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RESEARCH BRIEF

Leishmania mexicana: Uptake of Sodium Stibogluconate (Pentostam) and Pentamidine by Parasite and Macrophages

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Pentavalent antimonials in the form of sodium stibogluconate (Pentostam) or meglumine antimonate (Glucantime) are the primary therapeutic agents for leishmaniasis. Pentamidine is clinically effective but has been relegated to the status of a secondary agent because of toxicity. However, pentamidine is a widely employed antimicrobial because it is also efficacious in *Pneumocystis carinii* infections and in African trypanosomiasis. There are two stages of *Leishmania* spp.: the mammalian form (amastigote) which is an obligate intramacrophage microorganism and the insect vector form (promastigote). Both Pentostam and pentamidine are more active against intramacrophage amastigotes than extracellular promastigotes (Berman *et al.* 1980), and the fact that both drugs are clinical agents indicates that in general both drugs are more active against amastigotes than mammalian cells.

Uptake of drugs by cells can be via simple diffusion, facilitated diffusion, or active transport. Acquisition of radiolabeled Pentostam and pentamidine permitted this report of the uptake of these drugs by *Leishmania* spp. amastigotes and *Leishmania* spp. promastigotes and macrophages.

The strain used in these experiments, *L. mexicana* WR 227, was cultured from a patient who acquired cutaneous infection in Panama. The strain was determined to be *L. mexicana* by isoenzyme analysis (R. D. Kreutzer, personal communication). Promastigotes of WR 227 were maintained in Schneider's drosophila medium revised (GIBCO, Grand Island, NY, U.S.A.) with the addition of 20% heat inactivated fetal calf serum (Hendricks and Wright 1979). Promastigotes were grown to late log phase (40×10^6/ml), washed with HBSS (GIBCO), and resuspended in HBSS for experimentation. Amastigotes of WR 227 were obtained from amastigote-containing J774 macrophages by the method of Chang (1980) as modified (Berman *et al.* 1985). The macrophages were infected with promastigotes, maintained until the promastigotes transformed to amastigotes, and lysed to release the intracellular amastigotes. The amastigotes, purified by Percoll sedimentation, were washed and resuspended in HBSS for experimentation. J774 macrophages were also washed and resuspended in HBSS for experimentation. Experiments with all cell types were performed at the pH of HBSS (pH 7.4) because this pH is optimal for the macrophages and adequate for promastigote viability. The pH of the phagolysosome in which amastigotes reside is not known.

In experiments involving uptake of drug versus time, cells in HBSS were incubated with 1.6 m*M* ^{125}Sb in the form of Pentostam (^{125}Sb labeled) or 6.7 μM [^{3}H]pentamidine isethionate ([^{3}H]pentamidine). At appropriate time points, 0.2 ml of cell suspension containing 10×10^6 amastigotes, 14×10^6 promastigotes, or 3×10^5 macrophages was withdrawn and centrifuged (12,000*g* for 3 min) through silicone F-50 oil (General Electric, Waterford, NY, U.S.A.), and the pellet was scintillation counted. In experiments involving velocity of drug uptake versus drug concentration, the same number of cells in 0.2 ml was exposed to 1 m*M* ^{125}Sb and 0–49 m*M* cold Sb in the form of Pentostam or to 3 μM [^{3}H]pentamidine and 0–147 μM cold pentamidine, for 1 min. The cells were then pelleted and counted. Experiments with amastigotes and macrophages were performed at 35 C; experiments with promastigotes were performed at 26 C. Cellular volumes were approximated by comparing the volume of a large number of pelleted cells to known volumes of water. Protein determinations were made by the Lowry technique. The amount of 1×10^6 amastigotes constitutes 0.027 μl and 3.1 μg protein; 1

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TABLE I
In Vitro Antileishmanial Activity of [^{125}Sb]Pentostam and Unlabeled Pentostam

Pentostam	Concn (m*M*)	Promastigotes: % control 2 days after drug exposure	Amastigotes: % control 2 days after drug exposure
Cold Sb	1.2	90[a]	75[a]
	2.4	74[a]	62[a]
	4.0	60[a]	39[a]
^{125}Sb	1.2	76–85[b]	72–75[b]
	2.4	64–71[b]	62–63[b]
	4.0	42–55[b]	39–48[b]

Note. Leishmania mexicana WR 227 promastigotes or amastigotes were exposed to cold or radiolabeled Sb in the form of Pentostam for 4 hr, washed, and resuspended in promastigote culture media (Berman *et al.* 1985). After 2 days, the number of promastigotes in each drug treated culture was expressed as a percentage of the number of organisms in simultaneously cultivated controls.

[a] Values from Berman *et al.* 1985.

[b] Range of two experiments.

$\times 10^6$ promastigotes constitute 0.10 μl and 7.0 μg protein; 1×10^6 J774 macrophages constitute 20 μl and 300 μg protein.

[^{125}Sb]Sodium stibogluconate was custom synthesized by Amersham Corp. (Chicago, IL, U.S.A.) under the direction of Burroughs Wellcome Co. (London, UK) and had a specific activity of 0.375 mCi/mmole Sb. Because cold Pentostam consists of an unknown number of uncharacterized combinations of Sb and carbohydrates derived from gluconic acid, the purity of the radiolabeled product could not be compared to that of the cold drug by standard chemical means. Instead, the purity of the radiolabeled product was determined by bioassay using previously published procedures (Berman *et al.* 1985). The antileishmanial activity of radiolabeled Pentostam was similar to that of cold Pentostam for both amastigotes and promastigotes (Table I).

[^{3}H]Pentamidine isethionate (310 mCi/mmole) was custom synthesized by Moravek Biochemicals, La-Brea, CA, U.S.A. and its purity was verified by HPLC (C. Dickenson, personal communication). Quantities of pentamidine isethionate refer to quantities of the total salt; 57% of pentamidine isethionate is pentamidine base. Cold Pentostam was obtained from Burroughs Wellcome. Cold pentamidine isethionate was obtained from May & Baker (Dagenham, UK).

The uptake of 1.6 m*M* [^{125}Sb]Pentostam as a function of time by *L. mexicana* amastigotes, *Leishmania mexicana* promastigotes, and J774 macrophages is seen in Fig. 1a. During the first minute of drug exposure, there was an initial rapid uptake by three cell types. By 3 min of exposure, amastigotes concentrated Sb more than macrophages. By the end of the hour of incubation, the Sb concentration within amastigotes (49 m*M*: Concentration factor ≅30 compared to the external medium) was about three times that of both promastigotes and macrophages (14 m*M*: Concentration factor ≅9). Croft *et al.* (1981) found that after 24 hr, amastigotes and promastigotes concentrated [^{125}Sb]Pentostam by factors of 10–20 and 2–10, respectively. The uptake of ^{125}Sb as a function of concentration is seen in Fig. 1b. Although uptake at less than 1 min was not ascertained, the generally linear increase in uptake by all three cell types in these 1-min experiments ($R^2 \geq 0.97$ for each straight line) indicates that Pentostam is taken predominately up by cells by simple diffusion. Therefore, the ability of all cell types tested to concentrate Pentostam may be due to binding of the drug to macromolecules in the cells.

The uptake of 6.7 μ*M* [^{3}H]pentamidine as a function of time is seen in Fig. 2a. As with Pentostam, the [^{3}H]pentamidine uptake was initially greater for amastigotes and macrophages than for promastigotes (Fig. 2a). By the end of the hour of incubation, pentamidine was concentrated within amastigotes by a factor of 20 compared to the external medium and within macrophages and promastigotes by factors of 6.3 and 4.5 compared to the external medium. The uptake of pentamidine versus concentration indicates that drug uptake is predominantly by simple diffusion ($R^2 \geq 0.98$ for the straight lines in Fig. 2b). Thus, concentration of this drug with cells is probably due also to binding to macromolecules. The data with pentamidine are consistent with the previous determination of pentamidine uptake by *Trypanosoma brucei* stains. In one report, pentamidine was concentrated by a factor of 2.3–17 (Damper and Patton 1976a); in another report, the drug was taken up by linear diffusion by some of the strains tested (Damper and Patton 1976b).

The greater ability of amastigotes, compared to promastigotes and mammalian macrophages, to concentrate Pentostam and the generally greater ability of

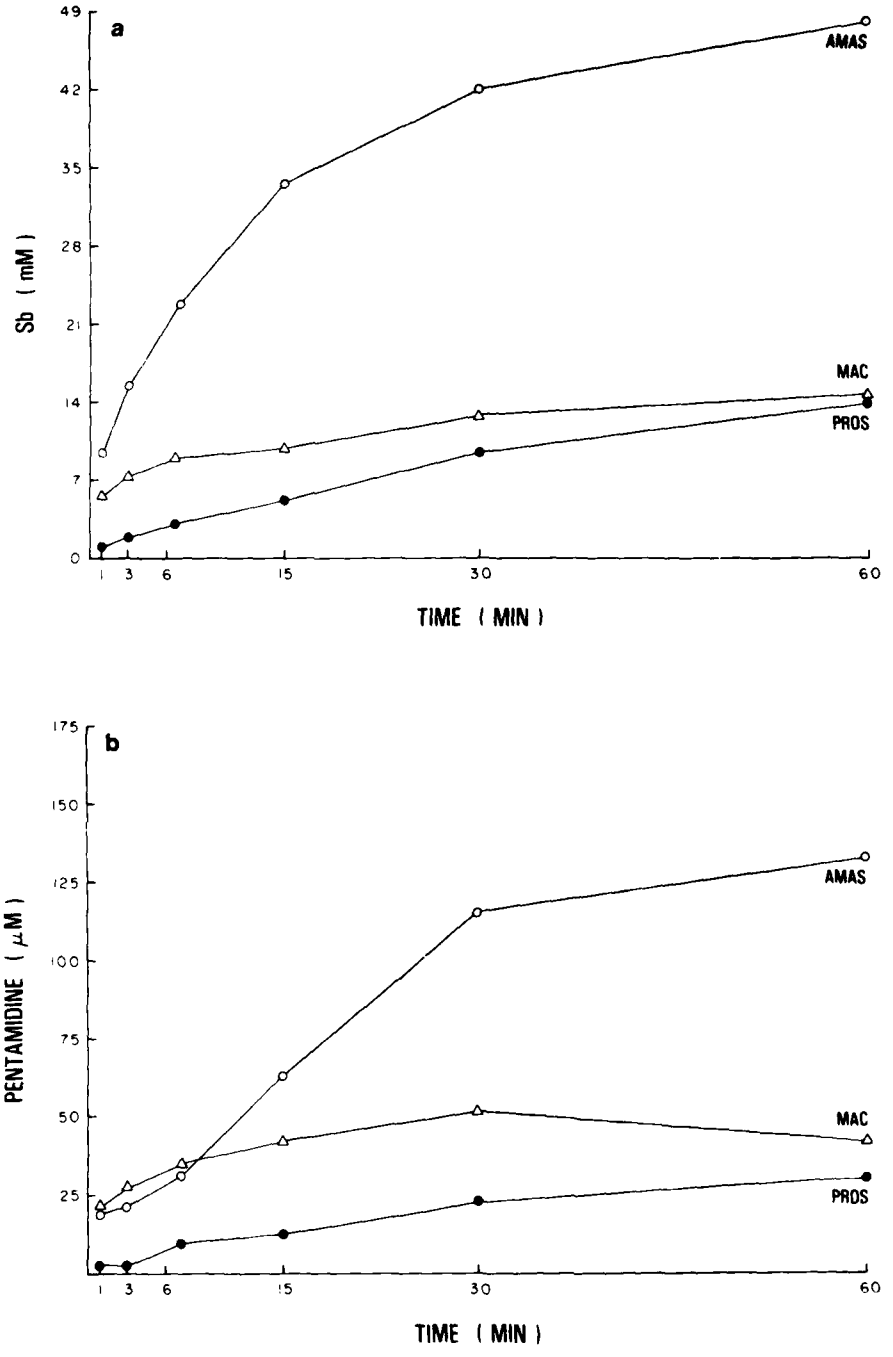

FIG. 1. Uptake of radiolabeled drugs by *Leishmania mexicana* amastigotes (AMAS), *L. mexicana* promastigotes (PROS), and J774 macrophages (MAC) over 60 min. (a) Uptake of [^{125}Sb]Pentostam. (b) Uptake of [^{3}H]pentamidine.

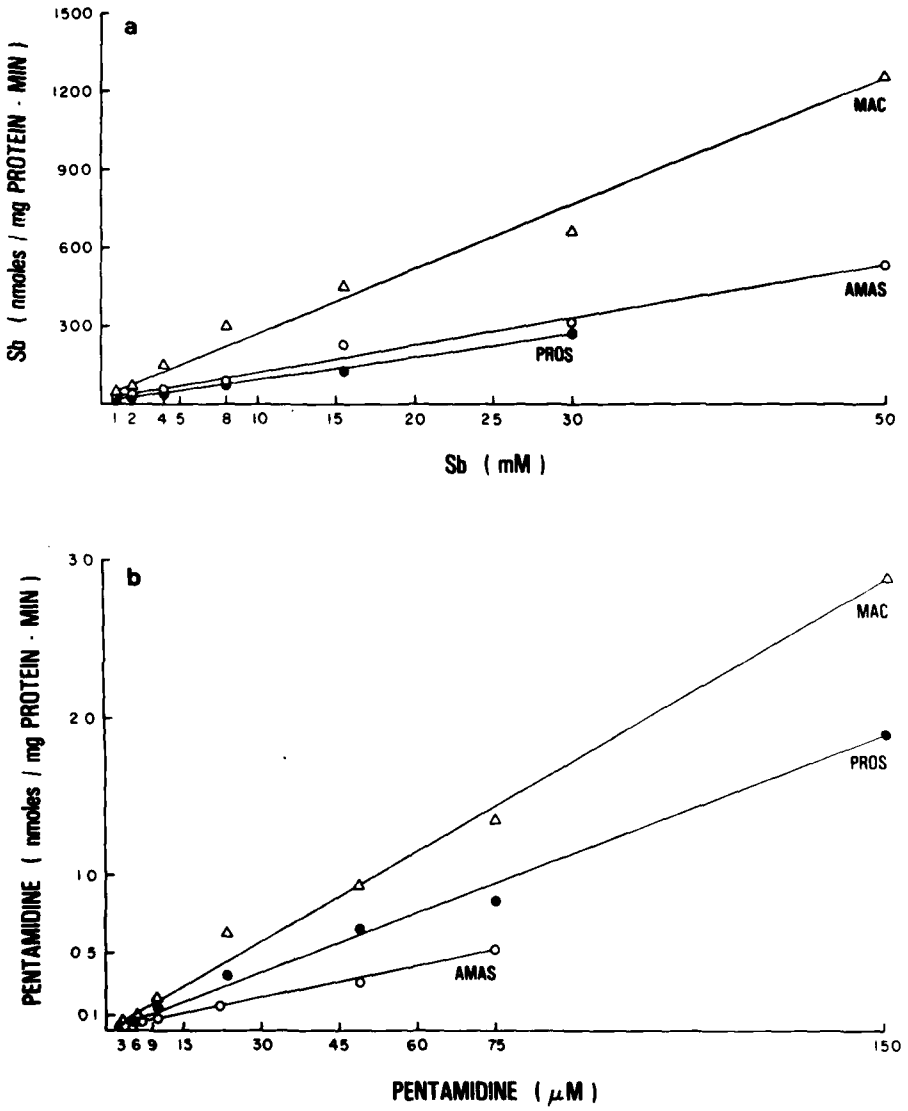

FIG. 2. Uptake of radiolabeled drugs by *Leishmania mexicana* amastigotes (AMAS) and promastigotes (PROS) and by J774 macrophages (MAC), versus concentration. (a) Uptake of [^{125}Sb]Pentostam. (b) Uptake of [^{3}H]pentamidine.

amastigotes to concentrate pentamidine suggest a mechanism that may contribute to the toxicity of these drugs to amastigotes compared to promastigotes and mammalian cells.

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