Effects of MS-8209, an Amphotericin B Derivative, on Tumor Necrosis Factor Alpha Synthesis and Human Immunodeficiency Virus Replication in Macrophages

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Amphotericin B derivatives, such as MS-8209, have been evaluated as a therapeutic approach to human immunodeficiency virus (HIV) infection. We show that MS-8209, like amphotericin B, increases tumor necrosis factor alpha (TNF-α) mRNA expression and TNF-α production and consequently HIV replication in human macrophages. These effects confirm the pharmacological risk associated with the administration of amphotericin B or its derivatives to HIV-infected patients.

Macrophages and related cells play a key role in pathological events, particularly in inflammatory processes associated with human immunodeficiency virus (HIV) disease. They are a major target cell for HIV, and proinflammatory monokines such as tumor necrosis factor alpha (TNF-α) increase HIV type 1 (HIV-1) gene expression and replication, via intracellular activation and autocrine and paracrine pathways (5, 14, 19). MS-8209 is a water-soluble derivative of amphotericin B (AmB) of lower cellular and animal toxicity and greater solubility than the parent compound (13). MS-8209 exhibits antiretroviral activity in mitogen-activated CD4+ T lymphocytes infected in vitro with HIV-1 (4, 11). MS-8209 exerts its antiviral action by inhibiting HIV entry into cells after CD4-gp120 interactions (13). AmB provokes marked overexpression of TNF-α in murine and human macrophages (10, 15, 17). In view of the deleterious effects of TNF-α on HIV replication, it was therefore of interest to investigate the possible effects of MS-8209 on TNF-α synthesis and HIV replication in macrophages.

We obtained human macrophages by 7-day differentiation of freshly isolated monocytes. Monocytes were separated from peripheral blood mononuclear cells using countercurrent centrifugal elutriation. Cells were cultured in RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum, 2 mM L-glutamine, and 1% triantibiotic mixture (penicillin, neomycin, and streptomycin). Cell culture medium was endotoxin free, as shown by the Limulus amebocyte lysate test. To study the effects of MS-8209 on TNF-α synthesis, monocyte-derived macrophages (MDM) were exposed to various concentrations of MS-8209 (0, 5, and 10 μM) for 24 h, during which TNF-α was measured in cell supernatants by enzyme-linked immunosorbent assay (ELISA), and its mRNA was quantified by reverse transcriptase PCR (RT-PCR) (1). To investigate the effects of MS-8209 on HIV replication, 1 million MDM were infected in vitro with 10,000 50% tissue culture infective doses of either the reference macrophage-tropic HIV-1/Ba-L strain or the primary HIV-1/DAS isolate. TNF-α and viral replication were measured throughout the culture in cell supernatants by ELISA and by dosing RT activity, respectively, as previously described (7). In these last experiments, cell culture medium and MS-8209 (0, 1, 5, and 10 μM) were renewed twice a week. MS-8209 dose dependently increased TNF-α synthesis and TNF-α mRNA expression during the first 24 h of MDM treat-

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FIG. 1. TNF-α mRNA expression and TNF-α production in uninfected MDM treated for 24 h with MS-8209. RNAs were quantified using a noncompetitive RT-PCR (1), and the cytokine was detected in cell culture supernatants by ELISA (Immunotech, Luminy, France). Results are expressed as the means ± standard deviations of three different culture wells. This experiment was performed using cells from one given donor, and identical results were observed with cells isolated from a second donor. Data were analyzed using an unpaired t test (Statview microcomputer software; Abacus Concept Inc., Berkeley, Calif.). Differences were considered to be significant at P < 0.05 (∗) or P < 0.01 ∗). GAPDH, glyceraldehyde-3-phosphate dehydrogenase.
The significant increase in HIV-1 replication was evaluated throughout the culture by the assay of RT activity using the RetroSys kit (Innovagen, Lund, Sweden). TNF-α was measured in cell culture supernatants of uninfected MDM by ELISA. Each point is the mean ± standard deviation of results from three different culture wells. This experiment was performed using cells from one given cell donor, and identical results were observed with cells from a second blood donor. Data were analyzed using an unpaired *t* test. Differences were considered to be significant at *P* < 0.01 (**) or *P* < 0.05 (*).

![Graph showing RT activity and TNF-α in culture supernatants of MDM treated with 1, 5, and 10 μM MS-8209.](http://aac.asm.org/)
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REFERENCES