Investigation of the Antituberculous Effect In Vivo of The New Aerosol Remedy

Dr. Zveryachenko Timur,
(The North Kazakhstan State University named after M. Kozybayev, Republic of Kazakhstan)
Introduction

The substance spited from buds of balsam poplar contains terpenoids: hydrocarbons make up about 70% and consist mainly of acyclic (Farnese) and monocyclic (type of bisabolan and type of humulan) sesquiterpenes. There are also present sesquiterpene alcohols in some quantity - farnesol (up to 12%) and bisabolol (up to 10%).

We have developed new pharmaceutical forms: aerosol for prevention of tuberculosis bacterioexcretion based on the fraction of terpenoids and pfokalin.

The new remedy was investigated in vitro concerning strains of tuberculosis (H37Rv and 2 antibiotic-resistant strains), and also strains of Staphylococcus aureus and Streptococcus pneumoniae. There was established the accurate cidal effect. There was also studied antitussic activity and safety of preparations in acute and long-term experiment. Preparations are safe at application in therapeutic dosages.
Summary

The antituberculous activity of new remedy was studied by the experiments on guinea pigs.

The best results were achieved in the group with a combination of traditional antibiotics and test remedy. Mass recovery of the remaining animals was faster, and compared with the group treated with only traditional antibiotics, the weight of animals of this group was about 14 g more by the end of the experiment. Also, as a result of treatment cough attacks were completely absent. 3 animals were completely healthy by the end of treatment, and the remaining 3 had minor lesions with minimal growth (+1) only in the inguinal lymph node closest to the site of infection. Also there was no mortality of the animals compared to the third groups treated with only antibiotics.

In the group by self-use of the test remedy mass reduction of animals significantly slowed down and was $97 \pm 5.2$ g by the end of the experiment. Although physical activity was reduced, and there was a depression at the same time the amount of beadma and cough attacks decreased significantly in comparison with the animals of the non-treated group and the culture growth of internal organs was 3+. But all animals were alive till the end of experiment.

Thus, we can conclude that the self-use (without antibiotics) in the studied dosage the test remedy has bacteriostatic action against Mycobacterium tuberculosis in the experiment in vivo, thus effectively reducing the amount of beadma and cough attacks, which helps to reduce bacterioexcretion.
**Object of the research**

The new remedy is a two-component emulsion of terpenoid fraction (essential oil) balsam poplar buds and pfokalin in water intended for use in aerosol form for the treatment of respiratory diseases and pulmonary tuberculosis and to prevent bacterioexcretion.

**The aims of the work**

The research of the antituberculous activity (strain H37Rv) by the experiments on guinea pigs.

balsam poplar  
*(Populus balsamifera L.)*

pfokalin
Materials and methods

The experiment was conducted on 16 males and 16 females guinea pigs (weighing 280-350 g). Animals were challenged with a museum strain of Mycobacterium tuberculosis N37Rv. Animals were divided into 5 groups (the 2nd Group included 8 animals, the others included 6):

1. Control uninfected animals.
2. Control infected animals without treatment.
3. Control infected animals, treated with conventional antibiotics (rifampicin - 10.0 mg / kg, isoniazid - 15.0 mg / kg per orally).
4. Experience infected animals treated with antibiotics (rifampicin - 10.0 mg / kg of isoniazid - 15.0 mg / kg, per orally) and with the test drug.
5. Experience infected animals, with the test drug treatment. Animals of groups 4 and 5 treated with test remedy (0.071 ml/kg 3 times per day) injected using the inhaler.
Animals of the first group (uninfected) during the whole experiment retained their normal motorial and behavioral activity. Feed intake corresponded to normal. During the experiment the animals weight increased by 90 ± 6 g.
Results. Group 2

Animals of the second group which received no treatment died within 36 days after infection. In the experiment, they had the full range of behavioral, morphological and microbiological pathological changes attributable to tuberculosis. Animals in this group suffered from infection most severely. Mass loss during the experiment was about 100 ± 3.1 g. There was observed the formation of a defect of the skin in animals of this group to the site of infection. At autopsy of dead animals there was observed plethora of internal organs, on the surface of the lungs, hepatic and spleen there were observed numerous grayish-yellow mons, in many places merging with each other. Right inguinal lymph nodes were significantly increased in size, on the discission there was creamy pus. Inoculation of the animals in this group showed a heavy growth of Mycobacterium tuberculosis (3 +) from all samples.
Results. Group 2

Picture 1 - Photo of lung of animal from 2nd group (×250)

Picture 2 - Photo of lungs and bronchi of animal from 2nd group (×40)
Results. Group 2

Picture 3 - Photo of liver of animal from 2nd group (×400)

Picture 4 - Photo of spleen of animal from 2nd group (×100)
Results. Group 2

Picture 5 - Photo of lymph node of animal from 2nd group (×40 and ×400)
Results. Group 3

In the third group (was treated with rifampicin - 10,0 mg/kg, isoniazid - 15,0 mg/kg, per orally) during the experiment only one female died on the 30th day after treatment.

Mass reduction of the survived animals was 70 ± 3,5 g. At thanatopsy 3 animals had no pathological changes in the internal organs. Homogenate culture of internal organs of animals of this group gave a single growth of *Mycobacterium tuberculosis* (single colony from 1 to 5). Two animals had isolated small hillocks of grayish-yellow color, with no tendency to merge on the surface of the lungs and liver. Organs culture of these animals showed a slight increase (1+) of all samples.

Thus, these data confirmed the high protective effect of the essential anti-TB drugs (isoniazid, rifampicin) in the treatment of tuberculosis.
Results. Group 3

Picture 6 - Photo of liver of animal from 3rd group (×40)
The best results were achieved in the fourth group with a combination of rifampicin (10.0 mg/kg) and isoniazid (15.0 mg/kg) with test remedy (0.071 ml/kg 3 times per day) injected using the inhaler. All animals of this group were alive till the end of experiment.

All the animals were active, did not refuse from the meals, hair loss was not observed. Since the beginning of the treatment there were observed the weight recovery by the animals, and by the end of the experiment the weight loss was only (56 ± 5 g). Skin defect in the area of infection was not found. Coughing attacks during the treatment stopped.

At autopsy of 3 animals had no pathological changes in the internal organs of pathological changes. 3 animals had inguinal lymph node to the site of infection 1,2 times larger.

High cure rates in this group were confirmed by microbiological studies: 3 animals had no growth of Mycobacterium tuberculosis, 3 animals with enlarged lymph nodes had growth of Mycobacterium only 1 +.
Results. Group 4

Picture 7 - Photo of lymph node of animal from 4th group (×40)
Results. Group 5

In the fifth group by self-use of the test remedy in a dosage of 0.071 ml/kg 3 times per day) injected using the inhaler all the animals survived till the end of the experiment. Mass reduction of animals significantly slowed down and was $97 \pm 5.2$ g by the end of the experiment. Although physical activity was reduced, and there was a depression at the same time the amount of begma and cough attacks decreased significantly in comparison with the animals of the 2nd group (non-treated).

All animals were identified with all the macroscopic and microscopic signs of disease. At autopsy, all animals of the fifth group had a plethora of internal organs, with the observed colonization of internal organs with tubercles gray-yellow mons, sometimes merging with each other. The culture growth of internal organs was 3+. 
Results. Group 5

Picture 8 - Photo of lung of animal from 5th group (×40)

Picture 9 - Photo of spleen of animal from 5th group (×40)
Results. Group 5

Picture 10 - Photo of liver of animal from 5th group (×40)

Picture 11 - Photo of lymph node of animal from 5th group (×40)
Conclusion

Thus, we can conclude that the self-use (without antibiotics) in the studied dosage the test remedy has bacteriostatic action against Mycobacterium tuberculosis in the experiment in vivo.

The rate of development of tuberculous process has fallen by more than 2 times compared with animals receiving no treatment.

It is advisable to explore higher doses of test remedy for self use.

In the complex therapy with antibiotics the test remedy effectively reduces the amount of begma and cough attacks, which helps to reduce bacterioexcretion. Also there was no mortality of the animals compared to the third groups treated with only antibiotics.