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Studies on antiparasitic agents: effect of the lactam nucleus substitution in the 2-position on the in-vitro activity of new 5-nitroimidazoles

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Abstract—The in-vitro antiprotozoal activity of a series of new 5-nitroimidazoles substituted in the 2-position via a lactam nucleus was studied. All these compounds exhibited a better effect than metronidazole and structure-activity relationships are discussed.

Metronidazole and related 5-nitroimidazoles are valuable drugs for the treatment of several protozoal diseases as well as for treating infections due to anaerobic bacteria and for the radiosensitization of hypoxic tumours (Breccia et al 1982; Nair & Nagarajan 1983; Docampo & Moreno 1984; Josephy & Mason 1985; Halliwell & Gutteridge 1986; Hubert-Habart et al 1987; Kernbaum 1988). The demonstration of the mutagenic and carcinogenic properties of certain nitroimidazoles (Breccia et al 1982; Walsh et al 1987; Vanelle et al 1990) and the possible clinical resistance in pathogenic micro-organisms (Breccia et al 1982) led us to investigate such new derivatives.

Materials and methods

Chemistry. 5-Nitroimidazole derivatives bearing a trisubstituted ethylenic double bond in the 2-position were prepared by reacting 1-alkyl-2-chloromethyl-5-nitroimidazoles with 3-nitrolactam anions under phase-transfer catalysis (Jentzer et al 1991). These new compounds were identified by spectral data and their purity established by controls on thin-layer chromatography and microanalysis.

Parasitology. In-vitro screening was carried out with *Entamoeba histolytica* (Rahman strain). This strain was grown in "Jones" liquid medium (Taylor & Baker 1968). Horse serum (100 mL L^{-1}) and rice starch were added to this medium. *Trichomonas vaginalis* (TVR 87 strain) was cultured in Trichomonas Medium (Oxoid CM 161), complemented with inactivated horse serum (80 mL L^{-1}) and chloramphenicol (0·125 g L^{-1}) (Audibert et al 1979).

The test compounds were first dissolved in dimethylformamide (at a concentration not affecting the parasites) then distributed to the culture tubes to obtain final concentrations of 50, 20, 10, 5, 2, 1, 0·5, 0·2, 0·1, 0·05, 0·02 and 0·01 mg L^{-1}. The minimal inhibitory concentration (MIC) of the different compounds was evaluated for each concentration and each parasite in quadruplicate.

For amoebae, 0·3 mL of culture containing 2×10^4 amoebae mL^{-1} were added to tubes containing 9·7 mL of complete medium and the compounds at the required concentrations. The MIC was determined after 48 h incubation at 37°C.

For Trichomonas, the tests were made under the same conditions; 0·2 mL of culture containing 20×10^4 Trichomonas mL^{-1} was added to 4·8 mL of complete medium with the compounds at the required concentrations. Control cultures were introduced in each test. Samples which gave negative results were submitted to a retroculture to confirm the absence of parasites.

The MIC values were evaluated against metronidazole as a reference drug.

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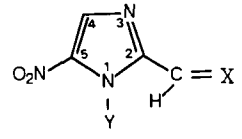
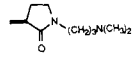
Results and discussion

Results from the in-vitro testing of antiprotozoal activity are presented in Table 1. When compared with metronidazole, derivatives 1 and 3 are 100-fold more effective against *Trichomonas vaginalis* and 50-fold more active against *Entamoeba histolytica*.

Table 1. In-vitro antiprotozoal activity of new 5-nitroimidazoles.



N°	X	Y	MIC (mg mL^{-1})	
			Entamoeba histolytica	*Trichomonas vaginalis*
1		CH_3	0.1	0.01
2		CH_3	0.5	0.05
3		CH_3	0.1	0.01
4		CH_3	0.5	0.05
5		CH_3	0.2	0.02
6		CH_2CH_2OH	0.5	0.05
Metronidazole	H_2	CH_2CH_2OH	5	1

The present study indicates the importance of the lactam group in the 5-nitroimidazole series. The replacement of cyclopentane or cyclohexane in the 2-position by *N*-methyl-2-pyrrolidinone or 2-piperidinone strongly increases the activity (Vanelle et al 1991). The size of the lactam is important: the presence of a seven membered ring (*N*-methyl-2-oxo-hexamethyleneimine) results in a loss of activity (4 vs 1 and 3). The introduction of a substituent (3-dimethylaminopropyl) on the nitrogen atom of the pyrrolidinone has a negative effect on the in-vitro potency (2 vs 1). The presence in the molecule of a sulphur atom instead of oxygen (2-thiopyrrolidinone nucleus instead of 2-pyrrolidinone) also leads to loss of activity (5 vs 1). Replacing the methyl group with a hydroxyethyl group in the 1-position is detrimental for activity (6 vs 1), as has been

demonstrated for other 5-nitroimidazole drugs (Cosar et al 1966).

These results suggest that the lactam nucleus is an important molecular moeity for antiprotozoal activity in the 2-substituted 5-nitroimidazoles.

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Gastrointestinal absorption of quaternary ammonium compounds correlated to their binding to small intestinal brush border membrane in rat

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Abstract—The relationship between absorption of quaternary ammonium compounds (QACs) from rat intestine and their in-vitro binding to isolated brush-border membrane has been examined, using a series of n-alkyltrimethylammoniums. The binding of these QACs gradually increased with each extension of unbranched hydrocarbon chain from octyltrimethylammonium to tetradecyltrimethylammonium. However, hexyltrimethylammonium and heptyltrimethylammonium failed to bind to the membrane. On the other hand, the disappearance of these QACs from rat jejunal loop also increased with the length of hydrocarbon chain over the range of 8·9 to 71·3%. A good correlation was found between binding to the brush-border membrane and disappearance from jejunal loop. From these results, it was suggested that the size of the hydrophobic part of a QAC molecule was a principal determinant of both absorption and membrane binding, and that the absorption of QACs, with an appropriate sized hydrophobic part, was closely associated with the degree of binding to the membrane.

To elucidate the intestinal absorption mechanisms of quaternary ammonium compounds (QACs), we have studied the transport characteristics of these drugs by the intestinal brush-border membrane vesicles, and found that the binding of QACs to the membrane is a first step in the specialized transport mechanism driven by physiological membrane potentials (Saitoh et al 1987, 1988a, b, 1989). We have also demonstrated that the structure of the hydrophobic part of various organic cations is a determinant factor of the binding activities (Saitoh et al 1990). It remains unclear, however, whether the differences in the binding among QACs would be reflected in their absorption behaviour. In the current study, we have developed a simple and sensitive assay for n-alkyltrimethylammoniums and examined in-vitro binding to the brush-border membrane, in-situ absorption, and the correlation between the two.

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Materials and methods

We expressed each n-alkyltrimethylammonium as the number of carbons of the n-alkyl group; hexyltrimethylammonium (C6), heptyl- (C7), octyl- (C8), nonyl- (C9), decyl- (C10), undecyl- (C11), dodecyl- (C12), and tetradecyl- (C14). C12 and C14 were purchased from Nakalai Tesque, Inc. (Kyoto, Japan) and Tokyo Kasei Kogyo, Co. Ltd (Tokyo, Japan), respectively. Other QACs were synthesized in our laboratory as reported previously (Saitoh et al 1990).

The procedure for isolating the brush-border membrane from rat small intestine and the binding study to the membrane were described previously (Iseki et al 1989; Saitoh et al 1990). The absorption experiment was carried out at pH 6·5 using the in-situ loop technique of Levine & Pelikan (1961). A jejunal loop (10 cm) was prepared in a male Wistar rat, 200–250 g, and the proximal ligature of the loop placed about 10 cm from the pylorus. After washing the loop gently with 10 mL of a modified Ringer solution (Schultz et al 1966), 50 μM QAC (1 mL) dissolved in the Ringer solution was injected into the loop. After 30 min, the contents of the loop were withdrawn as completely as