

ELIMINATION OF DRUG RESISTANCE OF STAPHYLOCOCCI BY TREATMENT WITH 5-NITROFURAN DERIVATIVES

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Cultivation of staphylococci in the presence of subbacteriostatic concentrations of 5-nitro-furan derivatives leads to loss of drug resistance of the strains. Resistance to antibiotics is lost most frequently after treatment with furagin. In some cases simultaneous loss of pathogenicity was observed in strains of Staphylococcus aureus which became sensitive to antibiotics.

Previous investigations [1, 2] have shown that certain 5-nitrofurans suppress transmission of the R factor in *Escherichia coli* during conjugation. The inhibitory effect of the nitrofurans was exhibited when they were added directly to the conjugating mixture, to broth cultures of the parent bacteria, and in the period of phenotypic expression of the R factor transmitted to *E. coli* strains. The writers have postulated that one mechanism by which nitrofurans inhibit transmission of the R factor is by the ability of this group of compounds to induce its elimination.

In the investigation described below, the elimination of drug resistance of polyresistant strains of staphylococci was studied after their treatment with nitrofurans in experiments in vitro.

EXPERIMENTAL METHOD

The following 5-nitrofurans were used in the experiments: furazolidone [N-(5-nitro-2-furfurylidene)-3-amino-2-oxazolidone], furacilin (5-nitro-2-furfurylidene semicarbazone), furagin (1-[β-(5-nitrofuryl)-2-acrylidene-amino]-hydantoin), furazolin [5-morpholinomethyl-3-(5-nitro-2-furfurylideneamino)-2-oxazolidone], obtained from the Institute of Organic Chemistry, Academy of Sciences of the Latvian SSR, furacrylin [1-(5-nitrofuracrylideneamino)-1,3,4-triazole], obtained from the Department of Organic Chemistry, Saratov University, and furadonin [N-(5-nitro-2-furfurylidene)-1-aminohydantoin], a commercial preparation.

TABLE 1. Comparative Action of 5-Nitrofurans on Stability of Drug Resistance of *Staphylococcus aureus* 1074 (Pe, Tc, Sm, Cm, Er) and *Shigella flexneri* 170, 2a (Tc, Cm)

Strain	% of colonies losing drug resistance under the influence of						
	con- trol	fura- gin	furacilin	furadonin	fura- zoli- done	fura- cry- lin	furazolin
1074	0	13	0	5	1	5	9
170, 2a	15	73	16	25	23	20	34

The elimination of drug resistance was studied in 11 strains of pathogenic staphylococci isolated from patients with staphylococcal infections in different parts of the body. Eight of the strains were resistant to tetracycline (Tc), streptomycin (Sm), penicillin (Pe), chloramphenicol (Cm), and erythromycin (Er), two were sensitive to Er, and one to Pe.

The control tests were carried out on 12 strains of intestinal bacteria to which resistance to Tc and Cm had been transmitted in preliminary experiments by conjugation with *E. coli* R⁺. These

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TABLE 2. Loss of Drug Resistance by Staphylococci under the Influence of Furagin (percentage of colonies losing resistance to single antibiotics or to a group of them)

Group investigated	Strain									
	71	737	824	1639	2184	Roma-shev	1753	571	1917	1740
Control	0	0	0	0	0	46	0	0	0	6
Experimental	0	2	0	35	0	54	0	0	5	60

bacteria (*E. coli* K-12, M-17; *Shigella flexneri* 5237, 1a, 170, 2a, 1020, 4a, 9726, 4b; *Shigella sonnei* 8062, 725; *Salmonella typhimurium* 79; *Salmonella typhi abdominalis* 4446) were obtained from the museum of the L. A. Tarasevich Government Control Institute for Medical and Biological Preparations. Strains *S. typhimurium* 1 and *S. typhi abdominalis* 274 were obtained from the bacteriological laboratory of the No. 2. Saratov City Hospital.

To eliminate drug resistance, 24-h agar cultures of the microorganisms were washed off with physiological saline and added ($2 \cdot 10^5$ bacterial cells/ml medium) to tubes with Hottinger's broth (pH 7.4), containing serial dilutions of the nitrofurans. After incubation for 24 h (intestinal bacteria) or 48 h (staphylococci) at 37°C, seedings were taken from the tubes containing the highest dilutions of the compounds just permitting growth of the test bacteria, and from the control tube (without compounds), on agar plates to obtain isolated colonies. By the impression method, the growing colonies of staphylococci were then transferred to dishes of nutrient agar containing Pe (10 units/ml), Er (10 units/ml), Sm (30 units/ml), Tc (20 µg/ml), and Cm (20 µg/ml). To detect intestinal bacteria which had lost their resistance to antibiotics, impressions were made on Endo's medium containing 25 µg Cm/ml or 30 µg Tc/ml. The results were read 24 h later. On the average not less than 100 colonies of each strain were studied.

EXPERIMENTAL RESULTS

The experiments showed that cultivation of staphylococci in the presence of subbacteriostatic concentrations of 5-nitrofurans leads to loss of resistance of some colonies of nearly all the microorganisms tested (Table 1 and 2).

The percentage loss varied within wide limits and depended both on the compound tested and on the individual characteristics of the strains. The strongest eliminating effect was observed by the action of furagin, followed by furazolin and furadonin.

Furagin (Table 2), in subbacteriostatic concentrations, caused loss of resistance in five of the 10 strains of staphylococci. Characteristically, resistance to several antibiotics was lost at the same time. Resistance to Er and Cm was lost most often, followed by resistance to Tc. The frequency of loss of resistance to other antibiotics was about equal, and the genetic determinants responsible for resistance to Pe and Sm were eliminated, as a rule, independently of each other. A high proportion of the sensitive colonies were mutants which lost their drug resistance to all the antibiotics tested at the same time.

Among the intestinal bacteria (control), reversion toward sensitivity to antibiotics was observed more often and in a higher proportion of cases than among the staphylococci.

Investigation of the biological properties of the colonies of staphylococci becoming sensitive to antibiotics as a result of treatment with furagin showed that many of them lost not only the characteristic of drug resistance, but also the ability to coagulate rabbit plasma, to produce hyaluronidase and lecithinase, and to ferment mannitol, while their deoxyribonuclease activity and their behavior toward a series of international staphylococcal phages were indistinguishable from those of the original strains. Loss of the characteristics of pathogenicity was observed mainly in strains losing their drug resistance to all antibiotics used in the tests, or to most of them.

These results thus indicate that 5-nitrofurans have the ability to induce elimination of drug resistance in polyresistant staphylococci. However, the mechanism of this effect, including the simultaneous loss of certain other properties by the staphylococci, is not clear. Nevertheless, if this effect of the nitrofurans is still present in vivo in man and animals, it means that compounds of this type can be used in medical practice in order to eliminate the drug resistance of the agents of certain infectious diseases.

LITERATURE CITED

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