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Differential effects of the (+)- and (−)-gossypol enantiomers upon *Entamoeba histolytica* axenic cultures

MARÍA TERESA GONZÁLEZ-GARZA*, STEPHEN A. MATLIN†, BENITO D. MATA-CÁRDENAS*, SALVADOR SAID-FERNÁNDEZ*‡,
Unidad de Investigación Biomédica del Noreste, División de Biología Celular, Instituto Mexicano del Seguro Social, Apto Postal 020-E 64720, Monterrey, Nuevo León, México, †Chemistry Department, City University, Northampton Square, London EC1V 0HB, UK, and ‡Centro Internacional de Biología Celular y Molecular, Edif. Kalos, Desp. 324, Monterrey, Nuevo León, México

Abstract—The in-vitro anti-amoebic effects of (±)-, (+)-, (−)-gossypol and emetine were tested against axenic trophozoites from five *Entamoeba histolytica* strains. The (−)-isomer was more active than the racemate and the (+)-isomer. These results indicate that the gossypol anti-amoebic activity is mainly due to its content of (−)-gossypol in all strains tested.

Amoebiasis, a parasitic disease caused by *Entamoeba histolytica*, is a major health problem in several developing countries (Sepulveda 1982; Walsh 1986). The untreated disease may progress to hepatic amoebiasis and other complications (Guerrant 1986). There are numerous anti-amoebic compounds used in medical practice, but most of them are toxic and in certain cases it is necessary to use a combination of two or more drugs in the treatment. Therefore anti-amoebic agents are required and it is important to continue the search for new, more potent and less toxic anti-amoebic drugs.

Gossypol acetic acid is a polyphenolic binaphthyl derivative. Its pharmacological properties are well studied, mainly due to its antifertility effects in men and males of several species (for an overview see Wu 1989), and to its in-vitro and in-vivo activity against diverse pathogenic agents, including the human immunodeficiency virus (Lin et al 1989). Gossypol is concentrated in the liver and in the colon, and excreted with the bile (Abou-Donia & Dieckert 1975). Accordingly, this drug could be a good anti-amoebic medication.

The natural compound is a mixture of (+)- and (−)-gossypol enantiomers. The second is the most active in infertility production in hamsters (Matlin et al 1988), cytotoxicity in tumoural cells (Joseph et al 1986) and antiviral activity against the human immunodeficiency virus type 1 (Lin et al 1989).

Recently, we found that the racemic mixture of gossypol has an in-vitro anti-amoebic effect, which is noticeably more potent than metronidazole, emetine and diiodohydroxyquinoline (González-Garza & Said-Fernández 1988). This toxic effect extends to several axenic *E. histolytica* strains (González-Garza et al 1989). The aim of this study was to define the specific contribution of each enantiomer to the anti-amoebic activity of gossypol.

Materials and methods

Amoeba. Trophozoites from HM-1:IMSS, HM-2:IMSS, HM-3:IMSS, HM-38:IMSS (abbreviated in the text as HM-1, HM-2, HM-3, HM-38, respectively) and HK-9 *Entamoeba histolytica* strains, were maintained axenically in PEHPS medium (Said-Fernández et al 1988) by serial culture. Briefly, culture tubes containing 11 mL of PEHPS were inoculated with 1000 trophozoites mL^{-1} and incubated at 36°C; the subcultivation frequency was every 4 days.

Assay method. The drug potency test was performed as described earlier (González-Garza & Said-Fernández 1988). Briefly, culture tubes with fresh sterile PEHPS medium, alone or with (in triplicate) 0·002–0·200 μM each of one of the following compounds: (±)-gossypol, (−)-gossypol, (+)-gossypol or emetine, were inoculated, with 1×10^3 amoebae mL^{-1}, which were derived from a reference culture in logarithmic growth. The tubes were incubated for 72 h at 36°C, cooled in ice water for 10 min and the number of trophozoites mL^{-1} in each tube determined with a haemacytometer. The results were estimated as the percentage of growth inhibition compared with the untreated controls and plotted as probit values as a function of the drug concentration (n = 9). The IC50 and 95% confidence limits were interpolated in the corresponding dose-response curve.

Statistical analysis. Student's *t*-test was used to determine the significant difference of results obtained with each one of the five *E. histolytica* strains treated with gossypol or each one of its enantiomers among data obtained with emetine. The slope and elevation of the amoebic dose-response curves were compared by analysis of covariance.

Chemicals. Racemic gossypol acetic acid from cotton plant (*Gossypium* sp.), (+)-gossypol isolated from *Thespesia pupulnea* (optically pure) and (−)-gossypol (all samples having > 99·5% chemical purity) from cotton plant were converted to diastereomeric Schiff's base derivatives using a chiral amine followed by preparative HPLC separation on a chiral amino acid bonded phase, as described elsewhere (Matlin et al 1987). Emetine was purchased from Sigma (St Louis, MO, USA) and other salts, reagent grade, were purchased from J.T. Baker de México (Xalostoc, México). Sterile bovine serum and PEHPS medium were prepared in our laboratory.

Results and discussion

The results are summarized in Table 1. (±)-Gossypol purchased from Sigma Chemical Co. showed a potent in-vitro anti-amoebic effect and a differential potency against the same axenic *E. histolytica* strains used for this study (González-Garza & Said-Fernández 1988). In the present work, we show that purified (±)-gossypol free from acetic acid (Matlin et al 1988), has a similar potency against the same five strains as those previously tested, and that both gossypol isomers are active against *E. histolytica* trophozoites.

The (−)-enantiomer is noticeably more active than (+)- and (±)-gossypol, and thus, the in-vitro anti-amoebic effects of racemic gossypol are considered to be mainly due to the (−)-enantiomer, the isomer responsible for the antifertility effects in male animals (Matlin et al 1988) and for antiviral activity in peripheral blood mononuclear cells against human immunodeficiency virus type 1 (Lin et al 1989). The mechanism of action of gossypol or its enantiomers is not yet clear. However, it has been reported that both gossypol isomers bind to proteins, including serum albumin, and that the protein-gossypol complexes are inactive (Lyman et al 1959). Accordingly, it has been suggested

Correspondence: M. T. González-Garza, Unidad de Investigación Biomédica del Noreste, División de Biología Celular, Instituto Mexicano del Seguro Social, Apto Postal 020-E 64720, Monterrey, Nuevo León, México.

Table 1. In-vitro anti-amoebic activity of (±)-, (+)-, (−)-gossypol and emetine against five *Entamoeba histolytica* strains.

	(±)-Gossypol		(+)-Gossypol			(−)-Gossypol			Emetine		
Strain	IC50 (μM)	Relative potency[b]	IC50 (μM)	Ratio[a]	Relative potency[b]	IC50 (μM)	Ratio[a]	Relative potency[b]	IC50 (μM)	Ratio[a]	Relative potency[b]
HM-1	0·018±0·001	1·00	0·235±0·024	0·08	1·00	0·010±0·001	1·80	1·00	0·170±0·038	0·11	1·00
HK-9	0·053±0·010	2·94	2·086±0·368	0·03	8·88	0·016±0·002	3·31	1·60	0·153±0·013	0·35	0·90
HM-3	0·064±0·007	3·56	0·225±0·038	0·28	1·00	0·038±0·002	1·68	3·80	0·113±0·015	0·57	0·66
HM-2	0·068±0·010	3·78	0·455±0·035	0·15	1·94	0·026±0·003	2·61	2·60	0·131±0·012	0·52	0·77
HM-38	0·071±0·006	3·94	1·085±0·120	0·07	4·62	0·029±0·003	2·45	2·90	0·082±0·012	0·87	0·48

±95% confidence limits. [a]IC50 of (±)-gossypol/IC50 of (+)-gossypol, (−)-gossypol or emetime. [b]Relative potency compared with HM-1.

that the relative lower activity of (+)-gossypol is due to its higher affinity for these protein molecules (Tanphaichitr et al 1988).

On the other hand, several of the gossypol effects on mammalian cells and parasites are due to an inhibition of NADH-dependent enzymes. Thus, it is not possible to rule out the possibility that there is a major specific affinity of (−)-gossypol for such classes of enzymes, and a major inhibitory effect.

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