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THE ACTION OF 5-BROMO-5-NITROTETRAHYDRO-1,3-OXAZINE DERIVATIVES ON *ENTAMOEBA MOSHKOVSKII*

by

BOGUMIŁA ORŁOWSKA, MARIAN MORDARSKI, DANIELA GÜRNE
and TADEUSZ URBAŃSKI

Department of Biosynthesis, Institute of Immunology and Experimental Therapy,
Polish Academy of Sciences, Wrocław
Institute of Organic Chemistry, Polish Academy of Sciences, Warsaw

The action of three new derivatives of nitrotetrahydro-1,3-oxazine on the cells of Entamoeba moshkovskii was studied. In low concentrations the compounds killed the cells of the amebae or inhibited their growth. 5-Bromo-5-nitrotetrahydro-1,3-oxazine exhibited the strongest antiprotozoal properties, acting cytostatically in concentrations as low as 5 μg/ml.

Although not pathogenic for man, *Entamoeba moshkovskii* is a convenient model for *in vitro* studies, especially when evaluating or searching for new antiprotozoal drugs. Morphologically, it resembles *Entamoeba histolytica*, but is much easier to cultivate and propagate on various artificial culture media [2]. Moreover, as CARNERI has shown [1], *Entamoeba moshkovskii* is sensitive to compounds active against *Entamoeba histolytica*.

In a previous communication it has been reported that some nitrotetrahydro-1,3-oxazine derivatives are protozoocidal for *Trichomonas vaginalis* already in very low concentrations [3].

The influence of these compounds on amebae is the subject of the present paper.

MATERIAL AND METHODS

The strain of *Entamoeba moshkovskii* used in the experiments was obtained from the Department of Parasitology of the Medical Academy in Łódź and was passaged at 26° on Pavlova's medium. The action of oxazine derivatives on amebae cells was determined as follows: to 5 ml of Pavlova's medium containing fixed concentrations of the studied compound, 40,000 trophozoites of *Entamoeba moshkovskii* were added, and the primary cultures were incubated at 26° (the optimal temperature for the growth of these protozoa). After 24 and 48 hours numbers of living trophozoites were counted in samples from the cultures in a Thoma-Zeiss chamber

with a phase-contrast microscope. After 24 and 48 hours subcultures were also made by inoculating 0·5 ml of the primary culture on fresh medium. After incubation at 26° for 120 hours, living trophozoites were counted in the subcultures.

The following oxazine derivatives were studied:

1) 5-bromo-5-nitro-3-benzyltetrahydro-1,3-oxazine;
2) 5-bromo-5-nitro-3-methyltetrahydro-1,3-oxazine hydrobromide;
3) 5-bromo-5-nitro-3-ethyltetrahydro-1,3-oxazine hydrochloride.

The chemical formulas of the compounds were given in a previous communication [3].

RESULTS AND DISCUSSION

The results are summarized in Table 1. As in the case of *Trichomonas vaginalis*, the studied compounds proved protozoocidal for amebae in low concentrations. The strongest action on *Entamoeba moshkovskii* was exerted by 5-bromo-5-nitro-3-benzyltetrahydro-1,3-oxazine. In the concentration 20 μg/ml, this compound killed the protozoa; no living cells were found in the primary or secondary cultures. At the concentration of 10 μg/ml the compound had a marked effect on the amebae; no trophozoites were found in the primary cultures, and secondary cultures set up after 48 hours of primary culture remained "sterile". Only a few protozoa cells were alive after 24 hours' incubation, giving growth in the

Table 1. Action of the oxazine derivatives on *Entamoeba moshkovskii*

Compound	Conc. (μg/ml)	Primary cultures		Secondary cultures	
		24 hrs	48 hrs	S_I	S_{II}
		Number of trophozoites $\times 10^3$/ml			
5-Bromo-5-nitro-3-benzyltetrahydro-1,3-oxazine	20	0	0	0	0
	10	0	0	17·5	0
	5	12·5	2·5	65	32·5
	2·5	30	20	6	81·7
5-Bromo-5-nitro-3-methyltetrahydro-1,3-oxazine hydrobromide	30	0	0	0	0
	20	2·5	7·5	22·5	25
	10	20	27·5	30	97
5-Bromo-5-nitro-3-ethyltetrahydro-1,3-oxazine hydrochloride	40	0	0	0	0
	30	0	0	32·5	+ *
	20	2·5	0	5	37·5
	10	35	60	60	47·5
Control	0	70	98	51·8	81·7

S_I = secondary culture set up after 24 hours' incubation of the primary culture;
S_{II} = secondary culture set up after 48 hours' incubation of the primary culture.
Results of the secondary cultures were counted after 120 hrs of incubation.
* Single living cells.

secondary cultures. In concentrations of 5 μg/ml the effect of the preparation was cytostatic. The number of protozoa after 24 hours' incubation at this concentration of the compound was about five times less than in the control culture, and after longer incubation continued to decrease. However, the surviving cells did not lose ability of reproduction, giving rise to normally developing populations. An inhibitory effect on the development of the amebae was observed at concentrations as low as 2·5 μg/ml; after 24 hours the number of trophozoites was one-half of the number in control cultures, and after longer incubation continued to decrease.

The hydrobromide of 5-bromo-5-nitro-3-methyltetrahydro-1,3-oxazine was somewhat less strongly protozoocidal. In concentrations of 30 μg/ml, this compound killed the amebae in cultures, and at lower concentrations exerted a cytostatic effect. At 20 μg/ml, the number of protozoa in the primary culture was about 30 times less than in the control culture, and this high difference was maintained during further incubation. A weak cytostatic action was observed at concentrations of 10 μg/ml of the medium.

The last of the studied compounds, the hydrochloride of 5-bromo-5-nitro-3-ethyltetrahydro-1,3-oxazine killed *Entamoeba moshkovskii* in the concentration of 40 μg/ml. Single cells survived, however, as demonstrated by secondary cultures set up after 48 hours' incubation of the primary culture. In the presence of 30 μg/ml the amebae failed to develop; the secondary culture set up after 24 hours' incubation, however, was normal, indicating that the cells were not damaged. Damage of the cells occurred after further incubation, resulting in smaller numbers of protozoa than in the control culture. Concentrations of 20 μg/ml inhibited the growth of the protozoa very strongly, but secondary cultures showed growth. Some inhibition of growth was observed even at 10 μg/ml.

It should be emphasized that 5-bromo-5-nitro-3-benzyltetrahydro-1,3-oxazine, which exhibited the strongest protozoocidal activity, was also the least toxic of the three oxazine derivatives. Moreover, it also exhibited selective action, being more active than the remaining compounds in relation to *Entamoeba moshkovskii*, but less strongly protozoocidal for *Trichomonas vaginalis* than the hydrobromide of 5-bromo-5-nitro-3-methyltetrahydro-1,3-oxazine.

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The author's address: Wrocław, ul. Chałubińskiego 4