COMPARISON OF BIOLOGICAL ACTIVITY OF LIPID FRACTIONS OF SILT MUD

S. V. Nizkodubova, A. A. Tabatskaya,
G. M. Nechai, V. A. Kishakovskaya,
V. G. Chernykh, Yu. M. Rogozin,
and E. Ya. Matis

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During the extensive economic exploitation of Siberia, a promising future development is the use of natural health resort factors — mineral waters, peats, therapeutic muds — outside the health resorts themselves. However the use, in particular, of natural native mud is not always possible or economically profitable. In some diseases, moreover, mud baths are contraindicated. Accordingly the search for and production of therapeutic mud preparations and the study of responses of the body to their application are of great importance. Mechanisms of the action of peloids and preparations based on them have not been studied in detail, yet there is no doubt that their efficacy is due not only to thermal and mechanical factors, but also to the influence of biologically active chemical components.

The object of this investigation was to isolate a complex of lipid organic substances from silt mud, to separate the lipids into fractions, and compare their biological activity.

EXPERIMENTAL METHOD

Lipids were isolated from native mud by repeated extraction with mixtures of alcohol with benzene or chloroform, without heating. To remove water the mud was first treated with acetone. Extracts of lipids in solvents were washed with water and dried, after which the solvents were removed by evaporation under reduced pressure.

Experiments were carried out on 60 male rate weighing 180-200 g, divided into six groups: 1) background, 2) experimental peritonitis, 3) peritonitis without subsequent treatment (control), 4-6) peritonitis + course (10 procedures) of ultra-sound and ultrasonic application (phonophoresis) of solutions of the chloroform and hexane fractions. For phonophoresis a 1% solution of lipid fractions in mineral oil was prepared in a dose of 35 mg/kg body weight (UTP-1 apparatus, frequent 830 kHz, intensity 0.6 W/cm^2, exposure 5 min by the labile method under constant conditions). Tests were carried out on animals in the initial state (background), 6 h after production of peritonitis, and at the end of the course of procedures. Peritonitis was induced by intraperitioneal injection of a 0.2% solution of silver nitrate, Morbid anatomical changes were assessed in the peritoneal cavity [7], glycogen [3], alkaline phosphatase [5], and peroxidase [2] were determined cytochemically in the blood leukocytes, and the mean cytochemical coefficient was deduced [6]. Humoral and cellular factors of nonspecific immunity were studied as the serum lysozyme concentration by a nephelometric method, the complement activity was determined by the 50% hemolysis test, and phagocytic activity of the leukocytes was estimated [1]. The partial pressure of oxygen (pO_2) and the redox potential (RP) were determined polarographically in blood and liver homogenates. Pieces of liver for histochemical analysis were fixed in appropriate fixatives and embedded in a paraffin wax. For a general survey sections 4-6 μ thick were stained with hematoxylin and eosin, with azure II-eosin, and by Van Gieson's method. Bile acids were detected histochemically in the liver by Forsgren's method and estimated semiquantitatively by counting 100 cells of the preparation by means of an Ehrlich's ocular.

Experimental Laboratory, Research Institute of Health Resorts, Ministry of Health of the RSFSR, Tomsk. Institute of Petrochemistry, Siberian Branch, Academy of Sciences of the USSR, Tomsk. (Presented by Academician of the Academy of Medical Sciences of the USSR N. V. Vasil'ev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 94, No. 7, pp. 54-55, July, 1982. Original article submitted December 23, 1981.

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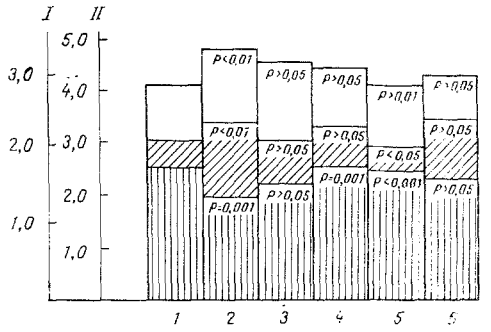

Fig. 1. Some cytochemical parameters of peripheral blood leukocytes under the influence of mud lipid fractions. 1) Background; 2) peritonitis; 3) peritonitis without treatment; 4) ultrasound; 5) phonophoresis of chloroform fractions; 6) phonophoresis of hexane fraction. Ordinate: I) mean cytochemical coefficient (MCC) of glycogen content (in conventional units), II) MCC of enzyme activity (in conventional units). Unshaded part of column represents glycogen, obliquely shaded part alkaline phosphatase, vertically shaded part peroxidase; P calculated by comparison with peritonitis.

EXPERIMENTAL RESULTS

Lipids with accompanying sulfur isolated from silt mud (57% water) accounted for 0.25-0.27% of the weight of the crude residue. The lipid concentrate was divided into two main fractions by treatment with hexane. The hexane-soluble, nonpolar fraction included 30-50% of the total lipids, whereas the fraction insoluble in hexane was the polar or chloroform fraction. Mainly hydrocarbons were concentrated in the monpolar part of the extract: paraffins, squalene, carotenes. The chloroform fraction was found to contain phospholipids, alcohols, sterols, fatty acids, and pigments belonging to tetrapyrrole classes.

The experiments showed that phonophoresis of the polar fraction had a marked anti-inflammatory, stimulating, and absorptive action. This was shown mainly by the pathological investigation of the peritoneal cavity: Complete absorption of most adhesions of the viscera was observed, toxic changes in the capillaries and signs of hemostasis, which were very distinctly seen 6 h after the formation of peritonitis and which still persisted to a considerable degree in animals of the control group, were reduced. In experiments with phonophoresis of the nonpolar fraction and ultrasound, adhesions were found in the subdiaphragmatic region with the liver and spleen and also in the region of the testes. Hemorrhages were present on the abdominal wall and in the intestine, with congestion of the parenchymatous organs. Under the influence of the factors studied the trabecular structure of the hepatic lobule was restored, and the lumen of the sinusoidal capillaries and central vein was dilated in places and congested with blood cells. Meanwhile, only after phonophoresis with the chloroform fraction was an increase observed in the number of hepatocytes with a high (45.8 ± 1.21, control 10.8 ± 3.64; $P < 0.01$) and average (44 ± 2.18, control 34.75 ± 3.88; $P < 0.02$) content of bile acids, which were irregularly distributed over the cytoplasm and fused into conglomerates, evidence of activation of bile acid synthesis [4].

Phonophoresis of the lipid fractions had a stimulating effect on the factors of nonspecific immunity, more especially under the influence of the polar fraction. For instance, the blood lysozyme titer was increased to 29.8 ± 2.30 C united ($P < 0.01$) and the complement titer to 21.6 ± 0.76 C units ($P < 0.02$), and the phagocytic activity of the leukocytes was increased to 33.2 ± 1.22% ($P < 0.02$); the corresponding figures in the control were 19.8 ± 2.80 and 11.2 ± 0.96 C units and 28.2 ± 1.97%. After a course of ultrasonic procedures the results did not differ significantly from the control. The results of the immunologic investigation were confirmed by the cytochemical determination of blood parameters (Fig. 1) and the state of oxidation—reduction. After phonophoresis with the polar fraction of lipid, increased functional activity of the blood neutrophils was observed and all values of the cytochemical parameters returned to their background levels. The intensity of oxygen uptake by

liver tissue was increased on average by 32-37% ($P < 0.01$) compared with the control. Determination of RP showed the same pattern as determination of pO_2.

It can thus be concluded from these investigations that the biological activity of the lipid extract of silt mud is due to substances concentrated in the polar part. Despite the presence of carotene, the hexane-soluble compounds had no significant biological action and, from the practical point of view, they can be regarded as inert components.

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ROLE OF THE MAJOR (H-2) HISTOCOMPATIBILITY SYSTEM IN THE RESPONSE OF MICE TO ETHANOL

S. V. Shoshina and A. I. Maiskii

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The search for genetic markers can be undertaken if lines of mice differing in localized regions of the genome are used as biological model [2]. These include congeneic resistant lines (CR lines) of mice, characterized by differences in the major (H-2) histocompatibility system. Convincing evidence of the role of this sytem in the regulation not only of immune, but also of neuromediator, endocrine, and metabolic processes, has been accumulated [7, 10]. Considering the importance of the latter in the pharmacodynamics of ethanol [4, 6], it was decided to study the response of CR lines of mice to a single dose and chronic administration of ethyl alcohol.

EXPERIMENTAL METHOD

Experiments were carried out on male mice weighing 20-35 g belonging to two pairs of CR lines, namely B10·R107, B10·RIII, and A/Sn, A·SW, differing in their H-2 locus, kept on a standard laboratory diet. The progenitors of the lines used in the experiments were obtained from the Research Laboratory of Experimental Biological Models, Academy of Medical Sciences of the USSR.

After a single intraperitoneal injection of ethyl alcohol in doses of 1.0 and 2.5 g/kg, changes in the locomotor activity of the animals were estimated 15, 30, and 60 min after injection in chambers with a photoelectric cell [9]. Parallel studies were made of the motor activity of control animals receiving isotonic NaCl solution. The experiments were carried out from 10 a.m. to 2 p.m., in a period characterized by its ability of the parameter chosen for recording for these particular rodents [3]. The response of mice of the CR lines to a narcotic dose of ethanol (4 g/kg body weight [11]) was determined in "dropping off time" tests, recorded as the time between administration of ethanol and loss of the correct body position reflex by the animals, and the "duration of alcohol sleep," i. e., the time after

Laboratory for the Search for and Study of Substances for Prevention and Treatment of Drug Addiction, Institute of Pharmacology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman.)

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