ELSEVIER

Acta Tropica 70 (1998) 119–122

ACTA TROPICA

Short communcation

Leishmania amazonensis infection is reduced in macrophages treated with guanine ribonucleosides

Selma Giorgio [a,*], Sandra C. Barão [a], Ohara Augusto [b], Jolie K. Kwee [b]

[a] *Departamento de Parasitologia, Instituto de Biologia, Universidade Estadual de Campinas, CP 6109 Campinas, São Paulo 13083-970, Brazil*

[b] *Departamento de Bioquimica, Instituto de Quimica, Universidade de São Paulo, CP 26077, São Paulo 05599-970, Brazil*

Received 31 October 1997; received in revised form 16 January 1998; accepted 28 January 1998

Keywords: Leishmania amazonensis; Macrophage; Guanosine analogues

The substitution of guanine ribonucleosides at the carbon 8 position produced a class of immunostimulatory compounds among which the best studied are 8-bromoguanosine (8BrGuo), 8-mercaptoguanosine, (8SGuo), 7-hydro-8-oxo-guanosine (8OxoGuo) and 7-allyl-8-oxo-guanosine (Goodman, 1991, 1995). These nucleosides activate a broad range of immunological functions such as differentiation of B cells, natural killer cell-mediated cytotoxicity, increase in cytokine production (TNFα, IFN and IL1) and both the respiratory burst and cytotoxicity of macrophages (Goodman and Weigle, 1983; Koo et al., 1988; Ojo-Amaize et al., 1990; Goodman et al., 1995). Recently, these compounds have been found to be active in mouse models for the treatment of viral infections as a cancer chemotherapeutic agent and as a vaccine adjuvant for immunization against tumors (Goodman, 1991, 1995; Bonnet and Robins, 1993). However, the mechanisms by which these compounds stimulate the immune response as well as the signaling pathways they use remain poorly understood (Goodman, 1995).

* Corresponding author. Tel.: + 55 19 7887651; fax: + 55 19 2893124; e-mail: sgiorgio@uol.com.br

PII S0001-706X(98)00012-6

Leishmania amazonensis infection is associated with benign cutaneous lesions and diffuse cutaneous disease. Its treatment has been based on the use of *N*-methylglucamine antimonate, sodium stibogluconate and amphotericin B, compounds responsible for severe toxic effects (Walton, 1987). The use of immunostimulators combined with these conventional drugs has been explored more recently (Chance, 1995). To the best of our knowledge, the effects of guanine ribonucleosides on *Leishmania* infection have never been reported in the literature. In this study, we investigated the effect of 8BrGuo, 8SGuo and 8OxoGuo on the cytotoxicity of macrophages against the parasite in a well established in vitro system to test the effects of drugs with leishmanicidal potential (Rabinovich et al., 1982; Chaudhuri et al., 1989; Cantos et al., 1993). Peritoneal macrophages from BALB/c or C57Bl/6 mice were removed, cultured and assayed for their leishmanicidal effect in the presence and absence of 8BrGuo, 8SGuo and 8OxoGuo. The nucleosides 8BrGuo and 8SGuo were obtained from Sigma, St Louis, MO and 8OxoGuo was synthesized in our laboratory (Kwee, unpublished data). Fig. 1 shows that the phagocytic index of macrophages pretreated with 0.3 mg/ml of 8BrGuo and then infected with *L. amazonensis* amastigotes was reduced to about 60% of the control values. At higher doses, up to 0.9 mg/ml, no further reduction of the phagocytic index was observed. Similar results were obtained in macrophages cultures treated with 8BrGuo before or after infection with both forms of the parasite (data not shown). Experiments with the parent compound guanosine were attempted but abandoned because it detached peritoneal macrophages from glass cover slips and inhibited

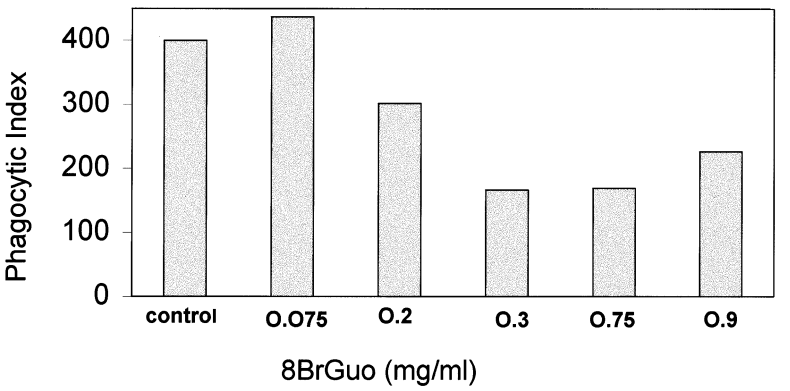

Fig. 1. Effects of 8BrGuo pretreatment on macrophage infected by *L. amazonensis*. About 5×10^5 peritoneal macrophages from C57Bl/6 mice were attached to round 13 mm diameter glass coverslips, rinsed in saline and placed on Costar plates containing 1 ml Iscoves medium with 5% inactivated fetal calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine, 100 mM hepes and 20 mM sodium bicarbonate. After 24 h, the cultures were incubated with 8BrGuo for 24 h before infection with a 3-fold excess of amastigotes for 1 h. The parasites were purified from BALB/c mice cutaneous lesions. Control cultures were set up in a diluent (0.01 M NaOH). Macrophages on glass cover slips were stained with Giemsa and examined microscopically 24 h after infection. For each glass cover slip about 250 cells were counted. The phagocytic index is the product of the percentage of infected macrophages times the average number of amastigotes per macrophage. The data are representative of six experiments.

Table 1
Effects of guanosine ribonucleosides on reduction of *L. amazonensis* infection in mouse macrophage[a]

Treatment	Percent of infected macrophages (%)	Number of amastigotes/macrophage
Control[b]	70.0	5.0
8OxoGuo	46.2	4.1
8Sguo	60.5	4.8
8BrGuo	34.4	4.2
8BrGuo +INF$\alpha\beta$	18.5	4.7
INF$\alpha\beta$	48.0	5.0

[a] Macrophages were obtained, cultured, infected, stained and counted as described in the legend to Fig. 1. The cell cultures were pretreated for 24 h with 0.15 mg/ml 8OxoGuo; 0.3 mg/ml 8SGuo; 0.75 mg/ml 8BrGuo; 0.75 mg/ml 8BrGuo + 100 U/ml INF$\alpha\beta$ (Sigma, St. Louis, MO); and 100 U/ml INF$\alpha\beta$. These are representative data of at least three experiments for each compound.
[b] Control cultures were set up in a diluent (0.01 M NaOH).

proliferation of macrophage cell line J774. Similar guanosine effects upon lymphocytes have been described before (van de Kraan et al., 1986). In contrast, 8BrGuo, 8SGuo and 8OxoGuo were not directly toxic to either macrophages or parasites (data not shown). The same protocol described in Fig. 1 was employed to test the effects of 8SGuo and 8OxoGuo (Table 1). The ribonucleoside 8SGuo was not capable to activate the leishmanicidal effect of macrophages whereas 8OxoGuo at concentrations ranging from 0.075 to 0.15 mg/ml caused a reduction in both the percentage of infected macrophages and the number of amastigotes per cell. Since guanine ribonucleosides were not toxic to cell and parasite cultures, we may conclude that 8BrGuo and 8OxoGuo induced macrophage activation. Interestingly, 8BrGuo (reduction of 50% in infected cells in relation to the control values) had a synergistic action with INF$\alpha\beta$ on macrophage activation against *Leishmania* (reduction to about 70% of infected macrophages in relation to the control values). The cytokines alone were unable to induce efficient parasite killing (the number of infected cells was reduced but not the number of parasites per cell) (Table 1). Evidence from other laboratories has defined 8BrGuo as an inductor of these INF (Koo et al., 1988; Goodman et al., 1995). Recently, Shankar et al. (1996) have shown the capacity of INFα and subactivating doses of LPS to induce a *L. major* killing response in macrophages. We can not exclude the presence of contaminating endotoxin in our assays (i.e. in amastigote preparations) that may supply the second signal that is needed for the macrophages to express leishmanicidal activity. It is likely that 8BrGuo primed macrophages by inducing INF$\alpha\beta$ and/or other lymphokines in quantities sufficient to kill *L. amazonensis* amastigotes. The stimulation of leishmanicidal function in macrophages described here and the broad range of immunostimulant capacities of guanine ribonucleosides (Goodman, 1991, 1995) suggest that these compounds may be useful modifiers of the immunological response to combat infections with intracellular pathogens.

Acknowledgements

This study was supported by funds from the following Brazilian agencies: FAPESP, CAPES and CNPq.

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