ELECTRON BEAM TECHNOLOGY FOR PRODUCTION OF NEW ANTI-TUBERCULOSIS DRUG

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Possibility of electron beam usage for immobilisation of hydrasidum of isonicotinic acid (HIA) on dextrane was studied to produce a drug for treatment of tuberculosis. As a result of irradiation of dextrane the processes of oxidation and formation of polysaccharide occur due to carbonyl groups which are capable to link isoniasidum. HIA immobilised on dextrane may be used as an effective and having prolonged action medical preparation for action on endo- and extracellular population of *Mycobacterium tuberculosis*.

INTRODUCTION

In recent years it is more and more often reported about usage of the electron accelerators for production of new medical preparations. Electron beam immobilisation is used for production of medical drugs based on biologically active substances. Enzyme complex produced by *Bacillus subtilis* is immobilised on polyethylenoxide by radiation grafting [1].

The report describes the technology for production of the new antituberculous preparation "Isodex" based on the well-known preparation "Tubazid" (or "Isoniazidum" - hydrazide of isonicotinic acid HIA). The radiation technology for production of medical drugs has the advantages over the methods of chemical synthesis because toxic reagents that will be then removed are not used. Usage of the electron beam technology permits to dispense with the expensive time- and labour-consuming processes of purification of a final product from intermediate chemical agents and products. The electron accelerators [10] are the most suitable sources because they provide high dose power rate, easy control and reproducibility of process.

TREATMENT OF TUBERCULOSIS

In connection with intensive increase in incidence of tuberculosis in the world development of new medicinal preparations that can effectively suppress growth of *Mycobacterium tuberculosis* in cells, blood and lymph is relevant. Until now this problem is far from resolution and for basic treatment of this disease the HIA is still used. HIA has a low molecular mass and in this connection the drug is quickly removed from a body. More than

80% of HIA is destroyed in liver and does not cause antimycobacterial effect. Therefore treatment of a patient having tuberculosis by HIA lasts for long time (1 year and more) with large frequency of toxic reactions.

The essence of the problems in treatment of tuberculosis is absence of the drugs equally effective for action on *Mycobacterium tuberculosis* circulating in blood and lymph and persisting in the cells of immune system (phagocytes). The persisting mycobacterium possess the high resistively towards the basic anti-tuberculous preparations (isoniazidum, rifampicinum, pyrazinamidum, etc.) because these preparations do not have the ability to be selectively accumulated in phagocytes containing persisting mycobacterium. So there is the necessity to develop the antimycobacterial preparations capable to be selectively "delivered" for action on endocellular tuberculous bacterial population. One of the ways to achieve this is immobilisation of the tuberculostatic preparation on lymphotropic polysaccharide.

For treatment of heavy forms of tuberculous in the experimental conditions, HIA immobilised on dialdehyde of dextrane with molecular mass 30-40 kDa (HIAD) has shown good effect. HIAD is occluded by macrophages selectively that allows effectively and prolonged action on an endocellular population of pathogenic organisms. The drug is less toxic then HIA, it has the prolonged antimycobacterial effect.

Despite of efficiency of HIAD in treatment of tuberculosis, till now remains an actual problem of its chemical synthesis as it is continuous, composite and is not adapted for pharmaceutical industrial technology. The main difficulty of chemical synthesis is purification of HIAD from HJO_4 and other toxic reagents by dialysis and methanol's sedimentation. The purpose of the this research was development of the radiation technology for production of antituberculous drug based on HIA immobilised on dextrane.

MATERIALS AND METHODS.

In the work we have used 10% solution of dextrane with M.m. 30-40 kDa from *Leuconostoc mesenteroides*. This polysaccharide is widely used as a blood substitute. Radiation activation of dextrane solution was achieved using braking gamma radiation on the electron accelerator ILU-6 [10, 11] (produced in Institute of Nuclear Physics of the Siberian Branch of the Russian Academy of Sciences). The electron energy was 2 MeV, dose power rate was 0.5 Mrad/s, the range of the investigated doses was 5-35 kGy. Due to action of generated free radicals dextrane was oxidated with formation of highly active carbonyl groups. The concentration of carbonyl groups in the irradiated solution of dextrane was assayed by reaction with 2,4-dinitrophenyl-hydrazine.

HIA was added into the electron beam treated water solution of dextrane and the reaction mixture was exposed at temperature of 100°C during up to 30 minutes to carry out the immobilisation process.

RESULTS AND DISCUSSION

Irradiation of dextrane solution by gamma radiation causes its activation and formation of polymer due to carbonyl groups. The exit of carbonyl groups has direct relation with the irradiation dose (see Fig.1). At the irradiation doses of more than 3,5 kGy the formation of hydrophilic gel was observed, and we failed to determine the concentration of carbonyl groups in it. We supposed that the molecular mass of dextrane can change as a result of irradiation process. Increase in the molecular mass of cross-linked dextrane more then 100 kDa may cause the undesirable biological effects connected with more long period of removing of polymer from a body. It is important because the molecular mass of dextrane and its pharmacokinetics are interdependent. The dose range chosen by us provides soft operating on dextrane and does not cause noticeable influence on its molecular mass.

For further research we have used solutions of dextrane irradiated in a dose of 35 kGy as thus the maximum carbonyl capacity is reached at the constant molecular mass. The simultaneously selected radiation dose allows to sterilise the final drug. In pharmaceutical industry of many countries for radiation sterilisation of medical drugs the radiation dose in range of 25 - 35 kGy is used. It allows easily to adapt production of HIAD for the modern industrial technology of radiation sterilisation of medical products.

For immobilisation of HIA on radiation - activated dextrane HIA is diluted in irradiated solution of dextrane up to the final concentrations from 4 up to 40 mg/mls. The prepared solutions were exposed at 100°C within 15 minutes for completion of immobilisation.

In the chemical technology for production of HIAD HJO_4, HJO_3, methanol, ethyleneglycol are used, and after activation of dextrane they must be carefully removed because they are toxiferous impurities. Such clearing essentially complicates the technology for production of HIAD (Fig. 2). Using the radiation method of activation these toxic impurities in dextrane are not required and therefore HIAD can be produced without complicated methods of cleaning. HIAD can be produced in one stage by irradiation of solution of dextrane containing HIA. However it is necessary to study the chemical and biological properties of products of HIA radiolysis that may be the object of our next study.

CONCLUSION

The new antituberculous preparation "Isodex" is the balanced mixture of free HIA (hydrazide of isonicotinic acid - the well-known preparation "Tubazid" or "Isoniazidum") and HIA immobilised on dextrane. acting on of *Mycobacterium tuberculosis* everywhere inside a body.

The medical testing of "Isodex" have shown its lower toxicity and prolonged action in comparison with "Tubazid" (preparation of HIA). The special feature of "Isodex" is its ability to be absorbed by macrophages which are the main places of persistence and vegetation of *Mycobacterium tuberculosis*. The duration of treatment is reduced and the efficiency of preparation usage is increased. The advantage of «Isodex» are as follows:

- The 6 months long treatment of chronic generalised tuberculous by Isodex shows decrease of phibrotic complications in liver in 4.5 times and phibrotic complications in lungs in 1.3 times in comparison with treatment by free isoniazide.
- Isodex remains in tissues (in vacuolar apparatus of macrophages - persistence place of mycobacterium) for a period in 18-20 times longer than isoniazide thus giving the possibility to accumulate the efficient concentration of the preparation (Fig. 3).
- Izoniazide doses of Isodex possesses toxicity in 5.5 times less than free izoniazide and its hepatotoxicity is in 1.6 times lower (Fig.4 and Fig.5).
- In case of intravenous or intraperitoneal injection twice a week Isodex provides the therapeutic activity equal to that of free isoniazide due to properties of dextrane capable to activate natural resistance mechanisms of a body, and this together with other pharmacodynamics of Isodex permits to decrease toxicity of treatment.
- It was shown on cellular cultures in vitro that during treatment by Isodex the confluence of phagosomes with lysosomes occurred in 1.4 time more frequently than in case of treatment by free isoniazide, so one of the main obstacles in the mechanism of persistence of mycobacterium - incompleteness of phagocytosis - was surmounted. It also determined high efficiency of Isodex.
- Isodex possesses bacteriostatic activity in blood equal to that of free isoniazide, and in vitro bacteriostatic activity of Isodex towards the persisting population of mycobacterium is sufficiently higher than that of free isoniazide.
- The obvious advantage of Isodex is that it is injected intravenously twice a week - it guarantees control and monitoring over the treatment procedure and permits to use it for a

walking patients. This is important because a sufficient part of the tuberculous patients are asocial persons and prisoners.

- All components of Isodex and the final product are sterilised during the production cycle - it is the great advantage for the industrial production. Free isoniazide is not sterile and can be used only for perroral reception - not for injections.

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