A BIOLOGICAL METHOD OF DETERMINATION OF PREPARATION 1314 Th (THIANIDE) IN THE BLOOD

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Among the antituberculous chemotherapeutic drugs synthesized in recent years, the preparation 1314 Th, the thioamide of α-ethylisonicotinic acid, which was produced in France, is of particular interest [3, 4]. An original method of synthesis of this drug, which we call thianide, has been developed in the Institute of Pharmacology and Chemotherapy of the AMN SSSR. Thianide was obtained in the form of a water-insoluble base and of a readily soluble hydrochloride. The activity of these two preparations is practically equal [1].

Thianide possesses a very narrow spectrum of activity mainly against the various species of mycobacteria of tuberculosis [1]. In the study of certain aspects of the mechanism of action of thianide, its distribution in the body and so on, the tubercle bacillus is very unsuitable for use as a test organism, and often impossible on account of its pathogenicity or, in particular, its slow growth

We sought a suitable test organism for these purposes among the group of acid-fast saprophytes. We found here a strain (M. chelonei Friedman) sensitive to thianide and growing on Sauton's and Hiss's media and in Hottinger's broth in the course of 2-3 days. We attempted to develop a method of determination of thianide in the blood using this microorganism.

The strain was grown on Helberg's medium at 37° for 4-5 days. To obtain material for seeding a culture was emulsified in physiological saline with glass beads, and diluted to 500,000,000 bacterial cells per ml according to the GKI bacterial standard.

In order to select the most suitable medium for titration of thianide, we determined the minimal suppressing concentration (MSC) of its hydrochloride on Sauton's and Hiss's media and on Hottinger's broth at pH 7.3. Friedman's bacillus was more sensitive to thianide on Hiss's medium and on Hottinger's broth (MSC 7.8-13.6 γ/ml), and the second of these was chosen, for the film formed on it was well defined after only 2 days. Trials with Hottinger's broth at pH 3.0, 4.0, 5.0, 6.0 and 7.3 showed that the best results were observed on broth at pH 5.0, and this was used in the later work.

The study of the effect of the dose of microorganisms on the results of titration showed that the most suitable dose was one of 1,000,000 bacterial cells per ml of medium. In this case, when the results were read on the second day, the MSC had a value of 7.8 γ/ml, and in other cases of 3.9 γ/ml.

In accordance with the aims of our work, we studied the effect of serum on the activity of thianide. Experiments were carried out with ox, horse, and rabbit sera, in two variants: the drug was dissolved in serum and then titrated in Hottinger's broth, or it was dissolved in physiological saline and titrated in Hottinger's broth to which 20% of serum had previously been added. In both cases the activity of the drug was lowered three- or fourfold. No significant difference was found in the degree of reduction of the activity of thianide in the two variants of the experiment or with the different sera.

In view of these findings, we decided to use trichloroacetic acid (TCA) for the determination of the thianide in the blood [2]. Experiments were carried out in which 2.7 ml of citrated rabbit's blood was mixed with 0.3 ml of thianide, dissolved in physiological saline. After exposure at 37° for 1 hour, to 3 ml of the mixture was added 1.5 ml of 20% TCA. The mixture was then shaken and centrifuged, and the activity of the supernatant fluid, neutralized with a 10% NaOH solution, was determined. As a control we used thianide in the same concentration as in the experiment, but dissolved in physiological saline. The results of the determinations in the experimental and control series were mainly in agreement, or differed by only one dilution.

In view of these findings, we determined the total thianide concentration in the blood of mice in accordance with the following scheme. Blood from the animals was collected in graduated centrifuge tubes containing sodium citrate in a dose of 16 mg per 1 ml of blood. After mixing the contents, we added 20% TCA to these tubes—0.5 ml to 1 ml of blood. The mixture was centrifuged for 20-30 minutes at 1500 rpm. After neutralization, the supernatant fluid was titrated in Hottinger's broth at pH=5.

To make the determination more accurate, we used serial dilutions in which the diluting factor was 1.5 instead of 2, and this gave clearer results. As a standard we used a solution of 1 mg thianide hydrochloride in 1 ml of physiological saline, and treated in the same manner as the blood.

As an illustration we give the following example. After injection of thianide hydrochloride intravenously into mice (weighing 20-22 g) in a dose of 1 mg per animal, 5 minutes after injection the blood concentration of thianide was 39 γ/ml, and 30 minutes after injection, 25 γ/ml.

The method described is not specific for blood alone, but may be used in principle for determination of the thianide content in other body fluids and tissues.

SUMMARY

The acid-resistant saprophyte Mycobacterium chelonei Friedman is sensitive to 1314 Th (Thianide)—an antituberculosis preparation. The authors developed a method of determining thianide in the blood with the aid of this microorganism.

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*Original Russian pagination. See C. B. translation.