On the Mechanism of Action of Tetracycline Antibiotics

V. The Effect of Oxytetracycline on the Reduction of Triphenyltetrazolium by Staphylocoeci and some other Microorganisms

V. KRČMÉRY and HELENA GRUNERTOVÁ
The State Veterinary Institute, Bratislava

Received June 27, 1963

ABSTRACT

Oxytetracycline-sensitive and resistant strains of Staphylococci and of Listeria monocytogenes and Pasteurella multocida were tested for differences in the reduction of triphenyltetrazolium (TTC) in the presence of glucose, acetate, lactate, pyruvate, glycerol, succinate, formate, malate, citrate, serine, glycine and asparagine. The sensitive staphylococci reduced TTC more actively than the resistant ones in the presence of glucose, acetate and serine. The resistant strains reduced TTC more actively in the presence of succinate, formate, glycine, pyruvate and malate. Oxytetracycline itself only inhibited the reducing activity greatly in the sensitive staphylococci. In the resistant ones, oxytetracycline only slightly decreased the TTC reduction. The insensitivity of the reducing activity of resistant staphylococci toward the effect of oxytetracycline indicates that this activity may be one of the sites of attack by this antibiotic. Ascorbic acid contained in the injection preparation of oxytetracycline interfered with the inhibitory effect of this antibiotic on the TTC reduction by staphylococci and actually increased the activity of reduction.

It was found earlier that oxytetracycline (OTC) inhibits catalase activity even after a 3—6-minute contact, both in sensitive and resistant staphylococci (Krčmery, Ferenčík & Kržanová, 1962) and brucellae (Krčmery, Ferenčík & Kellö, 1959) as well as the respiration of resting cells of brucellae and the utilization of some substrates of their energy metabolism (Nižnánsky et al. 1958). It has also been claimed that chlortetracycline did not exhibit any inhibitory effect on staphylococcus catalase (Krčmery et al. 1962). Even if the inhibition of catalase activity also represents interference by oxytetracycline with haem-oxidoreductases, the inhibition of catalase itself could hardly account for the mechanism of action of oxytetracycline as is the case with isonicotine hydrazide (Domagk, 1961; Krčmery, 1960).

The change in the reduction activity in the presence of certain substrates might indicate an effect on the corresponding dehydrogenases (Drobnič, 1959a, b). Therefore, the next step toward solving the problem of interference of tetracyclines with the enzyme apparatus of sensitive and resistant microorganisms was to test the effect on the reduction of TTC by some microorganisms, mainly staphylocoeci, in the presence of oxytetracycline.

The finding of the influence of a given compound on the reduction of TTC in the presence of a given substrate in intact bacterial cells should be supplemented by an investigation of its action on the corresponding isolated enzyme as well as by studying respiration activity. So far the investigation of TTC reduction by
intact microbial cells has been a suitable and, above all, simple means of screening in looking the enzyme system primarily attacked by the tetracycline antibiotics, in particular when the reduction is examined in the presence of each individual substrate. The application of TTC has stimulated considerable activity in research on bacterial dehydrogenases even if many unsolved questions still remain as to the mechanism of its participation in the reduction processes.

MATERIALS AND METHODS

Strains. The effect of oxytetracycline on TTC reduction was tested in two sensitive strains of staphylococcus (Staphylococcus aureus Oxford and Staphylococcus aureus 19—772) and in two polyresistant hospital strains (Staphylococcus aureus 2451 and V-11). Both sensitive strains were sensitive to the common antibiotics (tablet method) and to 0.1—0.5 μg. oxytetracycline/ml. (test-tube dilution method). The resistant strain 2451, kindly supplied by Dr. Bátoralá from the Institute of Epidemiology and Microbiology at Bratislava, was shown to be insensitive to penicillin, streptomycin, chloramphenicol and oxytetracycline by the tablet method and to 64 μg. OTC/ml. by the test-tube dilution method. The strain V-11, kindly provided by Dr. Jánoušková from the Regional Station of Hygiene and Epidemiology in Brno, was insensitive to penicillin, streptomycin and oxytetracycline by the tablet method and to 64—128 μg. OTC/ml. by the test-tube dilution method.

Some other sensitive and polyresistant strains of staphylococci were used for reduction tests with some substrates. A total of 5 other sensitive and 11 other polyresistant strains were used in the experiments. These strains reacted practically identically and for this reason the tables include only those where the whole set of reduction tests were carried out. In order to verify some previous findings, the Listeria monocytogenes Patterson 5214 strain, kindly supplied by Prof. F. Patočka and a Pasteurella multocida strain, obtained from autopsy material, were used.

Cultivation and preparation of cell suspensions. Staphylococci were transferred from a collection culture (depth inoculum first) into blood agar and then to meat-peptone agar from which the cells were washed down after a 24-hour incubation at 37° C with buffered physiological saline (0.033 M phosphate buffer containing 0.5% NaCl). The washed staphylococci were centrifuged for 20 min., at 13,000 r.p.m. and after washing and centrifugation, resuspended in the rinsing solution so that the final suspension absorbed about 75—80% light (Lange photometer, orange filter, 5 ml. cuvette). One ml. of this suspension contained approximately 2 × 10^10 ± 20% cells.

Determination of the TTC-reducing activity. The inhibition of TTC reduction by oxytetracycline was tested according to Drobnica (1959a, b, 1960) as described earlier (Kremlér, 1960) in a modification suitable for our purposes. The substrates used are shown in Table 1. After dissolving in Aqua pro injectione, the substrate solutions were autoclaved, with the exception of glucose which was always fresh-prepared aseptically. Oxytetracycline hydrochloride used was obtained through the kindness of Dr. V. Vlček from the Institute for Antibiotic Research at Roztoky near Prague. On comparing this crystalline preparation with the injection preparation of OTC (Oxymykoin Spofa used previously) it was shown that in all cases the injection OTC containing iscorbic acid caused nonspecific stimulation of TTC reduction (Figs. 3 and 4). In this connection the work of Dudani and Krishnamurti (1954) is of particular interest. 1 ml. of a standardized bacterial suspension was pipetted into each of twelve test-tubes. Similarly, 1 ml. of suspension which had been in contact with 100 μg. OTC/ml. for 120 min. was pipetted into another 12 tubes. 0.1 ml.