Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Vol. *87*, Suppl. III, 263-264, 1992

IN VITRO MUTAGENESIS DEFINES DRUG TARGETS IN ALDOLASE OF *PLASMODIUM FALCIPARUM*

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Multiple drug resistance in *Plasmodium falciparum* is currently a major obstacle for the drug based eradication of malaria. Development of drug resistance appears to be due to continuous drug application in malaria affected resorts which leads to genetic selection of resistant parasites. One can envisage two different types of resistance: either the drug target, which is frequently only poorly characterized or even unknown has undergone mutagenesis or a drug export machinery has been activated. Examples are dihydrofolate reductase and the overexpression of the multi-drug resistance genes, respectively. The number of parasites in a individual with a low parasitemia of 0.01% is approximately one billion. Given a natural mutation rate of 2×10^{-4} one can calculate that approximately 10^5 naturally mutated parasite circulate. However, mutations in essential genes would created non-viable parasites which are not further transmitted.

One such essential gene in *P. falciparum* is aldolase. It is highly conserved in all parasite isolates studied and *in vitro* mutagenesis has revealed that its product is highly sensible to subtle changes (mutations) in the primary sequence. Single amino acid insertions or deletions at random locations in the primary sequence, for example, lead to insoluble and consequently non-functional enzyme. Other point mutations have a marked influence on the enzyme kinetics. These findings suggest, that aldolase mutants are unlikely to appear under drug selection.

One drug target in *P. falciparum* aldolase are two lysine residues near the carboxy terminus which are not found in any other known aldolase including the human isoenzymes A, B or C. It was thus appealing to explore if these residues are required for *P. falciparum* aldolase function. It is important to note, that introduction of two lysines residues could simply reflect the tendency of *P. falciparum* to increase the A/T content of the genome: The most common codon for lysine is AAA.

Plasmodium falciparum aldolase is well expressed as an active enzyme in *Escherichia coli*. We could thus use *in vitro* mutagenesis in order to exploit a possible function of these parasite specific lysine residues. We decided to mutate the lysine residues to either asparagine or serine, because these amino acids occur at the equivalent position in vertebrate aldolase A. Surprisingly, both mutations have a drastic affect on the enzyme activity, substrate binding and inhibition by antibodies.

The enzyme activity or turnover speed, expressed as K_{cat}, shifted for both mutations virtually to the levels of the faster human enzyme. The substrate affinity, expressed as K_m, was clearly reduced probably due to the removal of two positive charges which may support binding of the polar substrate fructose-1,6-diphosphate (FDP).

We have reported earlier, that erythrocyte band III protein derived peptides are efficient inhibitors of both rabbit muscle or *P. falciparum* aldolase. However, inhibition of *P. falciparum* occurs at lower peptide concentrations suggesting a more specific interaction. The lysine residues appear to be involved in this process. For both mutations (see above) the specificity of inhibition is lost and the peptide concentrations needed for inhibition resemble those needed for the inhibition of rabbit muscle aldolase.

The data above strongly suggest, that the lysine residues mutated are important for the biological function of *P. falciparum* aldolase. It was therefore possible that they are part of the epitope recognized by polyclonal antibodies which completely and specifically inhibit the enzyme activity. We thus compared the

antibody inhibition of the mutant, wild type and rabbit muscle activity. As expected, rabbit muscle activity was not inhibited and the *P. falciparum* wild type and mutant inhibition was similar although the inhibition of the mutants was somewhat less efficient.

The described specific inhibition of *P. falciparum* aldolase at different levels shows that it is basically possible to find inhibitory drugs which are specific for the parasite enzyme. Those aldolase inhibitors could be designed at the molecular to specifically interact with the parasite specific, essential lysine residues. For modelling of such a drug we are currently exploring the three dimensional structure of *P. falciparum* aldolase.

NOTE

We have written his article solely for the proceedings of the meeting and we have therefore omitted detailed data and references in order to avoid complications with publication of the data as original articles. Details are however available on requests.