FCT Fundação para a Ciência e a Tecnologia
MINISTÉRIO DA CIÊNCIA, TECNOLOGIA E ENSINO SUPERIOR

PTDC/SAU-MII/099118/2008

The influence of bile acids on malaria liver infection

Malaria is one of the most prevalent infectious diseases worldwide. It is caused by a protozoan parasite from the genus Plasmodium and is transmitted through the bite of the female Anopheles mosquito. In order to reach the bloodstream in a form capable of causing disease symptoms, the parasite first undergoes an obligate and clinically silent developmental phase in the liver. Whereas important gaps subsist in our understanding of some fundamental processes that occur during the hepatic stage of infection, it is becoming increasingly apparent that, during their replication in the liver, Plasmodium parasites engage or subvert host cell resources in order to fulfil their developmental needs (1-3). Consequently, host factors are crucial determinants of the outcome of Plasmodium infection and, therefore constitute appealing targets for anti-malarial strategies.
Preliminary findings from our laboratory demonstrated that bile acids (BAs), strongly influence Plasmodium hepatic infection in vitro. The data unequivocally showed that several of the BAs tested markedly and reproducibly impaired the parasite's development inside human hepatoma cells. Recently, BAs have also been implicated in infection by the protozoa Cryptosporidium spp. (4) and by hepatitis C virus (5). Thus, the present proposal is aimed at UNDERSTANDING HOW BILE ACIDS INFLUENCE PLASMODIUM INFECTION IN THE LIVER AND INVESTIGATING THEIR POTENTIAL AS TARGETS FOR ANTI-MALARIAL INTERVENTION.
BAs are synthesized in hepatocytes and have recently been recognised as versatile signalling molecules endowed with systemic endocrine functions, emerging as important therapeutic targets for metabolic diseases (6,7). BAs signal through specific pathways to regulate not only their own synthesis, but also triglyceride, cholesterol, energy and glucose homeostasis. Two major BA-mediated signalling mechanisms have been described, arising from the fact that BAs are ligands for both the TGR-5 G-protein coupled receptor (GPCR), and nuclear hormone receptors, such as FXR, whose activation has been implicated in a number of important biological processes (8-12). Thus, we will investigate whether and how BA-mediated signalling pathways influence Plasmodium infection of the liver and unveil their potential as anti-malarial targets.
The synthesis of BAs accounts for the majority of cholesterol breakdown in the body. Moreover, BAs participate in cholesterol metabolism by functioning as hormones to repress the transcription of the rate-limiting enzyme in cholesterol biosynthesis. Importantly, cholesterol plays a crucial role during Plasmodium hepatic development presumably as a result of the outstanding parasite replication rate that occurs during this phase. Furthermore, we recently showed that the host scavenger receptor class B type I (SR-BI), which mediates cholesterol uptake by hepatocytes, plays a crucial role in the intracellular development of the Plasmodium parasite (13). Importantly, BAs have also been implicated in the modulation of SRBI expression (14). Thus, we will elucidate whether the regulation of cholesterol homeostasis by BAs contributes to their observed effect on Plasmodium development in the liver and clarify whether the demonstrated implication of SR-BI in Plasmodium development is related to BA metabolism.
In addition to their crucial role in cholesterol homeostasis, BAs have been implicated in other cellular processes that take place in liver cells, most notably, apoptosis (15). Apoptosis can be used by the host cell as a defence mechanism against intracellular pathogens. Conversely, as we previously demonstrated, inhibition of host cell apoptosis is advantageous for a pathogen such as Plasmodium to complete its development inside hepatocytes (16). We will therefore investigate whether BA-mediated (anti-) apoptotic effects contribute towards the marked effects that BAs exert on Plasmodium infection.
The proposed investigation of the mechanism(s) by which BAs interfere with Plasmodium development will be achieved by undertaking parallel state-of-the-art in vitro, ex vivo and in vivo approaches. Agonists, antagonists, immunofluorescence microscopy and RNA-interference (RNAi-) mediated knock-down of specific genes will be employed to dissect the mechanisms and pathways through which BAs influence infection both in vitro (hepatoma cell lines) and ex vivo (rodent primary hepatocytes). In vivo studies will be carried out in rodent models of malaria and will include state-of-the-art in vivo RNAi delivery techniques and, when appropriate, the use of transgenic lines of relevant knock-out mice.
Given the increasing relevance of BAs as clinical targets, unravelling their mechanisms of action during malaria infection holds immense importance from the points of view of both understanding fundamental aspects of host-pathogen interactions and unveiling novel anti-malarial targets.