

# COMPARATIVE ASSESSMENT OF SULFACRYLATE AND ETHYL ALCOHOL IMPACT ON THE FUNCTIONAL STATUS OF NEUTROPHILS AND MONONUCLEAR PHAGOCYTES DURING THE FORMATION OF NONPARASITIC HEPATIC CYSTS

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## Abstract

**Study Purpose:** To study the impact of sulfacrylate and ethyl alcohol on the functional status of neutrophils and peritoneal macrophages in animals with nonparasitic hepatic cysts.

**Methods:** The cysts were formed by implanting a foreign body followed by its removing and sclerosing the lunule by ethyl alcohol or sulfacrylate. Pharmacological, cytochemical and immunological methods of research were applied.

**Results:** Ethyl alcohol and sulfacrylate increase bactericidal, absorbing activity, oxidative metabolism, the content of myeloperoxidase, cationic proteins in neutrophils and peritoneal macrophages in animals with nonparasitic hepatic cysts.

**Conclusion:** Sulfacrylate eliminates the negative impact of the cystic process on the functional activity of neutrophils and macrophages more effectively than ethyl alcohol does.

## Introduction

It is known that neutrophils and mononuclear phagocytes not only perform the function of the body's first line of protection, take active part in the regulation of immune and inflammatory responses, hematopoiesis, anti-tumor and anti-microbial protection, affect the neuroendocrine regulation of homeostasis system, but also affect the processes of reparative liver regeneration [3, 7, 8, 10]. The most effective treatment method for nonparasitic cysts of the abdominal organs, including liver, is a combination of puncture and puncture-drainage method with sclerotherapy [1, 5, 6]. Various agents (such as alcohol, polydocanol, glycerol and others) can be used as sclerosants [5, 6, 9]. Therefore, the matter of importance is to explore the impact of sclerosants used in the treatment of nonparasitic cysts of the abdominal cavity on the functional status of neutrophils and macrophages.

## Objectives

In this respect, the objective of the research is a comparative study of the impact of sclerosants (ethyl alcohol (EA) and Sulfacrylate (SFC)) on the activity of polymorphonuclear leukocytes (PMNL) and peritoneal macrophages (PM) in animals with experimental nonparasitic hepatic cysts.

**Key words:** nonparasitic hepatic cyst, sulfacrylate, ethyl alcohol, neutrophils, mononuclear

phagocytes, microbicidal activity, oxidative metabolism, myeloperoxidase, cationic proteins, absorbing capacity.

### **Study areas and methods:**

The study was performed on 80 white non-inbred mature white male rats weighing 180 - 220 g, kept in standard vivarium conditions. To form the cysts in the liver tissue a polyvinylchloride (PVC) foreign body was implanted (for a 30 day period), followed by its removal. The animals were divided into 4 groups: group 1 - control group (intact animals), group 2 - animals with the cysts formed, groups 3 and 4 - animals with the cysts formed that received EA and SFC respectively in the cavity of the cyst.

The animals were kept in standard vivarium conditions with natural light mode on a standard diet for laboratory animals (State All-Union standard GOST R 50258-92), meeting the international recommendations of the European Convention for the Protection of Vertebrate Animals used in experimental studies, along with the regulations of laboratory practice when performing preclinical research in the Russian Federation (State All-Union standard GOST 51000.3-96 and 51000.4-96) and the Order of the Ministry of Health of the Russian Federation of 19.06.2003 №267 "On approval of the regulations of Good Laboratory Practice» (GLP).

We estimated the number of leukocytes, neutrophils, lymphocytes in the peripheral blood, the intensity of oxygen-dependent metabolism (spontaneous and induced NBT-test) in polymorphonuclear leukocytes (PMNL) and peritoneal macrophages (PM), their absorbing capacity, microbicidal activity (against fungi *Candida albicans*) under conditions of functioning and blockade (by sodium azide) of oxidative killing, myeloperoxidase (MP) activity and the content of cationic protein (CP) in polymorphonuclear leukocytes and peritoneal macrophages [4].

The results were recorded on the 60th and 90th days from the moment of cyst formation. The statistical data processing was performed using the methods of variation statistics [2], considering the difference  $p \leq 0,05$  as reliable. The data is presented as percentage of the control group values.

### **Results**

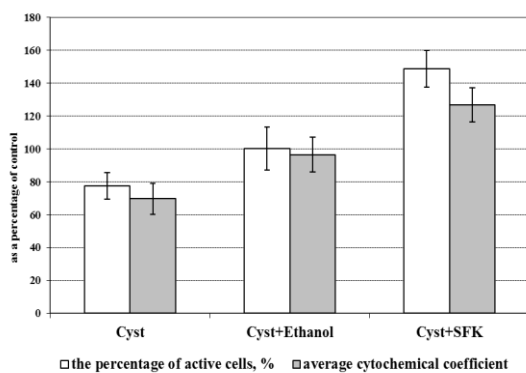
On day 60 (a formed cyst) the increase in number of leukocytes in peripheral blood (up to 120,7%) was observed, mainly due to the increase in number of lymphocytes. We also observed a drop in the intensity of oxygen-dependent metabolism (the induced NBT-test showed that the percentage of active cells (%A) amounted to 83,33%, the activation index (AI) - to 78,54%) and the almost two-fold reduction of MP activity in PMNL (the percentage of active (PA) PMNL amounted to 52,69%, and the average cytochemical coefficient (ACC) – to 43,43%). This is indicative of the suppression of both peroxidase-dependent and peroxidase-independent bactericidal activity mechanisms of PMNL, that was confirmed by a deep suppression of oxidative killing of PMNL (the

inactivation index amounted to 75,69%). We also noticed a decrease in both the number of PMNL involved in phagocytosis (to 80,0%) and their absorbing capacity (to 77,67%).

The inhibition of oxidative peroxidase-dependent mechanisms of peritoneal macrophages bactericidal activity was observed as well, which was confirmed by the decrease of myeloperoxidase activity: the percentage of myeloperoxidase-positive peritoneal macrophages amounted to 79,03% and the percentage of ACC – to 84,62%. As a result, the inactivation index of macrophages decreased to 83,99%.

On day 90 leukocytosis was retained (up to 147,71%) even at a steady increase of neutrophils and lymphocytes (up to 149,76% and to 143,47%). The decrease of oxidative killing of PMNL was also retained (the inactivation index amounted to 82,75%), mainly due to the inhibition of microbicidal peroxidase-dependent neutrophilic systems. This was evidenced by a drop in the activity of myeloperoxidase (ACC estimated at 69,66%), while the intensity of reactive oxygen species (ROS) formation (induced by the NBT-test) reduced insignificantly (the activation index amounted to 81,89%) (Fig. 1A).

**A**



**B**

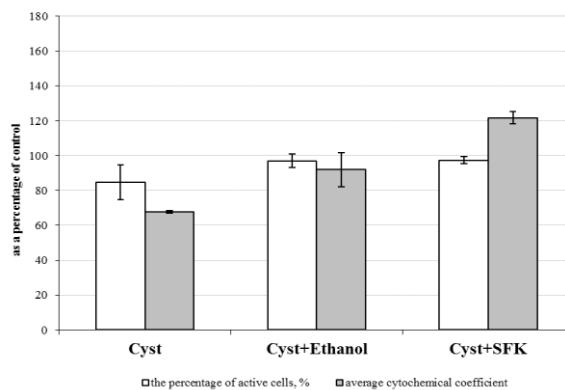


Fig. 1. Impact of ethyl alcohol and sulfacrylate on the content of myeloperoxidase (A) and cationic protein (B) in PMNL in experimental animals with hepatic cysts (the 90th day of observation)

The depressive impact of the pathological process in the liver on the functional state of the peritoneal macrophages escalated. There was observed a profound inhibition of oxidative mechanisms of bactericidal activity of the peritoneal macrophages (the inactivation index decreased to 70,43%) in the suppression of both peroxidase-dependent and peroxidase-independent killing mechanisms. This fact was evidenced by the decrease in the intensity of ROS formation in the induced NBT-test and the ongoing drop in the activity of myeloperoxidase in the peritoneal macrophages (the percentage of active macrophages was estimated at 63,86% and the ACC – at 70,75%) (Fig. 2A.). The increase in number of peritoneal macrophages involved in

phagocytosis with the drop of their absorbing activity (up to 73,65%) was retained as well.

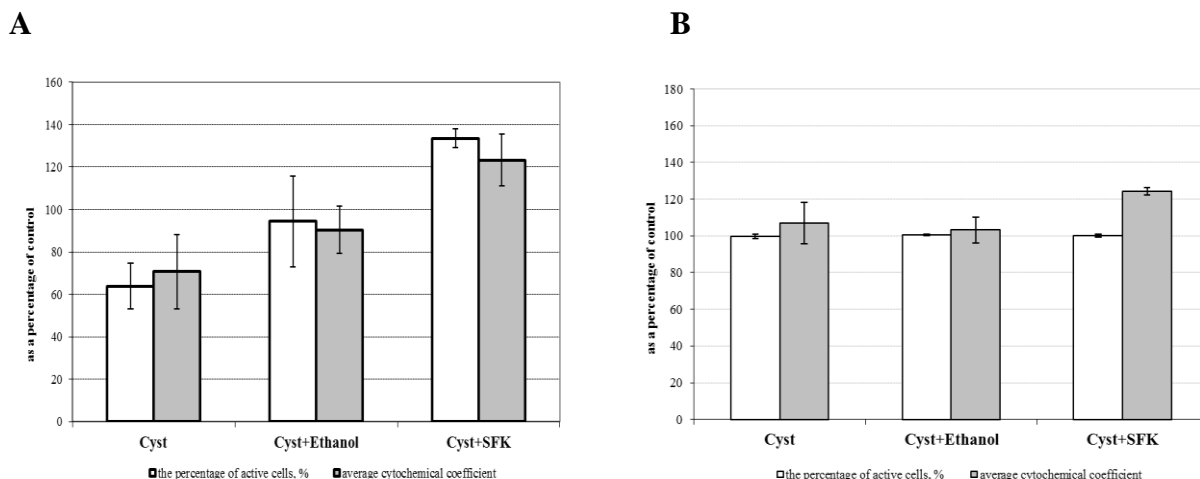


Fig. 2. Impact of ethyl alcohol and sulfacrylate on the content of myeloperoxidase (A) and cationic protein (B) in the peritoneal macrophages in experimental animals with hepatic cysts (the 90th day of observation)

The result of the studies showed that the formation of a cystic process in liver causes significant disorders in the functional state of PMNL and peritoneal macrophages. This is evident from the formation of leukocytosis, the inhibition of oxidative and nonoxidative killing mechanisms, the decrease of myeloperoxidase activity, cationic proteins, deep oxygen-dependent metabolism of cells and their absorbing capacity.

Using ethyl alcohol as a sclerosant reduced the intensity of leukocytosis (white blood cell number decreased from 147,71% to 125,91%) due to the reduction of neutrophil number to the norm while lymphocytosis was retained. Both oxygen-dependent and oxygen-independent bactericidal factor activity recovered (the inactivation index amounted to 91,67% and 84,13%, respectively). This fact was confirmed cytochemically by the increase of myeloperoxidase activity and the level of cationic proteins to the level of intact animals (Fig. 1A, B). However, the suppression of the absorbing capacity of PMNL was retained: the number of PMNL involved in phagocytosis stayed low, as well as their absorbing activity (77,50% and 71,84%, respectively).

Using ethyl alcohol normalized functional activity of the peritoneal macrophages. However, the intensity of oxygen-dependent metabolism of the peritoneal macrophages in the spontaneous NBT-test remained very high. We registered the recovery of the activity of peroxidase-dependent and peroxidase-independent killing mechanisms of macrophages (the inactivation index amounted to 89,65%). This was clearly seen from the recovery of ROS formation intensity (the induced NBT-test) and myeloperoxidase activity in peritoneal macrophages (Fig. 2, A). The absorbing capacity of the macrophages rose to normal.

Applying SFK corrected the functional activity of PMNL and peritoneal macrophages in

animals with nonparasitic hepatic cyst. SFK completely eliminated leukocytosis due to the steady reduction of neutrophils and lymphocytes number. Moreover, SFK not only restored but also activated oxidative and nonoxidative microbicidal systems of PMNL (the inactivation index amounted to 122,38% and 130,49%, respectively). This came with the increase of myeloperoxidase activity and the level of cationic proteins in PMNL (Fig. 1A, B). SFK normalized both oxygen-dependent metabolism and absorbing activity of PMNL.

SFK application also increased the functional activity of mononuclear phagocytes. Unlike ethyl alcohol, SFK normalized oxygen-dependent metabolism of peritoneal macrophages: the intensity of ROS in the spontaneous NBT-test decreased to normal and it recovered in the induced NBT-test. This proves the elimination of depression of peroxidase-independent killing mechanisms of macrophages by SFK. SFK not only recovered (as in the case of ethyl alcohol application), but also increased the myeloperoxidase activity in mononuclear phagocytes (Fig. 2A). As a result the inactivation index of peritoneal macrophages increased to 141,59%. Some increase of nonoxidative killing was also observed. This was accompanied cytochemically by the increase of the level of cationic proteins (Fig. 2B). SFK increased the absorbing activity of peritoneal macrophages to the level of that in intact animals.

### **Conclusions**

Thus, using ethyl alcohol and SFK for filling the cystic defect of liver significantly corrected the functional status of PMNL and peritoneal macrophages. SFK provided a better correction of the functional status of PMNL and peritoneal macrophages. We observed the increase of activity of oxidative and nonoxidative bactericidal mechanisms of phagocytes, the increase of oxygen-dependent metabolism and the absorbing capacity of PMNL and peritoneal macrophages.

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