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Review

Understanding malarial toxins

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ABSTRACT

Recognized since antiquity, malaria is one of the most infamous and widespread infectious diseases in humans and, although the death rate during the last century has been diminishing, it still accounts for more than a half million deaths annually. It is caused by the *Plasmodium* parasite and typical symptoms include fever, shivering, headache, diaphoresis and nausea, all resulting from an excessive inflammatory response induced by malarial toxins released into the victim's bloodstream. These toxins are hemozoin and glycosylphosphatidylinositols. The former is the final product of the parasite's detoxification of haeme, a by-product of haemoglobin catabolism, while the latter anchor proteins to the *Plasmodium* cell surface or occur as free molecules. Currently, only two groups of antimalarial toxin drugs exist on the market, quinolines and artemisinins. As we describe, they both target biosynthesis of hemozoin. Other substances, currently in various phases of clinical trials, are directed towards biosynthesis of glycosylphosphatidylinositol, formation of hemozoin, or attenuation of the inflammatory response of the patient. Among the innovative approaches to alleviating the effects of malarial toxins, is the development of antimalarial toxin vaccines. In this review the most important lessons learned from the use of treatments directed against the action of malarial toxins in antimalarial therapy are emphasized and the most relevant and promising directions for future research in obtaining novel antimalarial agents acting on malarial toxins are discussed.

Contents

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1. Introduction

1.1. World health impact of malaria

Malaria has been recognized a world health problem since antique. Until the mid-19th century, malaria was endemic in most countries, with an estimated 90% of the world's population living in malarious areas (Mendis et al., 2009). Efforts to eradicate malaria began in 1955 when the World Health Organization (WHO), encouraged by the early success of using the insecticide dichlorodiphenyl-trichloroethane (DDT) to eliminate the mosquito responsible for spreading malaria (see Section 1.2), initiated the Global Malaria Eradication Program. Consequently, malaria was eliminated permanently from many regions. During the 1970s and 1980s, international support for malaria control declined because of economic and financial crises and the disease reappeared in some areas. It was also realized that many insecticides, including DDT, are highly toxic to the environment and therefore unsuitable for use. 2007 saw a renewed impetus to eradicating malaria culminating in the publication of the Malaria Eradication Research Agenda (malERA) (Nájera et al., 2011) which resulted in expanded financing and implementation of malaria control programmes (WHO, 2012). Nevertheless, the disease remains a serious health problem. According to the WHO, about 3.3 billion people globally are currently at risk of being infected with malaria. The most endangered population is the one inhabiting sub-Saharan Africa. In 2013, an estimated 198 million cases of malaria occurred worldwide leading to 584,000 deaths.

1.2. Malaria is caused by parasitic protozoans

Malaria is caused by parasitic protozoans belonging to the genus *Plasmodium*. The life cycle of the *Plasmodium* parasite, a single cell microorganism, consists of a sexual cycle that takes place in the female anopheline mosquito, and an asexual cycle that occurs in, for example, humans (Fig. 1).

Five species of the *Plasmodium* genus—*Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae* and *Plasmodium knowlesi* – are known to cause malaria in man (WHO, 2014). The deadliest form of the disease, which predominates in Africa, is caused by *P. falciparum*, whereas the malaria induced by *P. vivax* is less dangerous but more widespread. Other species of parasites occur less frequently (WHO, 2012).

Symptoms of malaria become apparent 7–9 days after the mosquito infectious bite and include fever, shivering, headache, diaphoresis, dizziness, nausea, repeated vomiting, generalized convulsions and coma (Trampuz et al., 2003; Rang and Dale, 2008). The periodic episodes of fever, characteristic of malaria, are the consequence of the cyclic rupture of the red blood cells and subsequent release of merozoites and cell debris (Rang and Dale, 2008). The most dangerous species, *P. falciparum*, causes high levels of parasitemia, severe anemia, cerebral symptoms, renal failure, pulmonary edema and death. The reason for the higher pathogenicity of *P. falciparum*, as compared to other *Plasmodium* species, lies in its ability to infect red blood cells of any age, resulting in a high parasite burden and profound anemia. It also causes the infected erythrocytes to clump together, forming rosettes, and to stick to endothelial cells lining the capillaries. In this way, blood flow is obstructed, resulting in the production of high levels of cytokines that are harmful to the patient (Robbins et al., 2009). Like *P. falciparum*, *P. knowlesi* can also cause high levels of parasitemia, resulting in a severe form of the disease. However, because of its similar morphology to the less dangerous *P. malariae*, it is often misdiagnosed and not treated appropriately, which can have fatal consequences (Kantele and Jokiranta, 2011). *P. vivax*, *P. ovale*, and *P. malariae* are able to infect only young or old red blood cells that constitute only a marginal fraction of the red blood cell pool. For this reason, the symptoms of malaria caused by these three species are less severe and are characterized by low levels of parasitemia, mild anemia, and, rarely, splenic rupture and nephrotic syndrome (Robbins et al., 2009).

2. Malarial toxins

According to the medical dictionary, a toxin is a substance produced or excreted by a microorganism, plant or animal that is poisonous to other organisms. Beside African trypanosomes, which are thought to contain endotoxins, parasitic protozoa are not known to produce toxins similar to classic bacterial toxins, such as anthrax and botulinum toxins. They do, however, produce certain low-molecular-mass molecules that are responsible for at least some aspects of their pathology and can be regarded as toxins (Seed, 1996). In malarial pathogenesis there are two types of such molecules, commonly termed as malarial toxins, hemozoin and *Plasmodium* glycosylphosphatidylinositols (PGPIs) (Boutlis et al., 2005).

2.1. Hemozoin

During the intra-erythrocytic cycle, *Plasmodium* is capable of very limited *de novo* amino acid synthesis. A major source of amino acids for the parasite is the erythrocyte's haemoglobin (Rosenthal, 2001). During the blood stage of malaria infection (Fig. 1), the parasite internalizes and degrades large amounts of haemoglobin (Elliott et al., 2008) that is degraded, in a food vacuole of the parasite, to haeme and globin peptides by *Plasmodium* peptidases.