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LEISHMANICIDAL ACTIVITY OF AMINO ACID AND PEPTIDE ESTERS: AN UPDATE

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Amino acid esters, such as L-Leucine methyl ester (Leu-OMe), destroy intracellular and isolated *L. mexicana amazonensis* amastigotes by a mechanism which appears to involve trapping of the compounds and their enzymatic hydrolysis within acidified, lysosome-like parasite compartments. (1-4). As in the disruption of lysosomes by the compounds damage most likely results from rapid accumulation of more polar amino acid within the organelles. The requirement for enzymatic hydrolysis is supported by three main observations: a) among the few tritiated compounds examined, leishmanicidal esters were rapidly hydrolyzed by amastigotes, whereas inactive esters were not (5); b) isolated amastigotes preincubated with antipain or chymostatin, peptides which inhibit cysteine- and serine-proteinases, were protected from Leu-OMe toxicity (6); c) antipain reduced the hydrolysis of ^{3}H-Leu-OMe by the parasites (6).

The present communication will review progress made in the following areas: 1) Nature of the organelles and enzymes involved in the activity of the paradigm compound Leu-OMe. 2) Preliminary structure/activity correlations with dipeptide and Z-dipeptide esters. 3) Parasite destruction and reduced growth of mouse lesions injected with Leu-OMe.

1) Organelles and enzymes. Amastigotes of the mexicana species contain organelles, termed "megasomes", which display lysosomal enzyme markers and appear to contain a 31 kDa cysteine proteinase strongly inhibited by antipain and leupeptin (cf 7). Nearly 3 years ago, G.H. Coombs (Glasgow) proposed that megasomes are the amastigote targets of amino acid esters. This hypothesis gained support from two kinds of observations. First, it was directly shown that megasomes are acidified (8). Second, light and electron microscopic studies of amastigotes exposed for short time periods to sublethal concentrations of Leu-OMe, uncovered selective swelling and other morphological changes in the organelles. Such changes were inhibited by preincubation of the parasites with antipain (J.C. Antoine et al, submitted).

The approach chosen to determine the nature of the enzyme(s) responsible for ester hydrolysis, was to correlate, in parallel experiments, the protection of living parasites by class specific enzyme inhibitors from damage by Leu-OMe, with the selective inhibition of enzyme activities in lysates of the treated parasites. Valuable information was obtained with the peptidyl diazomethane Z-Phe-AlaCHN_2, a specific and irreversible cysteine proteinase inhibitor. Incubation of L. amazonensis amastigotes with small concentrations of the compound, not only prevented the killing of the parasites by

Leu-OMe, but selectively inhibited the major, low molecular weight (25-33 kDa) cysteine proteinase activity detected in gelatin-containing acrylamide gels (Alfieri, Shaw, Zilberfarb and Rabinovitch, submitted). These results will be presented by S.C. Alfieri in the course of this meeting.

The cysteine proteinase of L. amazonensis amastigotes is probably analogous to the 31 kDa proteinase purified from L. m. mexicana amastigotes (cf 7). A related, if not identical enzyme, has been purified to homogeneity from L. pifanoi axenic amastigote cultures by L. Rivas and D. MacMahon-Pratt (personal communication). Clarification of the subcellular distribution of the proteinase(s) will require the development of as yet unavailable methods for fractionation of amastigotes and isolation of megasome-rich fractions. It is expected that the purified enzyme(s) will be useful for the design of more active and selective leishmanicidal derivatives.

2) Preliminary structure activity correlations. Earlier studies indicated that the most active of the amino acid esters assayed on isolated amastigotes was that of Leu, followed by those of Trp, Met, Phe and Tyr. The ED_{50} ranged from 0.6 mM for Leu-OMe to 3.8 mM for Tyr-OMe. Esters of other amino acids (Ile, Val, Ala, Gly, Ser, His, Pro) were inactive. Thus, although most active esters contained hydrophobic amino acids, hydrophobicity alone could not account for the differences in parasite killing. The association of ester hydrolysis with leishmanicidal activity indicated that such differences should also reflect ester recognition by parasite enzymes (5). As previously shown in studies of ester toxicity for NK cells (9), dipeptide esters were more active than amino acid esters (e.g. the ED_{50} for Leu-Leu-OMe was 0.12 mM).

Leishmanicidal dipeptide esters contained at least one hydrophobic amino acid - Leu, Ile, Val, Phe or Trp. Interestingly, the most active was Val-Val-OMe, with an ED_{50} of 33 μM. The order of the amino acids was also important. For instance, the ED_{50} of Leu-Ala-OMe and Ala-Leu-OMe for amastigotes were, respectively, 0.4 and 2.2 mM. Finally, it was recently shown that t-butoxycarbonyl (BOC) or benzyloxycarbonyl (Z) N-derivatization markedly enhanced the leishmanicidal activity of dipeptide esters. Thus, the ED_{50} of BOC-Trp-Leu-OMe and Z-Val-Phe-OMe for isolated amastigotes were below 10 μM. This indicates that the weak base behavior conferred by a free amino group, is not essential for parasite destruction. It remains to be determined to what extent leishmanicidal activities of the compounds reflect a) rates of permeation into amastigote megasomes; b) rates of hydrolysis by the parasite proteinase(s); c) rates of amino acid or dipeptide efflux from the megasomes; and/or, d) toxicity of amino acid, dipeptides or their derivatives for megasomal membranes.

3) Parasite destruction and reduction in the growth of mouse lesions injected with L-Leu-OMe. In these experiments, BALB/c or C57Bl/6 mice were infected in one or both hind foot pads with *L. amazonensis* amastigotes. The ester in Hanks-MOPS, or diluent alone, were injected about 3 weeks later, when lesion growth was confirmed by caliper measurements. Injection of 50 μl of 100 to 400 mM Leu-OMe was followed by swelling which subsided within 4 days. In permissive BALB/c mice, lesion size remained nearly constant for 10-20 days after a single injection of Leu-OMe. Thereafter it resumed growth at rates similar to those of control paws. The difference in size of treated and control paws was still detectable 6 weeks after

Leu-OMe administration. Furthermore, fewer amastigotes were be recovered from treated than from control lesions. In the less permissive C57Bl/6 mice treated lesions remained constant in size for periods of 4 or more weeks. Pending other information, it is proposed that reduction in the growth of lesions resulted from killing of both parasites and host cells, as well as from the recruitment of other effector cells such as granulocytes. Studies in progress involve the intralesional administration of esters with selected lymphokines, monokines or immuno adjuvants.

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