CHANGE IN THE ACTIVITY OF ENZYMES UNDER THE ACTION OF DIOXIDINE AND FLORENAL

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UDC 615.281.015.43

A rational approach to the creation of antimicrobial preparations is based on the study of their influence on the specific peculiarities of the metabolism of the pathogen of the infectious process. However, only in the last decades have the successes of molecular biology permitted the disclosure of the molecular mechanisms of the action of a number of chemotherapeutic preparations, determining the selectivity of their action [1-3].

The study of the influence of drug preparations on the enzymatic properties permits the detection of vital differences in the metabolism of the action of new preparations and makes it possible to utilize them clinically more effectively [4].

The present article is devoted to a study of the action of new chemotherapeutic preparations, dioxidine (I) and florenal (II), on a number of enzyme systems. Both preparations were synthesized and studied at the S. Ordzhonikidze All-Union Chemicopharmaceutical Scientific-Research Institute (Head of the Laboratory Professor K. Yu. Novitskii, Head of the Laboratory Professor A. N. Grinev, Head of the Laboratory and Corresponding Member of the Academy of Medical Sciences of the USSR, Professor G. N. Pershin).

Dioxidine (I) — 1,4-di-N-oxide of 2,3-dihydroxymethylquinoxaline — is a new original chemotherapeutic preparation with a broad spectrum of action for the treatment of various forms of suppurative infection [5]. The mechanism of the action of the preparations, which can be classed as derivatives of di-N-oxides of quinoxaline, has been insufficiently studied, although there are data on the disruption of nucleic metabolism in the microbial cell under the influence of I, as well as its mutagenic action [6, 7].

In view of this, it seemed necessary to study the influence of I on certain enzyme systems of nucleic metabolism — DNA-dependent RNA polymerase, dihydrofolate reductase, and DNase.

The new antiviral preparation II — a bisulfite compound of 2-fluorenonylglyoxal — has a broad spectrum of antiviral action [8]. The biochemical mechanism of the antiviral action of fluorene derivatives has not been studied up to the present time.

EXPERIMENTAL SECTION

Determination of DNA-Dependent RNA Polymerase of *E. coli*. The method of isolation of the enzyme and determination of its activity were described earlier [9]. The synthesis of RNA and, consequently, the enzyme activity, were judged according to the incorporation of the label from [^{14}C]UTP into the acid-insoluble fraction. The incorporation of [^{14}C]UTP (in counts per 100 sec) into the control samples without the preparation was taken as 100%, and the percentage of the RNA polymerase activity in samples with the preparation was calculated. Exposure time of the enzyme with the preparation was 1 h.

Determination of Dihydrofolate Reductase — DFR (EC 1.5.1.3). Rat liver and a culture of *Staph. aureus* were used as sources of the enzyme. The DFR activity was determined by a colorimetric or spectrophotometric method [10].

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Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 14, No. 10, pp. 11-14, October, 1980. Original article submitted October 13, 1979.

0091-150X/80/1410-0674$07.50

TABLE 1. Action of I, III, and IV on DNA-Dependent RNA Polymerase of *E. coli*

Preparation	Concentration, M	Incorporation of [^{14}C]UTP, counts/100 sec*	Enzyme activity, % of control (according to the results of 5 experiments (P ± σ)
I		218 921	
		208 960	100
	10^{-5}	238 112	
		222 248	101.92±8.74
	10^{-4}	216 278	
		191 648	97,86±8.74
	10^{-3}	226 102	
		182 432	97,46±3,65
III		231 000	
	10^{-5}	186 426	96,49±2,83
	10^{-4}	217 120	
		199 569	97,07±2,37
		217 085	
	10^{-3}	202 840	99,12±3,18
IV	10^{-4}	1 012	
		1 008	0
	10^{-3}	1 304	
		1 117	0

*Results of one experiment.

Determination of the DNase Activity (EC 3.1.4.7). The conditions of determination of the activity of bacterial DNase in the presence of I were described in our earlier work [11]. The activity of crystalline pancreatic DNase (EC 3.1.4.5) (produced by Biokhimreactivy Olaine, Latvian SSR) was determined by a viscosimetric method. Composition of the incubation mixture: 0.2 ml of 0.05 M phosphate buffer pH 7.5; 1 ml of DNA solution (5 mg/kg); 0.5 ml of the incubate, consisting of equal volumes of the enzyme and the investigated preparation, dissolved in a 0.05% solution of gelatin.

Determination of the Neuraminidase Activity (EC 3.2.1.18). The source of the viral neuraminidase was influenza virus strain APR-8, obtained from allantoic fluid of infected chick embryos by differential centrifugation. The substrate was fetuin (from Koch-Light, England). The investigated preparations were incubated with the enzyme for 1 h at 37°C in 0.4 M phosphate buffer, pH 5.9, after which the substrate was added, and the mixture incubated for another 30 min under the same conditions. *Vibrio cholerae* neuraminidase from Serva (Sweden) was used as the bacterial enzyme. The activity of the enzyme was determined by the thiobarbituric method according to Warren [12].

RESULTS AND DISCUSSION

We studied the action of I and a biologically inactive compound — di-N-oxide of quinoxaline (III) — on DNA-dependent RNA polymerase of *E. coli*. The mechanism of the action of a number of antibiotics is associated with a disruption of the activity of DNA-dependent RNA polymerase, which carries out RNA biosynthesis on a DNA template [13, 14]. Rifampicin (IV) is a specific inhibitor of the initiation of transcription of bacterial DNA-dependent RNA polymerase [13]. We used IV as a control preparation.

The action of I on RNA synthesis was studied in a broad range of concentrations, including bacteriostatic concentrations (10^{-3}, 10^{-4} M). From Table 1 it is evident that I, just like III, practically does not change the activity of the enzyme in the indicated concentrations. At the same time, IV entirely suppressed RNA synthesis in these concentrations.

The influence of I on the activity of DFR in experiments with the enzyme of bacterial and animal origin was studied. DFR is a key enzyme in the synthesis of purines and pyrimidines, and the chemotherapeutic action of methotrexate, trimethoprim, and septrin is associated with its inhibition [2, 15]. In high concentrations (10^{-5}-10^{-7} M) in experiments *in vitro*, I did not cause any inhibition of the activity of DFR of bacterial and animal origin.

TABLE 2. Influence of I on the Activity of Staphylococcal and Pancreatic DNase

Enzyme	Concentration of preparation		
	$5 \cdot 10^{-4}$ M	$5 \cdot 10^{-3}$ M	$25 \cdot 10^{-3}$ M
	inhibition of DNase, % of control		
Staphylococcal DNase	52	57	100
Pancreatic DNase	20	40	66

TABLE 3. Influence of II and Its Derivatives on the Activity of Viral and Bacterial Neuraminidase

Preparation	Concentration, M	Influenza neuraminidase		V. cholerae neuraminidase	
		elimination of neuraminic acid in 30 min, μmoles	inhibition, %	elimination of neuraminic acid in 30 min, μmoles	inhibition, %
Control		45,7		56,0	
II	$5{,}8 \cdot 10^{-4}$	8,8	81,0	56,0	0
2-Dibromoacetylfluorenone	$2{,}5 \cdot 10^{-4}$	48,0	0	57,6	0
2-Dichloroacetylfluorenone	$3{,}5 \cdot 10^{-4}$	53,9	0	57,6	0
2-Acetylfluorenone	$4{,}5 \cdot 10^{-4}$	54,0	0	37,0	34,0
2-Dimorpholinoacetylfluorenone	$2{,}5 \cdot 10^{-4}$	38,2	17,0	17,6	69,0

In view of this, we conducted experiments to study the influence of the preparation on DFR biosynthesis in *Staph. aureus*. The culture of *Staph. aureus* was grown in the presence of I in a concentration ($5 \cdot 10^{-3}$ M) retarding the growth of the microorganisms. Under these conditions the activity of the enzyme was also unchanged under the action of the preparation.

A comparative study was made of the influence of I and III on the activity of bacterial and pancreatic DNase. Earlier we established [16] a substantial inhibiting effect of its analogs on bacterial DNase as well. A definite correlation was shown between the biological activity of derivatives of III and the inhibition of DNase [16]. A comparative study of the action of the preparation on bacterial and pancreatic DNase showed a higher sensitivity of the bacterial DNase to the action of I (Table 2).

The results obtained are evidence that I, in contrast to IV, does not affect RNA synthesis catalyzed by RNA polymerase. Therefore, the change in the nucleic metabolism observed in the bacterial cell under the action of I evidently is not associated with a disruption of RNA synthesis. Thus, on the basis of the data obtained we can conclude that the biochemical mechanism of the action of I on the bacterial cell differs from the action of IV.

The absence of inhibition of biosynthesis and the inhibition action of DFR permit us to assume that the biological activity, in contrast to sulfanilamide preparations, is not associated with a disruption of folate synthesis [17]. A peculiarity of the action of I and other derivatives of III is the suppression of the activity of bacterial DNase. The suppression of the activity of DNase in *Staphylococcus aureus* is accompanied by a disruption of the plasmocoagulase activity, the hemolytic activity, and evidently processes of toxin formation [16]. The ability of nucleases to "recognize" specific portions of nucleic acids and their participation in processes of DNA and RNA metabolism suggest that they have an important role in metabolic transformations occurring in the cell [18, 19]; therefore it can be assumed that the disruption of the DNase activity under the influence of I may be accompanied by a change in the processes of nucleic acid synthesis.

We studied the influence of II and other fluorene derivatives on the activity of influenza virus neuraminidase. The enzyme contained in the influenza virions performs a number of functions in the viral replication cycle [20]. As seen from Table 3, II in a concentration of $5.8 \cdot 10^{-4}$ M inhibits the enzyme by 80%, in contrast to biologically inactive analogs. II did not suppress the activity of *Vibrio cholerae* neuraminidase. The compound 2-dimorpholinoacetylfluorenone, with weak biological activity, inhibited the activity of bacterial neuraminidase by 69%.

Thus, it has been successfully established for the first time that the new antiviral preparation II inhibits the virus-specific enzyme neuraminidase and does not inhibit the bacterial enzyme. Biologically inactive derivatives of II do not inhibit the enzyme of influenza virus. It is known that influenza virus neuraminidase exhibits weak structural specificity and that its specificity differs from the specificity of bacterial neuraminidases [21]. Possibly II has an affinity precisely for the viral enzyme.

In general we should note that when the inhibiting action of the preparation on the enzyme is detected, an increased sensitivity of the enzyme to the preparation is manifested, which was shown for the examples of bacterial DNase in the case of I and influenza virus neuraminidase in the case of II.

The experimental data cited confirm the hypothesis that chemotherapeutic preparations can be used in research to establish fine differences in the metabolism of microorganisms and viruses.

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