EFFECTS OF ARBIDOL ON EXPERIMENTAL Escherichia coli, Salmonella typhi, AND Pseudomonas aeruginosa INFECTIONS IN MICE, AND ON THE CHEMOTHERAPEUTIC ACTIVITY OF DIOXIDINE

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Arbidol is the ethyl ester of 6-bromo-5-hydroxyl-4-dimethylaminomethyl-1-methyl-2-phenythiomethylindole-3-carboxylic acid hydrochloride monohydrate, a new antiviral agent developed at the TsKhLS – S. Ordzhonidze All-Union Science Research Chemicopharmaceutical Institute and the Science Research Institute of Radiology (Obninsk) [1]. Detailed studies of the antiviral activity and several aspects of the mechanism of action of arbidol have been carried out in the Laboratory for the Chemotherapy of Infections Diseases of the S. Ordzhonikidze All-Union Science Research Chemicopharmaceutical Institute by I. S. Nikolaeva, A. N. Fomina, N. I. Fadeeva, L. F. Stebaeva, and I. A. Leneva. Arbidol was approved by the Pharmacology Committee of the Ministry of Health of the USSR for the treatment of influenza types A and B infections; the drug has a number of advantages over remantadine, with a wider spectrum of activity and better tolerance.

Studies carried out by A. N. Fomina and I. S. Nikolaeva have also shown that, along with antivirial effects, arbidol has marked immunomodulating properties, which are primarily associated with its interferon-inducing activity and increases in the non-specific resistance of the body to experimental viral infections [2]. In addition, L. I. Ratnikova has shown that arbidol promotes cellular immune response in mice, increasing the number of activated phagocytes and increasing resistance of animals to infection with Salmonella typhimurium.

TABLE 1. Effect of Prophylactic Intraperitoneal Arbidol Given for Three Days Before Infection on the Course of Bacterial Infection in Mice (Septicemia Model)

Pathogen	Arbidol dose, mg/kg per day	Survival rate on day 10		p	Total duration of life	
		absolute*	%**		absolute*	%**
E. coli M-17	62,5	25/28	89	<0,01	250/280	89
	31,2	14/20	70	<0,01	145/200	72,5
	15,6	12/20	60	<0,01	131/200	65,5
	Control	5/30	16,7		51/300	17
S. typhi 4446	62,5	27/30	90	<0,01	272/300	90,7
	31,2	15/20	75	<0,01	155/200	77,5
	15,6	17/20	85	<0,01	172/200	86
	Control	9/30	30		99/300	33
Ps. aeruginosa 165	62,5	9/19	47	>0,1	93/190	49
	31,2	11/20	55	>0,1	111/200	55,5
	15,6	9/20	45	>0,1	90/200	45
	Control	7/20	35		70/200	35

Notes. *The numerator gives the number of mice surviving to the tenth day of the observation period; the number of days survived; the denominator gives the total numbers of mice in groups; the maximum possible number of days in the observation period. **As a percentage in relation to the maximum value.

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TABLE 2. Effect of Prophylactic p.o.Arbidol Given for Three Days Before Infection on the Course of Bacterial Infection in Mice (septicemia model)

Pathogen	Arbidol dose, mg/kg per day	Survival rate on day 10		p	Total duration of life	
		absolute*	%**		absolute*	%**
E. coli M-17	62,5	4/20	20	>0,1	42/200	21
	31,2	5/20	25	>0,1	52/200	26
	15,6	6/20	30	>0,1	63/200	31,5
	Control	5/30	16,7		51/300	17
S. typhi 4446	62,5	13/20	65	<0,05	134/200	67
	31,2	11/20	55	>0,1	117/200	58,5
	15,6	7/20	35	>0,1	82/200	41
	Control	9/30	30		99/300	33
Ps. aeruginosa 165	62,5	6/20	30	>0,1	60/200	30
	31,2	2/20	10	>0,1	27/200	13,5
	15,6	6/20	30	>0,1	60/200	30
	Control	7/20	35		70/200	35

Notes. * and ** – see notes to Table 1.

TABLE 3. Effect of Prophylactic Dosage with Arbidol for Three Days on the Efficacy of Chemotherapy with Dioxidine in Experiments Using Pseudomonas aeruginosa 165; Animals Received Dioxidine Once, 30 Minutes after Infection

Drug, dose per administration	Survival rate at day 10		p	Total duration of life	
	abs.	%**		abs.	%**
Dioxidine 16.5 mg/kg + arbidol 62.5 mg/kg	17/20	85	<0,05	170/200	85
Dioxidine 12.5 mg/kg + arbidol 31.2 mg/kg	18/20	90	<0,05	181/200	90,5
Dioxidine 12.5 mg/kg + arbidol 15.6 mg/g	18/20	90	<0,05	180/200	90
Dioxidine 6.25 mg/kg + arbidol 62.5 mg/kg	13/20	65	>0,1	130/200	65
Dioxide 6.25 mg/kg + arbidol 31.2 mg/kg	14/20	70	>0,1	140/200	70
Dioxidine 6.25 mg/kg + arbidol 15.6 mg/kg	14/20	70	>0,1	140/200	70
Dioxidine 12.5 mg/kg	9/20	45	–	91/200	45,5
Dioxidine 6.25 mg/kg	8/20	40	–	82/200	41
Control	7/20	35	–	70/200	35

Notes. * and ** – see notes to Table 1.

Further detailed studies of the properties of arbidol as an immunomodulator in the complex therapy of a variety of infections, especially bacterial, are therefore required. The aim of the present work was to study the effect of arbidol on the course of acute generalized bacterial infections in mice, induced by Escherichia coli, Salmonella typhi, and Pseudomonas aeruginosa; at effects of arbidol on the efficacy of dioxidine treatment was studied in mice with Ps. aeruginosa infections. Dioxidine was chosen because of the importance of finding ways to reduce the therapeutic doses of this agent, which is desirable because of its toxic effects.

MATERIALS AND METHODS

Experiments were carried out using 740 white mongrel mice of 15-16 using a previously described model of septicemia resulting from intraperitoneal infection [3]. In order to reduce the high level of intoxication and the acuteness of the infection, infective doses (ID) killing 60-80% of untreated control animals were used, depending on the pathogen concerned: the ID for E. coli strain M-17 was $7.5 \cdot 10^7$ colony-forming units (cfu), the ID for S. typhi strain 4446 was $1 \cdot 10^6$ cfu, and the ID for Ps. aeruginosa strain 165 was (0.5-1). 10^6 cfu. Arbidol, synthesized at the S. Ordzhonikidze All-Union Science Research Chemico-pharmaceutical Institute was used at daily doses of 62.5, 31.2, and 15.6 mg/kg. Doses were selected on the basis of previous results obtained in experiments with viral infections [2]. The effects of arbidol were studied in animals treated by different routes – animals recieved i.p. and p.o. arbidol for three days before infection in prophylactic experiments and were observed for ten days after infection. The efficacy of treatment was judged in terms of the survival rate of mice and the total duration of life. Statistical analysis of results was carried out using the χ^2 criterion. P values given in the Tables are the probabilities that there are no significant differences between the experimental and control groups.

RESULTS AND DISCUSSION

The results presented in Table 1 show that only 16.7% of untreated control mice survived colibacillary sepsis. The prophylactic use of i.p. arbidol at a dose of 62.5 mg/kg increased survival to 89%, while doses 2-4 times smaller than this gave survival rates of 60-70%. Thus, arbidol had a protective effect of 43-72% compared with controls. Intragastric dosage with arbidol produced only a tendency to increased resistance of animals to infection (Table 2).

In experiments with S. typhi, i.p. arbidol at doses of 62.5, 31.2, and 15.6 mg/kg prevented the deaths of 90%, 75%, and 85% of animals respectively, giving protective effects relative to the control group of 45-60% (Table 1). P.o. arbidol was less effective, and there was a clear dose-response relationship (Table 2).

In experiments with Ps. aeruginosa, i.p. arbidol gave only a slight increase in resistance of mice to infection, despite the relatively high survival of animals in the control group (Table 1). P.o. arbidol did not increase the resistance of mice to this organism (Table 2).

Since s.c. doses of dioxidine at a dose of 12.5 mg/kg given along with prophylactic arbidol (independently of the dose of arbidol) gave significant increases in survival, to 85-90.5%. The use of dioxidine at the lower dose of 6.25 mg/kg given along with prophylactic arbidol also increased survival compared to the control group, but the difference was not significant (Table 3).

Thus i.p. prophylaxis with arbidol for three days before infection produced significant increases in the resistance of mice to infection with E. coli M-17 and S. typhi 4446, while there was virtually no protective effect against Ps. aeruginosa. P.o. treatment with arbidol was less effective, or even ineffective (depending on the pathogen species), which may be associated with lower absorption. Preliminary data obtained in collaboration with L.I. Budanova on plasma arbidol concentrations in mice after p.o. and i.p. dosage have shown that the arbidol concentration after i.p. dosage is 1.5-2 times higher than after p.o. dosage.

These studies provide further reasons for studying the immunomodulating effects of arbidol on models of bacterial, fungal and protozoal infections, and for evaluating these effects on the chemotherapeutic properties of the drug. Such studies should take into account the difficulties associated with the features of particular pathogens, and the effects of ineffective dose size and details of the infectious process in control groups. It is also important to study the effect of arbidol on infections which are already established. Further studies on arbidol pharmacokinetics are also needed, along with evaluations of the extent of its immunomodulating effects when given by different routes. There is considerable interest in creating a form of this agent suitable for treating established infections.

The significant increase in the chemotherapeutic activity of dioxidine, produced by background prophylaxis with arbidol, represents a further step in the study of the combined effects of these two agents, aimed at reducing the therapeutic dose of dioxidine. It is

also important to evaluate the effects of arbidol on the chemotherapeutic effects of other antimicrobial drugs.

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HYDROXYAZOBENZENES: SYNTHESIS AND ANTIMICROBIAL ACTIVITY

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Some hydroxyazobenzenes have rather distinct antimicrobial and bactericidal properties [1-3].

During investigations of biological activities of derivatives of hydroxyazobenzenes we have prepared some hydroxyazobenzenes of the general formula:

$$XC_6H_4N{=}NC_6H_nY$$

in which X=H(I, II), 4-Br(III–IV), 4-COOH(VI, VII), Y= =2-OH-3-NO_2(I, III), 2-OH-3,5-Br_2(II), 2-OH-5-NO_2(IV), 2-OH-3,4,5-Br_3(V), 4-OH(VI), 2,3,4-$(OH)_3$(VII)

The compounds were prepared by azo coupling reaction of aniline derivatives with phenol derivatives.

The purity of the prepared compounds was checked by TLC on an unfastened layer of activity stage II Al_2O_3 in various systems (Table 1).

Prepared hydroxyazobenzenes I-VII (see Table 1) are crystalline compounds, well soluble in alcohol, chloroform, ethanol, and acetone, and poorly soluble in water.

The structure of the prepared compounds was demonstrated by elemental analyses and IR spectral data. Data of elemental analyses corresponded with calculated values.

2-Hydroxy-3-nitroazobenzene (I) was prepared by reacting a solution of 9.3 g (0.1 mole) of aniline in 100 ml of water and 25 ml of conc. hydrochloric acid with a solution of 7.0 g of sodium nitrite in 20 ml of water (0-5°C) and subsequent addition of a solution of 13.8 g

TABLE 1. Physicochemical Characteristics of Hydroxyazobenzenes

Compound	Yield, %	mp, °C (recrystallization solvent)	R_f (solvent system)	Empirical formula	IR spectrum		
					–N=N–	halogen	–OH
I	82.3	58	0.52 (benzene–ethylene–heptane 10:1:1)	$C_{12}H_9N_3O_3$	1460	—	3400
II	62.9	48—50	0.61 (benzene–ethanol–heptane)	$C_{12}H_8Br_2N_2O$	1400	750	3450
III	68.1	114—116 (ethanol)	—	$C_{12}H_8BrN_3O_3$	1450	750	3400
IV	51.0	108—110	0.57 (benzene–ethanol–heptane)	$C_{12}H_8BrN_3O_3$	1460	760	3410
V	41.4	62–64 (ethanol)	—	$C_{12}H_6Br_4N_2O$	1410	740	3400
VI	95.9	252 (dec.) (ethanol)	—	$C_{13}H_{10}N_2O_3$	1495	—	3400
VII	87.5	68(ethanol)	—	$C_{13}H_{10}N_2O_5$	1500	—	3410

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