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Inhibition of intraerythrocytic development of *Plasmodium falciparum* by proteinase inhibitors

K.A. Rockett, J.H.L. Playfair, F. Ashall[+], G.A.T. Targett[°], H. Angliker* and E. Shaw*

Dept. of Immunology, University College and Middlesex Hospital Medical School, 40-50 Tottenham Street, London W1P 9PG, [+]Dept. of Pure and Applied Biology, Imperial College of Science, Technology and Medicine, Prince Consort Road, South Kensington, London SW7 2BB, [°]Dept. of Medical Parasitology, London School of Hygiene and Tropical Medicine, Keppel Street, London, WC1E 7HT, England and **Friedrich Miescher-Institut, Postfach 2543, CH-4002 Basel, Switzerland*

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A group of inactivators of cysteinyl proteinases which function by covalent bond formation have been examined for their ability to inhibit the development of *Plasmodium falciparum* within red blood cells. The most effective of these caused inactivation of the parasite near 10^{-8} M concentration. The range of inhibitory action varied with peptide structure in a manner characteristic of affinity labels for proteinases suggesting that the target of inhibition was an unidentified proteinase, probably of the cysteinyl type, but different from cathepsins B and L.

Plasmodial inhibition; Antimalarial; Proteinase inhibitor

1. INTRODUCTION

The study of plasmodial proteinases is currently under investigation in connection with various features of the life-cycle of the parasite [1-5] including erythrocyte invasion [1], intra-erythrocytic events such as hemoglobin digestion [2,4,5] and merozoite maturation [3]. The use of specific inhibitors to obtain evidence for the physiological role of these proteinases and conceivably to lead to therapeutic applications is a logical extension of these studies. The success of this approach will depend on the specificity of the inhibitors since host cells employ similar proteinases for useful functions. Considerable progress has been made in the development of inactivators that function by affinity labelling, using peptide sequences which satisfy the specificity of a target proteinase but are also capable of forming a covalent bond at the active center. Peptide derivatives that irreversibly inactivate serine and cysteinyl proteinases include peptidyl chloromethanes [6], fluoromethanes [7-13], diazomethyl ketones [14-18] and peptidyl methyl sulfonium salts [19,20]. Variation in the peptidyl portion may provide selectivity of inactivation among closely related proteinases. This may be difficult to achieve within a common mechanistic family, but exploration of inhibitor structures has revealed useful enzyme differences, including topographical variations [17,18].

Correspondence address: K.A. Rockett, Dept of Immunology, University College and Middlesex Hospital Medical School, 40-50 Tottenham Street, London W1P 9PG, England

We found previously that certain peptidylfluoromethanes, and in particular, one containing the novel amino acid imidazolylnorvaline, ImNva [12] are able to lyse the infectious but not the non-infectious forms of African and South American trypanosomes in vitro (Ashall, Angliker and Shaw, in preparation). We subsequently examined a group of proteinase inhibitors of the peptidyl fluoromethane, diazomethane, and peptidylmethyl sulfonium salt classes for a possible effect on the intraerythrocytic development of the human malarial parasite, *Plasmodium falciparum*, with the results described below.

2. MATERIALS AND METHODS

Cultures of human A+ erythrocytes at a haematocrit of 5% in a standard culture system [21] were infected with *P. falciparum* strain

Table 1

Effects of proteinase inhibitors on intraerythrocytic development of *P. falciparum*

Proteinase inhibitor	Source	Concentration producing 50% inhibition of growth (nM)
Cbz-Leu-TyrCH_2F	11	59
Cbz-Phe-ImNvaCH_2F	12	80
Cbz-Tyr-AlaCH_2F	★	1390
Cbz-Phe-AlaCH_2F	7	3380
Ala-Phe-LysCH_2F	8	4940
Cbz-Ala-PheCH_2F	10	15670
Cbz-Phe-AlaCHN_2	14	8790
Cbz-Phe-Ala$CH_2S^+(CH_3)_2$	19	1950
Cbz-Phe-Arg$CH_2S^+(CH_3)_2$	20	21080

★ In preparation

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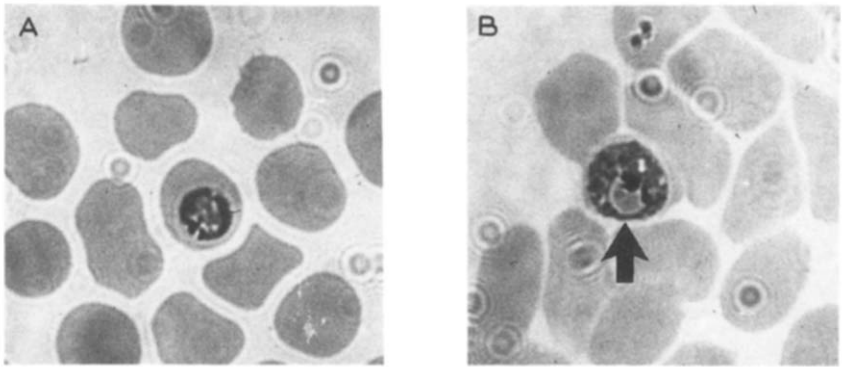

Fig.1. Effect of Cbz-Phe-ImNva-CH_2F on the morphology of intraerythrocytic *P. falciparum*. A, control culture; B, culture after treatment with 40 nM of the inhibitor for 4 h. An enlarged food vacuole in the trophozoite is arrowed.

NF54. Parasites were synchronized using 5% sorbitol [22] and cultured for an additional one or two cycles. When parasites reached the ring stage, culture volumes were adjusted to a parasitaemia of 0.5–1% at a haematocrit of 5%, then incubated with proteinase inhibitors at 37°C in an atmosphere of 5% CO_2/5% O_2/90% N_2. After 24 h, tritiated hypoxanthine (4 μCi/ml) was added to the cultures and after an additional 24 h, erythrocytes were harvested and incorporation of radioactivity was determined and used as an estimate of the rate of parasite multiplication [23].

3. RESULTS AND DISCUSSION

Nine synthetic proteinase inhibitors were examined, chiefly peptidyl fluoromethanes, but two other types were also included, a diazomethane and a pair of sulfonium salts. In the P_1 position [24] both neutral and positively charged amino acids were included since the specificity of proteolysis is often directed to this residue. Two of the inhibitors were particularly effective in inhibiting development of intraerythrocytic *P. falciparum* (the first two in table 1). The concentrations required to produce 50% inhibition of parasite division were similar to those of chloroquine, a widely used antimalarial drug (which for this strain has an IC_{50} of 1.28×10^{-8} M).

Morphology changes accompanying the inhibition of intraerythrocytic development of *P. falciparum* included enlargement of food vacuoles within the parasite (fig.1). It may be that the proteinase inhibitors produce these effects by inhibiting digestive proteinases within parasite food vacuoles. Independent observations made with one of the less effective inhibitors of the present study, Cbz-Phe-AlaCH_2F, led to a similar interpretation of its action [5]. However, in that study it was concluded that the proteinase affected may be cathepsin-L like. In fact, such a possibility would suggest a lack of selective toxicity.

An important characteristic of affinity labelling reagents such as those in table 1 is the influence of peptide sequence. With an unknown proteinase, the variation in susceptibility to inhibitors of different sequences provides information about the specificity of the proteinase, analogous to examining a group of synthetic substrates. The results in table 1 suggest that the most susceptible plasmodial proteinase is not cathepsin L-like, although they may belong to the same gene family, since cathepsin L is 10 times more susceptible to an inhibitor containing the Cbz-Phe-Ala- sequence than one with Cbz-Leu-Tyr- [18], whereas in table 1 the fluoromethane with the latter sequence is more than 50 times more effective, indicating quite a different binding site. This is promising with respect to developing inhibitors selective for an important plasmodial proteinase but without action on host cell function, thereby permitting treatment of human malarial infections in vivo. While it is not yet certain that the inhibition we describe is due to inactivation of a proteinase, it is very likely, since the fluoromethane group is unreactive and forms covalent bonds largely as a result of complex formation with a target enzyme [8]. The use of such reagents in radioactive form should permit the identification of the inactivated protease and characterization of its specificity in the usual manner.

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