Available online at www.sciencedirect.com
SCIENCE DIRECT
Electrochimica Acta 51 (2006) 5103–5111
ELECTROCHIMICA Acta
www.elsevier.com/locate/electacta

Molecular modeling of the voltammetric oxidation at a glassy carbon electrode of the antimalarial drug primaquine and its prodrugs succinylprimaquine and maleylprimaquine

Mauro A. La-Scalea [a,*], Carla M.S. Menezes [a,**], Guilherme C. Matsutami [a], Michelle C. Polli [a], Sílvia H.P. Serrano [b], Elizabeth I. Ferreira [a]

[a] *Lapen, Laboratório de Planejamento e Síntese de Quimioterápicos Potencialmente Ativos Contra Endemias Tropicais, Departamento de Farmácia, Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, Av. Prof. Lineu Prestes, 580 Bl. 13 sup., 05508-900 São Paulo, Brazil*
[b] *Departamento de Química Fundamental, Instituto de Química, Universidade de São Paulo, Av. Prof. Lineu Prestes, 748 Bl. 2 sup., 05508-90 São Paulo, Brazil*

Received 29 May 2005
Available online 11 May 2006

Abstract

The 8-aminoquinoline primaquine (PQ) is the only antimalarial drug used as tissue schizonticide and relapsing malaria. Antichagasic activity was also reported. Nevertheless, as it also shows serious side effects, prodrugs such as succinyl and maleyl derivatives have been proposed to decrease its toxicity. Although PQ mechanism of action has not been completely elucidated, the promotion of oxidative stress is an advanced hypothesis that could explain its activity in both plasmodia and trypanosome parasites. The oxidation of PQ and its prodrugs, maleylprimaquine (MPQ) and succinylprimaquine (SPQ), was studied by cyclic voltammetry using glassy carbon electrode. All compounds were oxidized in aqueous medium, with the charge transfer process being pH-dependent in acidic medium and pH-independent in a weak basic medium, being the neutral form more easily oxidized. This indicated that the protonation of the nitrogen atoms displays a determinant role in the voltammetric oxidation, being both prodrugs more easily oxidized than PQ protonated forms, in the order: SPQ < MPQ < PQ. For a better understanding of this behavior, a molecular modeling study was performed using the AM1 semi-empirical method from Spartan 04 for Linux (v.119, Wavefunction Inc.). The medium pH showed to be fundamental not only to the electronic density of the quinoline ring but also to the rearrangement of the nitrogen side chain. The electronic density of primaquine non-protonated quinoline ring is higher than that in its protonated and diprotonated species. Also, the use of prodrugs and the degree of saturation of the carriers (maleic or succinic acid) interfere with this feature. SPQ and MPQ have a slight increase in the quinoline electronic density in comparison to PQ. Nevertheless, the carrier in the side chain of SPQ is closer to the quinoline ring than it is in MPQ, which accounts for the higher electronic density in the former. The most significant effect occurs in the correspondent protonated forms of the nitrogen quinoline. The application of molecular modeling study associated to voltammetric techniques showed to be an important way to understand the redox mechanism of electroactive drugs. These results may be related to a biological activity and can be useful to future primaquine derivatives design.

Keywords: Primaquine; Primaquine prodrugs; Electrooxidation; Deprotonation; Molecular modeling

1. Introduction

The electrooxidation of primaquine diphosphate was studied by cyclic voltammetry using glassy carbon [1,2] and platinum [3] electrodes. The cyclic voltammogram of this drug showed only one irreversible anodic wave controlled by diffusion, involving two electrons in the rate-determining step of the electrode reaction [1]. Theoretical and experimental results indicated the existence of a relationship between the primaquine dissociation equilibrium and its electrooxidation process, and the quinoline ring displayed a fundamental role in both phenomena [1]. Moreover, the diprotonated form of primaquine was readily oxidized to a radical where the unpaired electron was delocalized over the

* Corresponding author. Tel.: +55 11 3091 3793.
** Corresponding author.
E-mail addresses: scalea@usp.br (M.A. La-Scalea), casmenezes@yahoo.com (C.M.S. Menezes).

0013-4686/$ – see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.electacta.2006.03.085

aromatic ring [2–4] and, working in alkaline medium, a cation radical was obtained as an intermediate in primaquine oxidation [2].

Primaquine is the only antimalarial drug used as tissue schizonticide and in relapsing malaria. It acts on latent or hipnozoite forms of *Plasmodium ovale* and *Plasmodium vivax*, in the liver of the host, and its combination with blood schizonticide can be completely curative [5]. On the other hand, primaquine shows serious side effects and its mechanism of action has not been totally elucidated [6–8]. One of the advanced hypotheses to explain the mechanism of action of primaquine involves free radicals formation, that would increase the oxidative stress in the parasite [4,9]. This oxidative action was based on the cycle primaquine redox, where some of its metabolites indicated the formation of hydrogen peroxide and the corresponding quinone-imine derivatives as the main products under physiologic conditions. Simultaneously, drug-derived radicals and hydroxyl radicals were detected [4,9].

Since the toxicity of primaquine could be related to the oxidation of NADPH [10], Thornaley et al. [11] concluded that this drug complex specifically with the cofactor, facilitates the electron transfer to oxygen generating free radicals. Bisby [12] studied the formation of this complex and the primaquine reduction involving one electron by pulse radiolysis. Augusto et al. [13] observed this same behavior with NADH and they detected free radicals during the enzymatic oxidation.

Primaquine showed also antichagasic activity, probably through oxidative stress and/or oxidative action of its metabolites [14]. Based on that and considering the inhibitory activity of nitrofurazone on trypanotione reductase, an enzyme found in *T. cruzi* but not in the host, mutual prodrugs of both drugs were synthesized either directly or by using a spacer group, such as succinyl, and dipeptides as well [15]. Therefore, trypanotione reductase would be inhibited by nitrofurazone and an increase in the oxidative stress provoked by primaquine could be much more effective, resulting in the death of the parasite. Primaquine peptide prodrugs, synthesized as intermediates of these mutual prodrugs, were also assayed and most were active in vitro in LLC-MK2 cell cultures infected with *T. cruzi* trypomastigotes [14]. Besides the improvement of the antichagasic activity, the molecular modification (defined as latentiation) of primaquine was proposed to decrease its toxicity.

The electrochemical methods have been quite useful in quantitative determination of drugs, as well as in the study of their mechanism of action [16]. This paper introduces a complementary approach to our previous work with primaquine [1], comparing voltammetric behavior of the drug with two of its prodrugs, maleyl (MPQ) and succinyl (SPQ) (Fig. 1), and determining the influence of pH medium in the potential values obtained by cyclic voltammetry. Furthermore, a molecular modeling was employed for explaining the structural and electronic differences as a consequence of the molecular modifications of primaquine and the deprotonation process of the studied compounds. In fact, molecular modeling has been a valuable tool for the proposal of useful models for studying structure features and forecasting their relationships with the biological activity [17]. The association between molecular modeling methods and voltammetric techniques has shown to be an important way to understand the redox mechanism of electroactive drugs and the results herein described may be extensive to biological activity and useful to future primaquine derivatives design.

2. Experimental

2.1. Chemicals

The stock solution (0.02 mol/l) of primaquine diphosphate (PQH_2^{2+}) (Itacá Laboratórios) and its prodrugs were prepared by direct dissolution in deionized water. The pH study was accomplished with universal buffer starting from the misture of phosphoric, acetic and boric with NaOH [18]. All solutions were prepared using analytical grade reagents from Merck and purified water from a Barnted Nanopure UV system.

2.2. Succinylprimaquine synthesis

Succinylprimaquine (SPQ) synthesis was carried out in methanol using 0.038 mol of primaquine diphosphate and tri-

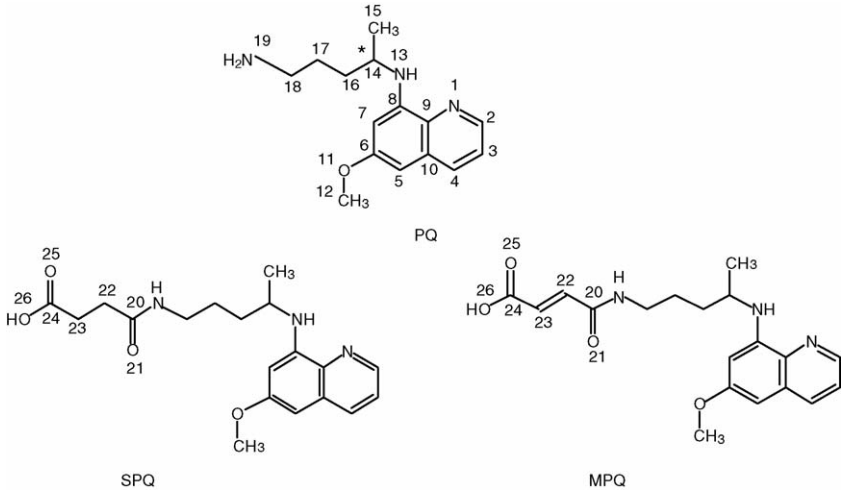

Fig. 1. Primaquine (PQ) and its prodrugs succinylprimaquine (SPQ) and maleylprimaquine (MPQ).