207%, respectively, relative to intact control Under the correction of chemically induced endotoxicosis by the DERM, the concentration of CRP in the serum of rats was characterized by a tendency to decrease relative to the affected animals. Thus, on the 5th and 7th month of the study, the concentration of the determined indicator decreased by 25% and 41%, respectively, relative to the control pathology.

It is known that cytokines have pleiotropic biological effects on different cell types, mainly by participating in the formation and regulation of protective reactions. Protection at the local level develops by forming a typical inflammatory reaction after the interaction of pathogens with pattern-recognizing receptors (key receptors of innate immunity) with the subsequent synthesis of pro-inflammatory cytokines. Cytokines, which are synthesized in the inflammatory focus, affect virtually all cells involved in the development of the inflammatory process, including neutrophils, macrophages, fibroblasts, endothelial and epithelial cells, and T- and B-lymphocytes [21, 22].

The effect of long-term administration of DMH on the cytokine profile of rat serum was investigated in the experiment. A probable increase in the content of pro-inflammatory cytokine (IL-6) against the background of a decrease in the content of anti-inflammatory interleukin (IL-4) was established.

Interleukin-6 is a pro-inflammatory cytokine that participates in the natural process of realization of the inflammatory reaction. Its action is aimed at stimulating the proliferation, differentiation and functional activation of cells involved in the formation of the immune response [14].

After the lesion of rats with a toxic agent, there was an increase in the content of IL-6 in the serum of animals in 1,5, 2,6 and 3,6 times in the 3rd, 5th and 7th month of the experiment, respectively, in relation to control animals (table 2). In rats, whom the extract was administered in parallel with DMP, a decrease in the content of proinflammatory cytokine was observed at all times of the study.

IL-4 limits the production of macrophages under conditions of normal reaction of such proinflammatory cytokines as IL-6 and IL-1β, so its sufficient synthesis ensures balanced development and timely limitation of the inflammatory process.

After the 3rd month of carcinogenesis modeling, the content of IL-4 was significantly

reduced by 16% compared with control animals, which indicates the progression of the inflammatory process (table 3). After 5th and 7th months of DMH administration, there was an even greater decrease in the content of this indicator in the serum of rats by 39% and 60%, respectively, relative to the intact control group, which indicates the dynamics of the severity of the inflammatory process.

The use of DERM in DMH pathology led to an increase in the content of the anti-inflammatory cytokine IL-4 in the serum of white rats and ensured its growth throughout the study.

Proliferation of immunocompetent cells is a necessary step in any immune response. The normal proliferative response is determined primarily by the production of TNF-α – the most important cytokine of the first phase of the immune response, which is an inducer of T and B-cell immunity. Therefore, the study of this cytokine is especially important for a fuller understanding of the immune mechanism with the development of oncopathology [15, 23].

The development of the oncological process in the body of animals with prolonged administration of the toxicant is evidenced by a significant increase in the level of TNF-α in the serum of white rats (table 4). A probable increase in cytokine levels was detected in the control pathology group after the 2nd month of the lesion compared to the control animal's group. By the end of the experiment, the level of TNF-α in the serum of animals with oncopathology increased in 6,9 times relatively to control.

Under the influence of DERM, a probable decrease in the level of TNF-α in the serum of animals was observed as early as the 3rd month of modeling the oncoprocess relative to the control pathology group. Thus, at the 7th month from the beginning of the study, the level of the studied cytokine decreased in 1,8 times relatively to the affected animals.

Discussion

Prolonged damage of rats by 1,2-DMH leads to a statistically significant decrease of CRP in serum, which may indicate its regulatory effect and ability to facilitate the inflammatory response in simulated oncopathology. It is known that the level of CRP has prognostic value as a marker of the intensity of the inflammatory process. Increased serum levels indicate the severity of the disease. In addition, the content of CRP is an informative indicator in monitoring the effectiveness of treatment of inflammatory processes. This is confirmed by the results of our study, because the probable decrease in CRP content with long-term use of DERM on the background of simulated carcinogenesis, indicates the effectiveness of this pharmacological drug [14, 17].

Cytokines are involved in the regulation of many processes, but their role in the regulation of hematopoiesis, inflammation and immune response is particularly important [16].

Lesions of rats by 1,2-DMH for 30 weeks led to a probable increase in the content of pro-inflammatory cytokine IL-6 and a decrease in the level of anti-inflammatory cytokine IL-4 in the serum of animals with oncopathology, indicating a cytokine profile imbalance due to prolonged administration of the toxicant. A significant increase in the content of IL-6 in the first months of the study indicates the development of inflammatory processes in the early stages of chemical intoxication, which deepen with the prolongation of the DMH introduction [14].

The carcinogenic effect of DMH is evidenced by the growth in the dynamics of one of the main triggers of systemic inflammation – TNF, which belongs to the anti-inflammatory cytokines and is an important low molecular weight mediator of intercellular interactions. TNF can have both protective and destructive effects on the body. The latter can occur in severe hall processes and autoimmune diseases [15, 22].

Prolonged administration of DERM in parallel with the carcinogen confirms the anti-inflammatory and oncoprotective effect of the studied extract, which is to normalize the cytokine profile, in particular to reduce the content of pro-inflammatory cytokines IL-6, TNF and increase the content of anti-inflammatory cytokine IL-4 relative to intact animals.

Conclusions

Experimentally proved a significant increase in the content of C-reactive protein, proinflammatory interleukin 6 and tumor necrosis factor on the background of reduced production of anti-inflammatory interleukin 4 in the serum of rats

with chemically induced cancer, which certainly indicates the progression of inflammatory processes in animals with long-term administration of 1.2-dimethylhydrazine hydrochloride.

Dry extract of Reishi mushrooms has an effective influence on the content of C-reactive protein, pro- and anti-inflammatory cytokines in the serum of animals with prolonged administration of 1,2-dimethylhydrazine hydrochloride. This indicates the anti-inflammatory and oncoprotective properties of the studied pharmacological drug, which are manifested, in our opinion, due to the biologically active substances contained in them, in particular polysaccharides and terpenoids.

The data obtained as a result of the experimental work indicate the prospects for further study of the dry extract of Reishi mushrooms in order to include it in the treatment of patients as an anti-inflammatory and oncoprotective agent in the early stages of the oncological process.

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Table 1. The content of C-reactive protein in the serum (mg/l) of rats affected by 1,2-DMH and after application of the Reishi mushrooms extract (M ± m; n = 80)

Indicators	Groups of animals		
	IC	CP	CP+DERM
1 month	3,79±0,18	3,86±0,26	3,65±0,19
2 month	3,79±0,18	4,13±0,16	3,87±0,26
3 month	3,79±0,18	5,05±0,19*	4,21±0,25
4 month	3,79±0,18	6,46±0,24*	4,71±0,19**
5 month	3,79±0,18	7,22±0,28*	5,43±0,18**
6 month	3,79±0,18	9,41±0,23*	6,13±0,26**
7 month	3,79±0,18	11,62±0,25*	6,91±0,32**

Note. Here and in the following tables * - probable changes between the rate of intact control and DMH-affected animals, ** - probable changes between the rate of DMH-affected and treated animals

Table 2. The content of IL -6 in the serum (pg/l) of rats affected by 1,2-DMH and after application of the Reishi mushrooms extract (M ± m; n = 80)

Indicators	Groups of animals		
	IC	CP	CP+DERM
1 month	3,14±0,14	3,34±0,15	3,10±0,19
2 month	3,14±0,14	3,88±0,25*	3,25±0,21
3 month	3,14±0,14	4,75±0,22*	3,54±0,13**
4 month	3,14±0,14	6,02±0,32*	3,91±0,21**
5 month	3,14±0,14	8,07±0,47*	4,78±0,26**
6 month	3,14±0,14	9,40±0,33*	5,09±0,27**
7 month	3,14±0,14	11,40±0,54*	5,66±0,20**

Table 3. The content of IL -4 in the serum (pg/l) of rats affected by 1,2-DMH and after application of the Reishi mushrooms extract (M ± m; n = 80)

Indicators	Groups of animals		
	IC	CP	CP+DERM
1 month	1,34±0,06	1,39±0,08	1,42±0,07
2 month	1,34±0,06	1,27±0,08*	1,30±0,06
3 month	1,34±0,06	1,13±0,06*	1,27±0,07**
4 month	1,34±0,06	0,99±0,06*	1,22±0,07**
5 month	1,34±0,06	0,82±0,10*	1,17±0,08**
6 month	1,34±0,06	0,68±0,06*	1,12±0,07**
7 month	1,34±0,06	0,53±0,07*	1,07±0,05**

Table 4. TNF-α level (ng/l) in the serum of rats affected by 1,2-DMH and after application of the Reishi mushrooms extract (M ± m; n = 80)

Indicators	Groups of animals		
	IC	CP	CP+DERM
1 month	1,08±0,09	1,21±0,11	1,24±0,09
2 month	1,08±0,09	1,39±0,10*	1,28±0,11
3 month	1,08±0,09	1,89±0,10*	1,42±0,11**
4 month	1,08±0,09	2,91±0,18*	1,91±0,10**
5 month	1,08±0,09	4,56±0,19*	2,74±0,16**
6 month	1,08±0,09	6,11±0,29*	3,36±0,36**
7 month	1,08±0,09	7,44±0,25*	4,05±0,28**