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Antiparasitic Properties of Diploceline, a Quaternary Alkaloid from *Strychnos gossweileri*

Monique Gasquet[1], *Joëlle Quetin-Leclercq*[2,3], *Pierre Timon-David*[1], *Guy Balansard*[1], *and Luc Angenot*[2,3]

[1] Faculté de Pharmacie, Université d'Aix-Marseille, 27, Boulevard Jean-Moulin, F-13385 Marseille Cedex 5, France
[2] Institut de Pharmacie, Faculté de Médecine, Université de Liège, rue Fusch, 5, B-4000 Liege, Belgium
[3] Address for correspondence

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Diseases caused by protozoa are responsible for a considerable mortality throughout the world, more particularly in the tropics. Few drugs are currently available to treat such infections and certain phenomena of resistance may even occur, leading to an urgent need for new selective agents (1). In this paper, we report the results of studies on diploceline, a quaternary alkaloid isolated from the root bark of *Strychnos gossweileri* Exell. (2). Previous works have shown that this alkaloid is devoid of cytotoxic activity (3) even at 200 μg/ml, but has antimicrobial properties on *Streptococcus* species (*S. haemolyticus*) at a relatively high dose (0.5 mg/ml) (4). The discovery of antiparasitic activities in quaternary alkaloids, such as berberine and derivatives (1), led us to check whether diploceline was active against *Entamoeba histolytica, Plasmodium falciparum*, or *Trichomonas vaginalis* cultured *in vitro*. Furthermore, we also studied its mutagenic or antimutagenic effects (inhibition of the mutagenicity of both benzo[*a*]pyrene and smoker urine).

The results indicate that diploceline is devoid of any mutagenic or antimutagenic effect at 2.5 mg/ml. Besides, it does not produce any inhibition of *P. falciparum* growth at 25 μg/ml (maximum tested concentration). Nevertheless, diploceline is active at 25 μg/ml on *T. vaginalis* and at 50 μg/ml on *E. histolytica* (Table **1**).

Table 1 Antiparasitic activities of diploceline.

Parasites \ Dose (μg/ml)	100	50	25	10	5	1	0.5
E. histolytica	++	++	+	–	–	–	–
P. falciparum	N.T.	N.T.	–	–	–	–	–
T. vaginalis	++	++	++	–	–	–	–

–: No inhibition; +: significant inhibition; ++: all parasites killed; N.T.: not tested because toxic at 50μg/ml.

This activity is relatively weak as compared to metronidazole whose M.I.C. values in the same experimental conditions on *E. histolytica* and *T. vaginalis*

are respectively 5 and 0.5 μg/ml. Moreover, metronidazole produces mutagenic effects on bacteria and is relatively ineffective in eradicating *E. histolytica* cysts or minuta trophozoites from the gut lumen (1). In fact, the activity of metronidazole is inhibited by oxygen which is present in low amounts in the gut lumen. Diploceline probably has another mode of action and might prove useful in eradicating cysts from the gut lumen. Furthermore, diploceline retains its quaternary characteristics at any pH and consequently undergoes little resorption in the alimentary canal. Such a property would allow it to achieve high concentrations in the intestine without producing important systemic effects. However, it seems rather difficult to establish a quantitative comparison between the activity of diploceline and the activity of other indole alkaloids described in the relevant literature (5–7) since different strains and a distinct methodology have been used.

Nevertheless, we believe that it might be interesting to perform other tests, both *in vitro* and *in vivo*, in order to elucidate its mode of action, verify its activity *in vivo*, and analyse its potential toxicity.

Materials and Methods

Diploceline was isolated from the root bark of *Strychnos gossweileri* Exell. and purified according to the methods previously described (2). It is used in its chloride form.

The amoebicidal activity of diploceline was measured on *Entamoeba histolytica* (rahman strain) *in vitro* (8) in Jones' liquid medium. Tubes were seeded with 0.3 ml of medium containing 2×10^4 amoebas/ml. The minimum inhibitory concentration (M.I.C.) was determined after a 72-hour incubation at 37 °C. Negative tubes were subcultured in order to confirm the absence of parasites.

The trichomonacidal activity of diploceline was evaluated on *Trichomonas vaginalis* (strain TVR 87) in liquid medium (OXOID-Trichomonas medium-CM 161) (9, 10). The M.I.C. was determined as described above for the amoebicide effect.

The antimalarial activity of diploceline was studied on *Plasmodium falciparum* (strain FCC2-chloroquine-sensitive) cultured *in vitro* in wells at 37 °C in an air/CO_2 incubator (6 %) as described by Trager and Jensen (11, 12). The medium was RPMI 1640 supplemented with HEP buffer, glucose and 10 % of human serum. The inhibition of proliferation was measured in continuous culture (13) during 48 hours. In each multiwell, 3 untreated wells gave the maximum proliferating rate. Chloroquine sulfate was used as a reference and its ED_{50} was 0.035 μg/ml.

The potential mutagenic effects of diploceline were analysed according to the Ames test (14) as modified by De Meo et al. (15). The strains used were TA97, TA98, TA100 and TA102 *Salmonella typhimurium* with or without addition of a metabolic fraction (S9 MIX) extracted from a rat liver induced by Aroclor 1254.

The antimutagenic effect was measured with benzo[*a*]pyrene and smoker urine as standard mutagens. The Ames' test was used on TA98 *S. typhimurium* with the S9 MIX metabolic fraction.

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