SIgN Immunology Seminar

Prof Richard K. Haynes

Department of Chemistry, The Hong Kong University of Science and Technology & Unit for Drug Research and Development, North-West University, South Africa

Artemisinins – Intracellular Oxidants par Excellence: Targeting Reduced Flavin Cofactors of Malaria Parasite Enzymes

Host
Dr Laurent Renia
Singapore Immunology Network, A*Star

Date
Monday,
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Time
4pm – 5pm

Venue
SIgN Seminar Room,
Immunos Building
Level 4
Biopolis

Artemisininsin conjunction with longer half-life quinoline and arylmethanol anti-malarial drugs currently represent the most effective means of treating malaria. However, in spite of intensive investigations, the mechanism of action of artemisinins has remained controversial. Antimalarial activity is ascribed to the intercession of C-radicals derived by reaction of artemisinins with ferrous iron, either in heme, or as a kinetically free species, within the digestive vacuole of the malaria parasite. The C-radicals are presumed to alkylate 'vital biomolecules', a deposition that thus far remains unverified.

Artemisinins synergize the action of the antimalarial drug methylene blue (MB) and other redox active drugssuch as naphthoquinones. MB for example is reduced in an intracellular environment to leucomethylene blue (LMB) by the NADPH-flavoenzyme disulfide reductases glutathione reductase (GR), thioredoxin reductase (TrxR) and lipoamide dehydrogenase, or by the NAD(P)H-flavin oxido-reductase flavin reductase (Fre). The LMB is then oxidized by oxygen to MB with generation of cytotoxic reactive oxygen species (ROS).Recycling of the MB results in enhanced consumption of NADPH required for normal functioning of the reductases. Thus, the basis for synergism between artemisinins and MB is consistent with our hypothesisthat artemisinins enhance redox cycling of MB and the other redox active drugs within the malaria parasite by rapidly oxidizing their reduced conjugates. Production of NADPH via the rate-limiting G6PD pathway is thereby unable to meet the requirements of the flavin disulfide reductases in controlling the build-up of ROS.

By using NADPH-Fre to generate LMB, we show that the latter is indeed rapidly oxidized by artemisinins and other antimalarial peroxides to MB. Further, the dihydroflavin conjugates of lumiflavine, riboflavin (RF) and crucially, the flavin cofactor FAD of the flavin disulfide reductases generated with NAD(P)H-Fre are also rapidly oxidized to the parent flavins. These and other findings confirm the idea that artemisinins potently exacerbate oxidative stress within the parasite. Our results are consistent with the known ability of artemisinins to modulate oxidative stress and are not dependent upon 'activation' by ferrous iron to generate the dreaded 'bioactive' C-centred radical. Further, we show that the destruction of reduced flavinco factors by artemisinins is consistent with the known antagonism to antimalarial activities *in vitro* between quinolines and artemisinins, and the additivity-synergism between artemisinins and arylmethanols such as quinine and lumefantrine.

Overall, the proper understanding of mechanism must enable rational application of artemisinins not just for treatment of malaria, but also to other pathogens that rely on suppression of oxidative stress for survival.