LITERATURE CITED

1. V. D. Antonenkov, S. V. Popova, and L. F. Panchenko, Vopr. Med. Khim., No. 6, 79 (1983).
2. V. D. Antonenkov and L. F. Panchenko, Byull. Éksp. Biol. Med., No. 11, 550 (1986).
3. L. F. Panchenko, S. V. Pirozhkov, S. V. Popova, and V. D. Antonenkov, Byull. Éksp. Biol. Med., No. 4, 407 (1987).
4. V. G. Tsyplenkova, Byull. Éksp. Biol. Med., No. 7, 112 (1988).
5. V. G. Sharov, P. B. Jennings, and H. K. Hawkins, Arkh. Patol., No. 10, 35 (1980).
6. V. D. Antonenkov and L. F. Panchenko, Int. J. Biochem., **20**, 823 (1988).
7. I. Balogh, E. Somogyi, P. Sotonyi, et al., Z. Rechtsmed., **90**, 7 (1983).
8. R. F. Beers and J. W. Sizer, J. Biol. Chem., **195**, 133 (1952).
9. S. Fairhurst, D. J. Barber, B. Clark, and A. A. Horton, Biochim. Biophys. Acta, **752**, 491 (1983).
10. R. C. Hibbs, V. J. Ferrans, W. C. Black, et al., Am. Heart J., **69**, 766 (1965).
11. M. Kino, J. Molec. Cell. Cardiol., **13**, 5 (1981).
12. O. H. Lowry, N. J. Rosebrough, A. F. Farr, and R. J. Randall, J. Biol. Chem., **193**, 265 (1951).
13. L. F. Panchenko, S. V. Pirozhkov, S. V. Popova, and V. D. Antonenkov, Experientia (Basel), **43**, 580 (1987).
14. J. Sedlak and R. H. Lindsay, Analyt. Biochem., **25**, 192 (1968).
15. V. G. Tsyplenkova (V. G. Tsiplenkova), A. M. Vikhert, and N. M. Cherpachenko, J. Am. Coll. Cardiol., **8**, No. 1, 22A (1986).
16. A. M. Vikhert, V. G. Tsyplenkova (V. G. Tsiplenkova), and N. M. Cherpachenko, J. Am. Coll. Cardiol., **8**, No. 1, 3A (1986).

EXPERIMENTAL STUDY OF ORIENTED ANTIBIOTIC TRANSPORT IN SUPPURRTIVE-INFLAMMATORY DISEASES OF THE LIVER AND BILIARY TRACT

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The relatively high frequency of suppurative-inflammatory complications in surgery of the biliary tract, in which they are the main cause of death, despite the widespread use of modern antibiotics, makes the search not only for new preparations, but also for more effective methods of administering them, an urgent problem [2, 3]. The attention of research workers in recent years has been drawn to the development of methods of oriented transport of therapeutic and diagnostic preparations directly into a pathological focus [1, 7]. Oriented transport of antibiotics directly into the zone where they exert their chemotherapeutic action would allow the systemic toxicity of these substances to be reduced, while enhancing their therapeutic effectiveness. These problems have not been studied experimentally. The urgency of the problem also is due to the fact that none of the existing methods of antibiotic administration enables a therapeutic concentration of the preparation to be maintained for a long time in the liver and portal system [5]. As regards the treatment of inflammatory diseases of the liver and biliary tract, the use of erythrocyte ghost carriers is particularly interesting, for it has been shown [7] that they are ingested by erythrophagocytic cells of

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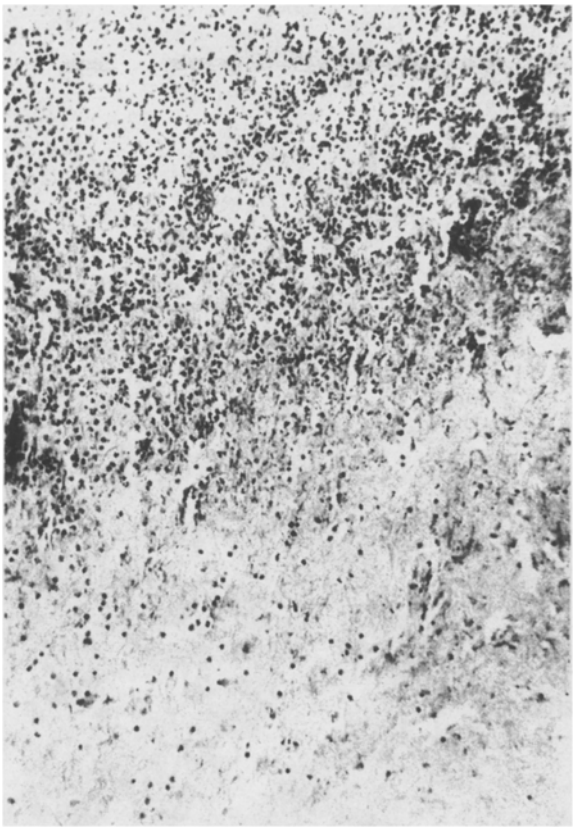

Fig. 1. Necrosis and phlegmonous inflammation of gall bladder wall. Here and in Figs. 2 and 3: hematoxylin and eosin. 160×.

the liver (stellate endotheliocytes). From the point of view of biological compatibility, it is advantageous to use autologous erythrocyte ghosts.

The aim of this investigation was to study the stability of antibiotic retention by autologous erythrocyte ghost carriers in the blood stream and the possibility of using them in the treatment of suppurative-inflammatory diseases of tie liver and biliary tract.

EXPERIMENTAL METHOD

Experiments were carried out on 25 mongrel dogs weighing 12-20 kg in which a model of destructive cholecystitis was produced surgically. For this purpose, after premedication (droperidol 0.5 mg/kg, diphenhydramine 1.5 mg/kg, analgin 50 mg/kg), a midline laparotomy was performed under thiopental anesthesia (10 mg/kg), the gall bladder was punctured, bile withdrawn from it, and a 7% solution of acetic acid injected into its lumen in a volume of 0.1 ml/kg body weight. The acetic acid solution was withdrawn after 5 min and a 20% fecal suspension injected into the lumen of the gall bladder in a volume of 0.7 ml/kg body weight. The criteria of development of acute cholecystitis were as follows: the presence of the corresponding clinical symptoms (apathy, raised body temperature, vomiting, guarding of the abdominal muscles, refusal to eat) and the results of blood tests. The final conclusion regarding the character of the changes in the gall bladder was based on visual and histologic investigations. The surface of the gall bladder 48 h after creation of the model was dull and covered with fibrin. The walls were strongly infiltrated and, consequently, thickened and tense. Purulent or mucopurulent contents were present in the lumen of the gall bladder. The mucous membrane was dull, sometimes with areas of hyperemia and hemorrhage. The microscopic picture was one of phlegmonous-gangrenous cholecystitis (Fig. 1). As a rule colonies of microorganisms could be identified in the wall of the gall bladder.

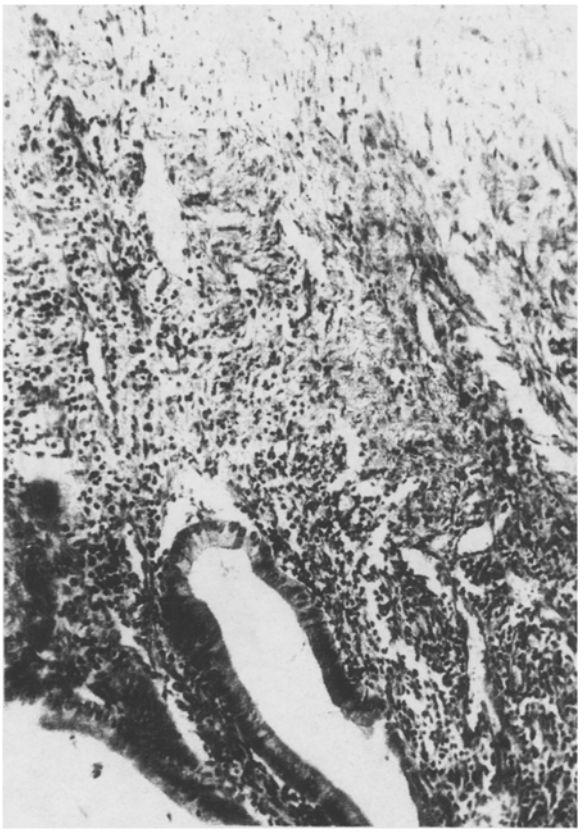

Fig. 2. Focus of inflammatory leukocytic infiltration of mucosa of gall bladder.

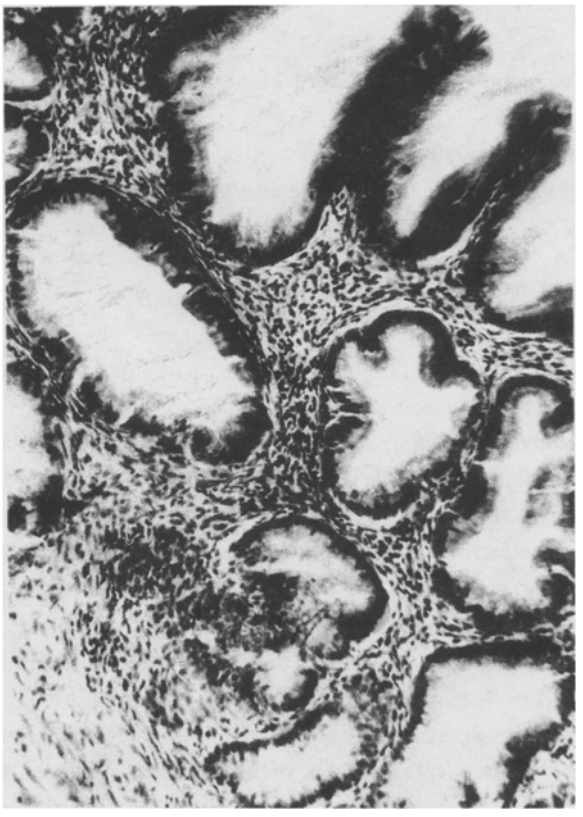

Fig. 3. No signs of inflammation present. Normal picture of histologic structure of mucosa of gall bladder.

TABLE 1. Concentration of Kanamycin Administered by Different Methods (in μg/g)

Mode of administration	Liver			Blood serum		
	24 h	48 h	72 h	24 h	48h	72 h
Traditional	0	0	0	3,7±0,8	0	0
Oriented transport	4,2±0,2	1,9±0,2	0	0	0	0

In the experimental model thus created, all the animals thus developed destructive cholecystitis after 48 h.

From the results of the total blood count a simplified hematologic index of the toxic manifestations was calculated by an appropriate equation [3]. The blood tests were carried out before creation of the model of acute cholecystitis, 48 h after its creation, and also during and after the course of treatment. The animals began to be treated with kanamycin 48 h after creation of the model of acute cholecystitis. Depending on the method of treatment the animals were divided into two groups: control and main groups. Kanamycin (30 mg/kg) was injected intravenously once a day into animals of the control group. In the main group, kanamycin (30 mg/kg), incorporated into autologous erythrocyte ghosts by the method of hypoosmotic hemolysis [6, 7], was injected once daily, intravenously. Thus in the main group, oriented transport of the antibiotic was carried out into the liver.

The method of incorporation of the antibiotic by hypoosmotic hemolysis into erythrocyte ghosts was modified a little in our experiments. After removal of the blood plasma, the erythrocytes were washed twice with physiological saline by centrifugation at 3000 rpm for 5 min at 4°C. Seven volumes of distilled water, cooled to 0°C, was added to the erythrocyte residue, and the contents were centrifuged at 8000 rpm for 25 min at 4°C. Seven volumes of antibiotic, dissolved in distilled water cooled to 0°C, was added to the resulting erythrocyte ghosts. The suspension was incubated for 20 min at 4°C, after which one-ninth of the volume of 9% sodium chloride solution was added to restore the integrity of the membrane of the erythrocyte ghosts, and the sample was incubated for 30 min at 37°C. After incorporation of the antibiotics into the erythrocyte ghosts, they were injected intravenously. The dose of antibiotic was calculated on the assumption that 30% of the preparation was incorporated into erythrocyte ghosts.

To study the stability of retention of the antibiotic by the erythrocyte ghosts and its desorption from them in the blood stream, special experiments were carried out with gentamicin. Erythrocyte ghosts with incorporated gentamicin were washed twice with physiological saline and then incubated in autologous plasma at 37°C for 25 min.

The course of antibiotic therapy lasted 5 days in the two groups. The animals were withdrawn from the experiment 24 h after the last injection of the antibiotic and killed in accordance with the ethical rules, laparotomy was repeated, and the state of the gall bladder and liver assessed visually; the gall bladder was investigated histologically. The concentration of the antibiotic in the liver tissue of the dogs was determined 24, 48, and 72 h after the last injection of the antibiotic by the agar diffusion method [4]. *Bacillus subtilis* ATCC 6633 was used as the test microorganism.

EXPERIMENTAL RESULTS

Experiments with desorption of the antibiotic from erythrocyte ghost carriers (using gentamicin) showed that the gentamicin concentration in the supernatant after the first washing of the erythrocyte ghosts with physiological saline was 0.4 ± 0.03%, falling after the second washing to 0.04 ± 0,007%. The gentamicin concentration in the blood plasma after incubation of the erythrocyte ghosts in it was 0.2 ± 0.008%, i.e., it was minimal. The gentamicin concentration in the erythrocyte ghosts themselves, after washing twice and incubation in autologous blood plasma, was 2.3 ± 0.01% compared with 3.1 ± 0.3% in the erythrocyte ghosts of the control group, in which the concentration of the antibiotic was determined immediately after a single washing of the ghosts. The facts described above demonstrate the adequate resistance of the erythrocyte carriers to desorption of gentamicin from erythrocyte ghosts in the blood stream and the possibility of their use for oriented antibiotic transport.

Comparative analysis of the traditional intravenous method of injection of antibiotics and of oriented transport in erythrocyte ghosts showed that the latter method is highly effective. For instance, in the main group of animals after only 2 days of treatment the dogs' body temperature was back to normal, they became more active, and ate food. The control animals remained apathetic and febrile and were not interested in eating. The hematologic index of the toxic manifestations of the dogs of the main group was 14.8 ± 5.1 before creation of acute experimental cholecystitis, 124.3 ± 67.4 after creation of the model, and back to normal at 13.9 ± 3.3 after the end of the course of treatment. Meanwhile, in the control group the hematologic index of toxicity showed no tendency to return to normal, and amounted to 17.8 ± 11.2, 42.2 ± 21.9, and 134.4 ± 80.5 respectively.

Pharmacokinetic studies revealed considerable differences in the kanamycin concentration in the liver tissue depending on the method of administration of the antibiotic (Table 1).

It will be clear from Table 1 that kanamycin, when given by the traditional method, was not determined in the liver tissue at the times of investigation. When kanamycin was administered by oriented transport directly into the liver in erythrocyte carriers, it was found after 24 and 48 h in the liver tissue but was absent from the blood serum. This is an important fact in connection with reducing the systemic toxicity of the antibiotic, while maintaining an adequate concentration of it for a long time actually in the liver and biliary tract.

The facts described above established a sound pharmacokinetic basis for the efficacy of oriented antibiotic transport in erythrocyte ghost carriers into the liver and indicated that its use is indicated in the treatment of suppurative-inflammatory diseases of the liver and biliary tract.

Visual assessment of the gall bladder and liver revealed that in dogs of the control group the gall bladder as a rule was surrounded by adhesions, the serous membrane was dull, and residual inflammatory changes were present. In three animals abscesses and foci of infiltration could be seen around the gall bladder. In dogs of the main group, adhesions also were present around the gall bladder but the serous membrane of the gall bladder was smooth and shiny, and no residual inflammatory changes could be detected. The results of the visual examination were in agreement with histological changes in the gall bladder wall. With the traditional method of administration of kanamycin (Fig. 2) foci of leukocytic infiltration of the mucosa, penetrating a little way into the muscular coat, were found in the wall of the gall bladder. At the same time numerous areas of lymphocytic infiltration were seen. With oriented transport of kanamycin into the liver (Fig. 3) virtually no residual inflammatory changes could be found in the gall bladder wall.

Analysis of these results thus demonstrates the resistance of erythrocyte ghosts to possible desorption of the antibiotic into the blood stream. The results of general clinical, pharmacokinetic, and morphological investigations described above indicate that oriented transport of an antibiotic into the liver can be used in the treatment of suppurative-inflammatory diseases of the biliary tract. The fact that this method is more effective than the traditional intravenous method of injection of the antibiotic demonstrates that the introduction of this method into clinical practice is indicated.

LITERATURE CITED

1. T. P. Gening, I. I. Kolker, and Zh. Sh. Zhumadilov, Antibiotiki, No. 11, 867 (1988).
2. A. S. Ermolov, V. A. Ivanov, E. E. Udovskii, and E. F. Mironer, Khirurgiya, No. 2, 137 (1986).
3. I. I. Kolker and Zh. Sh. Zhumadilov, Prevention of Postoperative Suppurative-Inflammatory Complications [in Russian], Alma-Ata (1988).
4. S. M. Navashin and I. P. Fomina, Rational Antibiotic Therapy (reference book) [in Russian], 4th edn., Moscow (1982).
5. V. S. Savel'ev, V. A. Gologorskii, B. R. Gel'fand, et al., Antibiotiki, No. 2, 143 (1989).
6. R. F. Baker and N. R. Gillis, Blood, **23**, No. 2, 170 (1969).
7. G. Ihler, Pharm. Ther., **20**, 151 (1983).