PN-ABM-447

AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D.C. 20523

DATE: 6/13/88

MEMORANDUM

TO: AID/PPC/CDIE/DI, room 209 SA-18

FROM: AID/SCI, Victoria Ose VO

SUBJECT: Transmittal of AID/SCI Progress Report(s)

Attached for permanent retention/proper disposition is the following:

AID/SCI Progress Report No. C7-171

Doc. Report - Aug 87 - Feb 88

Attachment

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US-ISRAEL CDR PROGRAM Project # C7-171

MODULATION OF MEMBRANE TRANSPORT: A BIOCHEMICAL APPROACH TO THE CHEMOTHERAPY OF MALARIA

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Scientific Report: August 87-February 88

The continuing effort of the present research program is the search for new approaches to the chemotherapy of malaria. The general aim of the project is the delineation of functional and structurally-associated alterations that the host membrane undergoes as a result of intraerythrocytic growth of Plasmodium falciparum . The immediate aim is to assess the functional role played by permeation pathways induced in red cell membranes by malaria parasites , define the pathways both in physiological, biochemical and especially immunochemical and pharmacological terms , develop chemical and immunochemical agents for the specific blockade of the pathways and for their chemical identification and assess their potential application as antimalarial agents or evaluate their membrane targets also as

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potential targets for a malaria vaccine.

In the period covered by this report we focused on three major subjects related to the above mentioned areas fo research. First, we shall deal with them separately and subsequently, we shall describe how all will be joined so as to materialize some of the goals we set out to accomplish.

A. Development of specific blockers for permeation pathways induced in erythrocyte membranes by intracellular malaria parasites

In the first phase of this project we searched for potential covalent modifiers of the new pathways, with the aim of defining particular amino acid groups that might involved in the protein associated function (Breuer et al. 1987). Out of a very long list of agents covering a broad spectrum of specificities , we came out with an amino specific agent which blocked the pathways at relatively low concentrations but at relatively high pH > 8.5. The covalent modifier 4,4'-diisothiocyano-2,2'-stilbene disulfonate, was used also as a labelling agent (in radioactive form) in conditions whereby the band 3 polypeptides were protected (i.e. , in the presence of excess disulfonic stilbenes (non-covalent analogs). The site of action of these otherwise impermeant agents was on membrane components facing the inner

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surface of the host cell membrane, thus demanding penetration of the agents into the cells for subsequent blocking. The proteins labelled by the modifier in the most specific conditions were of 120 Kd, 63 Kd and 51 Kd. The proteins were labelled from the trophozoite stage and onward, in parallel with the appearence of the transport pathways. The chemical groups which are targets of the NCS-stilbene disulfonates are lys groups associated with parasite derived proteins.

Recognition of the limits of tha above approach forced us the pursuit for an affinity label of t
sun the last two semesters of the project we completed the phase of screening a variety of bioflavonoids as potential blockers of the pathways and the phase of assessing their potential antimalarial activity . Based on our mechanistic definition of phlorizin's mode of action (Kutner et al., 1987), we designed structural analogs of phlorizin and established their structure-activity relationship (Silfen et al., 1988). We selected the 4-isothiocyano derivative of phlorizin as a potential affinity label of the permeation system on the basis of its relatively high blocking capacity, lack of interference with other permeation systems and the fact that phlorizin protects the putative permeation system from covalent modification by this agent. We have recently raised an anti-phlorizin antibody and are presently engaged in the identification of the putative targets of phlorizin-NCS action on the host membrane of parasitized

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cells. (This route of identification was chosen because of the relatively small number of phlorizin binding sites found per parasitized cell and because of the possibility of using these immunochemicals as tools for the chemical isolation of the drug [modified] targets). An additional aspect which resulted from the SAR study was the discovery of a new series of natural agents (phlorizin-like) with demonstrable in vitro antimalarial activity, of which cosmetin (from parsley leaves) is the best representative. Unlike phlorizin, those agents have no inhibitory effect on the Na-glucose cotransporter of mammalian epithelial cells, thus opening the road for their in vivo use in animal studies.

In parallel to the biopharmacological studies, we continued our search for malaria immune sera with pore-blocking capacity and develop for that purpose functional assays sensitive down to the ul cell level. As most of the sera used (from Thailand and Gambia) were immunochemically (by RIA) surface unreactive with cells parasitized with our P. falciparum strains (an expected result from the known antigenic diversity of surface antigens), we attempted and succeeded to expose cryptic epitopes by modulating the cholesterol (CHS) contents of the membrane (Baruch and Cabantchik, 1988). Present efforts are focused on the development of immunochemical probes for the putative pore components using human immune sera, rabbit sera and monoclonal antibodies developed against membranes from CHS enriched malaria parasitized cells to be selected for their pore

blocking capacity.

Publications

Kutner,S., Breuer,V.W., Ginsburg,H. and Cabantchik,Z.I. (1986). On the mode of action of phlorizin as an antimalarial agent in *in vitro* cultures of *Plasmodium falciparum*. Biochem. Pharmacol. 36: 123-129.

Breuer,V.W., Kutner,S., Silfen,J., Ginsburg, H. and Cabantchik,Z.I. (1987). Covalent modification of the permeability pathways induced in the human erythrocyte membrane by the malarial parasite *Plasmodium falciparum*. J. Cell. Physiol. 133: 55-63.

Silfen,J., Yanai, P. and Cabantchik, Z.I. (1988). Bioflavonoid effects on *in vitro* cultures of *Plasmodium falciparum*. Inhibition of permeation pathways induced in the host cell membrane by the intraerythrocytic parasite. Biochem. Pharmacol. (submitted).

Baruch,D. and Cabantchik,Z.I. (1988). Passive modulation of antigenic expression in the surface of normal and malaria infected erythrocytes. J. Exp. Med. (submitted)