Acta Scientific MEDICAL SCIENCES (ISSN: 2582-0931)

Volume 8 Issue 8 August 2024

Research Article

# Experimental Assessment of the Oncoprotective Properties of Low-Molecular Chitosan and its Effects on Cytostatic and Radiation Myelosuppression

**AV Troitsky[1]*, TN Bystrova[1], RA Knyazev[1], NV Trifonova[1], AA Rozhkov[2], NA Mamontov[2], KA Rozhkova[2] and BA Zhigadlo[2]**

*[1]Federal Research Centre for Fundamental and Translational Medicine, Novosibirsk, Russia*

*[2]Innovative Health Technologies LLC, Novosibirsk, Russia*

***Corresponding Author:** AV Troitsky, Federal Research Centre for Fundamental and Translational Medicine, Novosibirsk, Russia.

**DOI:** 10.31080/ASMS.2024.08.1894

**Received:** June 24, 2024

**Published:** July 31, 2024



## Abstract

The effects of low-molecular chitosan were studied in experimental models of leukopenia in mice caused by cyclophosphamide and gamma radiation. The oncoprotective effect of low-molecular chitosan on the solid lymphosarcoma model of mice was studied. Low-molecular chitosan has been shown to have an oncoprotective effect and to effectively compensate for leukopenia caused by cytostatic and gamma radiation.

**Keywords:** Chitosan; Cytostatic and Radiation Leukopenia; Cancer Protection

Currently, targeted therapy is the most promising method of treatment of malignant neoplasms. It is mainly used as a second-line therapy, along with classical methods of chemotherapy and radiation therapy. Of the areas of targeted cancer therapy, the areas for the creation of monoclonal antibodies and low-molecular biologically active substances, tyrosine kinase blockers, folic acid receptors and serine/threonine kinase blocker are most intensively developed [1-5]. There is another direction of targeted therapy, which is based on the selective activation of the cellular link of immunity, primarily macrophages. Macrophages and T-lymphocytes are the primary, genetically determined link for the elimination of tumour cells through natural pathophysiological mechanisms. In Japan, the preparation "Lentinan", created on the basis of β -1,3-glycans of the basidial mushroom Lentinus edodes, is still used. β-1,3-glycans are specific activators of macrophages through glycane receptors localized on the surface of the cell membrane [6-8]. As a result of this activation, dynamic transformation of tissue macrophages phenotypes from M1 to M2 and Mox is possible. The broad spectrum of cytokines produced by macrophages of different phenotypes is able not only to create high cytotoxic potential in the tumour growth zone, but to provide a systemic anti-inflammatory effect and detoxication resulting from the breakdown of tumour tissue. If you add to the effects of β -1,3-glycans their ability to stimulate leukopoiesis at the level of the bone marrow, they can act not only as anti-tumour drugs, but also as a means to compensate for cytostatic and radiation myelosuppression in standard schemes of radiotherapy of malignant tumours. Recently, as an analogue with the properties of β-1,3-glycan basidial mushrooms began to be considered natural amino polysaccharide chitosan. It also exhibits immunomodulating

properties against the cellular link of immunity, but also has antimicrobial properties and can be obtained in a highly purified form, unlike β-1,3-glycans from basidial mushrooms, which contain many pyrogenic and allergenic polyphenols. In the manufacture of anti-tumour drugs, chitosan is mainly used as a target carrier of the active antitumor substance, which can be represented by both cytostatic and monoclonal antibodies [9]. According to the study of the direct cancer-protective action of chitosan, there are currently practically no data. Also, there is no data on the effect of chitosan on leukopoiesis in conditions of chemoradiation myelosuppression, which will allow to assess the prospects of its use for the treatment of complications in standard regimes of cancer therapy.

The aim of this paper is to evaluate the oncoprotective effect of low-molecular chitosan on the model of transplanted malignant tumour in vivo and to investigate the effectiveness of the therapeutic effect of chitosane in leukopenia induced by cytostatic and gamma radiation.

## Materials and Methods

### Cytostatic leukopenia

15 outpoured nonlinear laboratory ICR (CD-1) mice (males) with an average body weight of 20-22 g were divided into 3 groups of 5 animals in each group. Cytostatic leukopenia was modelled with a single intra-abdominal administration of all groups of cyclophosphane (CF) solution at a rate of 250 mg of CF per 1 kg of body weight of the animal. 10 minutes after the administration of CF, the first group of animals (control) was administered intra-abdominal saline solution (250 μl), and the second and third (experimental) groups of animals were given a single intra- abdominal and subcutaneous administration of 250 μl of 0.1% water solution of low-molecular chitosan (m.M. up to 50 kDa). Starting from 4 days to 7 days of the experiment including animals experimental groups replaced drinking water with 0.1% aqueous solution of low-molecular chitosan. In all three groups, the total number of leukocytes in 1 ml of peripheral blood was measured daily. The significance of differences in the number of leukocytes between the groups was assessed using the non-parametric Kramer-Wellch criterion. The differences were considered to be statistically significant at $p < 0,05$.

### Post-radiation leukopenia

10 outpoured nonlinear laboratory ICR (CD-1) mice (males) with an average body weight of 20-22 g were divided into 2 groups of 5 animals in each group. Moderate post-radiation leukopenia was simulated with a single brake gamma radiation exposure at a dose of 0.9 Gy on an ILU-10 pulse linear accelerator. After irradiation, the experimental group replaced the drinking water with 0.1% aqueous solution of low molecular chitosan. In both groups, the total number of leukocytes in 1 ml of peripheral blood was determined for 6 days. The significance of differences in the number of leukocytes between the groups was assessed using the non-parametric Kramer-Wellch criterion. The differences were considered to be statistically significant at $p < 0,05$.

### Study of the anti-tumour activity of low-molecular chitosan on a transplanted tumour model *in vivo*

A cyclophosphamide-resistant solid form of mouse lymphosarcoma RLS 40 was used as a transplanted tumour. For the formation of a solid tumour, 22 out-breaded nonlinear laboratory ICR (CD-1) mice (males) were transplanted into the RLS 40 lymphosarcoma of mice. Passages were carried out into the outer upper right thigh in a quantity of $1x10^6$ tumour cells per person in a 0.1 ml saline solution. All mice were divided into 3 groups. The first group (control), 8 individuals – after the passage of tumour cells, starting from the second day, day after day for 28 days administered intra-abdominal 0.1 ml of 0,85% saline solution. The second group (experimental 1, evaluation of cancer protection), 8 individuals – after the passage of tumour cells, starting from the second day after day for 28 days administered intra-abdominal 0.1 ml of 0.1% aqueous solution of low-molecular chitosan. The third group (experimental 2, evaluation of therapeutic effect), 6 individuals – after passing tumour cells, starting from 7 days on a daily basis for 21 days administered intra-abdominal 0.1 ml of 0,1% aqueous solution of low-molecular chitosan. The mass and volume of the solid tumour in all 3 groups were determined 28 days after euthanasia of mice (cervical vertebrae dislocation) and extirpation of the tumour.

## Results of the Study

### Cytostatic leukopenia

An assessment of the dynamics of the drop in total leukocytes in the blood, based on the results of the study, shows that 0.1% water solution of low-molecular chitosan (NH), administered after intra-abdominal injection of cyclophosphane, have a clearly pronounced preventive effect against cytostatic leukopenia. At the same time, for early periods up to 3 days of the experiment,

the preventive effect of NH in cytostatic leukopenia is more pronounced with intra-abdominal administration of NH than with subcutaneous administration of NH. By the 4th day of the experiment, the values of total leukocytes in the blood in the second and third groups were compared. This may indicate that with intra-abdominal administration of NH, it immediately enters the systemic bloodstream and within 1 day is excreted, and with subcutaneous administration of NH in slightly smaller doses, but gradually, within 3 days, diffuses from the subcutaneous tissue into the systematically bloodstream, providing constant background stimulation of leukopoiesis. Starting from 4 days of the second and third group of animals, the effect of leukopoiesis restoration was studied with enteral administration of NH. As can be seen from the results obtained in animals of group 2 the restoration of the total content of leukocytes in peripheral blood occurs more effectively. By the 7th day of the trial, the peripheral blood leukocytes in this group had fully recovered, whereas by 7 days of the experiment, the total peripheral blood leucocyte content had been slightly reduced by 8% compared to the baseline, while in the control group, the reduction in the total blood leukocyte levels by 7 day of experiment remained at 31% from the base level. Thus, the study found that NH has therapeutic efficacy in cytostatic leukopenia, and its effectiveness was more pronounced when enterally administered (Table 1). Based on the results of the study, it can be argued that enteric administration of NH, starting from 2 days after the administration of cytostatic, can be a very promising way to compensate for cytostatic myelosuppression and can significantly improve the effectiveness of treatment and quality of life of cancer patients.

| | Control group | | Experimental group 1 | | Experimental group 2 | |
|---|---|---|---|---|---|---|
| **Blood collection time** | **The number of leukocytes in the blood, x$10^9$ ml ± SE** | **Reduction, %** | **The number of leukocytes in the blood, x$10^9$ ml, ± SE** | **Reduction, %** | **The number of leukocytes in the blood, x$10^9$ ml, ± SE** | **Reduction, %** |
| Before Introduction | 4,76 ± 0,8 | | 3,4 ± 0,3 | | 4,05 ± 0,95 | |
| After 1 day | 1,66 ± 0,7 | 65% | 1,36 ± 0,3 | 60% | 2,3 ± 0,5 | 43% |
| After 2 days | 1,2 ± 0,2 | 75% | 1,2 ± 0,1 | 65% | 1,65 ± 0,2 | 59% |
| After 4 days | 0,6 ± 0,1 | 87% | 0,8 ± 0,16 | 76% | 0,8 ± 0,2 | 80% |
| After 5 days | 1,46 ± 0,3 | 69% | 1,46 ± 0,3* | 57% | 1,2 ± 0,3** | 70% |
| After 6 days | 1,9 ± 0,4 | 60% | 1,8 ± 0,4 | 47% | 2,3 ± 0,8 | 43% |
| After 7 days | 3,28 ± 0,6 | 31% | 3,9 ± 1,06 | 0% | 3,7 ± 1,06 | 8% |

**Table 1:** The number of leukocytes in the blood in mice after intra-abdominal administration of cyclophosphane at a dose of 250 mg per 1 kg of body weight. Control group, trial group 1 - subcutaneous administration of 0.1% aqueous solution of NH at a dose of 250 µl/mouse, trials group 2 - intra-abdominal administration of 0,1% aqueous solution of NH at a dosage of 250 µl/mouse.

* After 4 days, a 0.1% aqueous solution of NH was added to the drink

** After 4 days, a 0.1% aqueous solution of NH was added to the drink

## Post-radiation leukopenia

There is a pronounced therapeutic effect from the use of NH in the compensation of post-radiation leukopenia of moderate severity (Table 2). In the oncological practice, post-radiation leukopenia of moderate and mild severity is generally found, so the modeling of leukopenia when exposed to 100% of the vertex of the body of mice with inhibitory gamma-radiation at a dose of 0.9 Gy is closest to the common complications from the blood side of radiation therapy in cancer patients.

| | Control group | | Experimental group | |
|---|---|---|---|---|
| **Blood collection time** | **Leukocyte count, x10$^9$ ml ± SE** | **Reduction, %** | **Leukocyte count, x10$^9$ ml ± SE** | **Reduction %** |
| Before irradiatin | 4,56 ± 1,2 | | 3,62 ± 0,7 | |
| After 1 day | 2,48 ± 0,5 | 46 | 2,26 ± 0,4 | 38 |
| After 2 days | 3,16 ± 0,6 | 30 | 3,0 ± 0,6 | 17 |
| After 4 days | 3,34 ± 0,9 | 27 | 3,06 ± 0,6 | 15 |
| After 6 days | 3,94 ± 0,8 | 14 | 3,42 ± 0,7 | 6 |

**Table 2:** The number of leukocytes in mice after irradiation at a dose of 0.9 Gy. The trial group after irradiation in the drink added 0.1% aqueous solution NH.

## Testing of the anti-tumour activity of low-molecular chitosan on the model of the transplanted tumour

In Table 3 presents the results of the study of the oncoprotective and therapeutic effects of NH on the solid form lymphosarcoma model of mice RLS 40. As can be seen from the presented results NH have a pronounced oncoprotective effect on the experimental model of solid form lymphosarcoma of mice. The volume and weight of the solid tumour in trial group 1 was almost twice as low as in the control group. The therapeutic effect of the use NH on this model is weak, which may be due to low vascularization of solid tumours.

| **Control** | | **Experiment 1 (assessment of the oncoprotective effect)** | | **Experiment 2 (evaluation of therapeutic action)** | |
|---|---|---|---|---|---|
| Tumor weight, g | Tumor volume см$^3$ | Tumor weight, г | Tumor volume см$^3$ | Tumor weight, г | Tumor volume см$^3$ |
| 3,69 | 2,212 | 1,01 | 0,942 | 2,18 | 2,029 |
| 2,39 | 1,282 | 2,05 | 1,567 | 2,78 | 1,923 |
| 1,78 | 1,429 | 0,02 | 0,534 | 4,99 | 3,432 |
| 2,71 | 1,811 | 1,62 | 1,037 | 3,87 | 1,847 |
| 5,73 | 5,014 | 3,11 | 2,403 | 2,78 | 2,492 |
| 1,44 | 1,609 | 3,08 | 1,847 | 3,9 | 2,513 |
| 4,3 | 3,721 | 1,88 | 1,371 | | |
| 2,8 | 3,133 | 2,85 | 2,403 | | |
| 3,105 ± 1,4 | 2,53 ± 0,47 | 1,952 ± 1,08 | 1,51 ± 0,24 | 3,417 ± 1,03 | 2,37 ± 0,25 |

**Table 3:** Results of the study of the oncoprotective and therapeutic effect of NH on the model of solid form lymphosarcoma of mice RLS 40.

## Conclusions

The improved data allow to consider low-molecular chitosan as a very promising aid in the treatment of malignant tumours by traditional chemotherapy methods. Compensating cytostatic and radiation leukopenia by taking a water solution of low-molecular chitosan can be an effective and safe way of reducing the complications of chemotherapy and improving the quality of life of cancer patients. Preliminary data on the oncoprotective effects

of low-molecular chitosan suggest that it is considered a safe prevention of cancer, especially in the early stages, but this requires further research on various models of transplanted tumours.

## Bibliography

1. Vermorken Jan B. "Cetuximab: Its unique place in head and neck cancer treatment". *Biologics Targets and Therapy* 7.1 (2013): 77-90.

2. Zhang J., *et al*. "Erlotinib for advanced hepatocellular carcinoma. A systematic review of phase II/III clinical trials". *Saudi Medical Journal* 37.11 (2016): 1184-1190.

3. Greenhalgh J., *et al*. "Erlotinib and gefitinib for treating non-small cell lung cancer that has progressed following prior chemotherapy (review of NICE technology appraisals 162 and 175): a systematic review and economic evaluation". *Health Technology Assessment* 19.47 (2015): 1-134.

4. Bouabdallah K., *et al*. "Temsirolimus in the treatment of mantle cell lymphoma: frequency and management of adverse effects". *Current Opinion Oncology* 25 (2013): S1-12.

5. Ambrosio AJ., *et al*. "Vintafolide (EC145) for the treatment of folate-receptor-alpha positive platinum-resistant ovarian cancer". *Expert Review of Clinical Pharmacology* 7.4 (2014): 443-450.

6. Zhang Y., *et al*. "Lentinan as an immunotherapeutic for treating lung cancer: a review of 12 years clinical studies in China". *Cancer Research Clinical Oncology* 144.11 (2018): 2177-2186.

7. Motta F., *et al*. "Mushrooms and immunity". *Autoimmune* 117 (2021): 102576.

8. Taek Joon Yoon., *et al*. "The effects of β-glucans on cancer metastasis". *Anti-Cancer Agents in Medicinal Chemistry* 13.5 (2013): 699-708.

9. Sabrin H Albeituni and Jun Yan. "The effects of β-glucans on dendritic cells and implications for cancer therapy". *Anti-Cancer Agents in Medicinal Chemistry* 13.5 (2013): 689-698.

10. Gorshenin DS., *et al*. "The use of chitosan and its derivatives in immunotherapy of malignant neoplasms". *Immunology* 41.5 (2020): 470-478.