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AMOEBICIDAL ACTIVITY OF CG-10213 GO AND OTHER NITROIMIDAZOLES AGAINST AXENICALLY GROWN *ENTAMOEBA HISTOLYTICA*

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The discovery that *Entamoeba histolytica* can be grown in cultures without any other living associate, in medium TP-S-1 (Diamond, 1968), opened a new era in the studies on *E. histolytica*, and made it possible to investigate accurately and precisely the effect of various amoebicidal drugs on it. Before this, most of such investigations involved the use of cultures in which *E. histolytica* was grown with various bacterial associates in diphasic medium. In such systems it was impossible to ascertain whether the drug was directly acting on the amoeba or indirectly by killing the associated bacterial flora, in the absence of which the trophozoites of *E. histolytica* would not grow.

Dutta and Yadava (1972) studied the effects of various amoebicides against axenically grown *E. histolytica strain* NIH-200 in modified TP-S-1 medium containing 0.2 percent L-cysteine hydrochloride without ascorbic acid. Das and Prasad (1973) used the same media for growing the amoebae, but unlike Dutta and Yadav (1972) who dissolved the drugs in physiological saline and then autoclaved them, dissolved the drugs in DMSO or DMF. Neal (1978) reported the activity of several amoebicidal drugs *in vitro* against axenically grown *E. histolytica* (Strain NIH-200), and reported the end-points representing ED_{50} values of these drugs.

In the present study, we report the direct amoebicidal activity of a new, third generation antiprotozoal drug, CG-10213 GO (Satranidazole), synthesized at this Research Centre (Nagarajan *et al.*, 1982), against axenically grown *E. histolytica* (Strain HK-9). This drug, also a nitroimidazole derivative, carries an imidazolidinone moiety at position 2, and differs from other marketed nitroimidazoles in having a nitrogen atom rather than carbon attached to position 2 of the nitroimidazole template.

A new medium TYI-S-33 (Diamond, Harlow and Curnick, 1978) was used for growing axenic strain of *E. histolytica*. The pH of the medium was adjusted to 6.8; 8.5 ml of complete sterile medium was dispensed in each screw-cap tube (Borosil 125 mm × 25 mm). An inoculum of 1.0 ml containing 100,000 amoebae from 4-5 days old cultures in active log phase of growth was then added to each tube, with a long sterile pasture pipette, giving a final amoebae count of 10,000 amoebae/ml. Approximately after one hr, 0.5 ml of drug dilutions were added to each tube and mixed. In a set of experiments, for each drug dilution duplicate cultures were run. Drug dilutions were prepared in 0.5% saline, autoclaved and adjusted to neutral pH. In control tubes, 0.5 ml of saline was added and all the tubes were incubated at 35.5°±1°C for 72 hr. in vertical position. The amoebicidal endpoints were recorded by direct examination of

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the bottom of culture tubes under an inverted microscope. The highest dilution at which no motile, elongated amoebae were seen was considered as amoebicidal endpoints (minimum lethal concentration, MLC). In doubtful cases subculturing was done (Das and Prasad, 1973).

Results presented in the Table I show that amoebicidal endpoint of CG-10213 Go is 1.0 μg/ml which is lower than metronidazole and seconidazole (1.9 μg/ml) and tinidazole and nimorazole (3.9 μg/ml), suggesting its marginal superiority. However, ornidazole also showed amoebicidal endpoint at 1.0 μg/ml. Das and Prasad (1973) used DMSO and DMF for dissolving the insoluble drugs. But in our experience we found that even as low as 10^{-4} μl/ml concentration of DMSO impaired the growth of amoebae, and hence in this study drug dilutions were prepared by autoclaving.

TABLE I.

Sl. no.	Drug	MLC (μg/ml)
1.	CG-10213 Go.	1.0
2.	Metronidazole	1.95
3.	Tinidazole	3.9
4.	Seconidazole	1.95
5.	Ornidazole	1.0
6.	Nimorazole	3.9

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