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Possibilities of fungal biodegradation of antimalarial and anticancer primaquine in the environment

Igor A. Parshikov[1] and Evgeny I. Zaraisky[1]

[1]Institute of Applied Mechanics, RAS, Moscow, Russia 125040

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ABSTRACT

After human and veterinary therapy primaquine is not completely removed at municipal wastewater treatment plants and therefore are discharged into the environment. We studied changes of the structure of primaquine by fungus *Beauveria bassiana* widely present in nature. Two peaks of metabolites were observed by HPLC in extract of incubation mixture. Proton nuclear magnetic resonance spectra and electrospray ionization mass spectra analysis of the products confirmed the formation of *N*-acetylprimaquine, 24.5% of total extract peak area at 265 nm and *N*-formylprimaquine, 2.8% of total extract peak area at 265 nm.

Key words : *Biodegradation, Acetylation, Formylation, Beauveria bassiana*

Introduction

Primaquine is 8-aminoquinoline drug has been used against malarial disease (Graves *et al.,* 2018; Parshikov *et al.,* 2018a) for over 50 years. Recently, primaquine has also been used for the treatment of cancer (Choi *et al.,* 2016).

Primaquine, obtained after the therapy of humans and animals, is not completely removed at the treatment plant and, therefore, is discharged into the wastewater (Heberer, 2002). Wastewater treatment processes were often not designed for removing compounds from wastewater (Kolpin *et al.,* 2002). Organic waste can turn into new and more resistant compounds that can be released in addition to the original.

Many researches were dedicated to bioconversions of primaquine for studies of pathways of transformations this drug in living organisms and by microorganisms.

Different metabolites of primaquine are produced during metabolism in animals and humans. The widespread metabolite in living organisms and in microorganisms cells is carboxyprimaquine (Hufford *et al.,* 1983). Another isolated major microbial metabolite of primaquine is *N*-acetyl derivative (Hufford *et al.,* 1983). This metabolite is produced by almost all species of *Streptomyces*. Other ways of primaquine transformation in living organisms are desalkylation, formation of *N*-hydroxyderivatives, 5- and 6-hydroxyderivatives and 6-metoxy-8-hydroxyaminoquinoline etc (Vale *et al.,* 2009).

We have investigated the transformation of primaquine by the fungus *Beauveria bassiana*. This fungus widely present in nature and was capable for transforming many organic compounds (Parshikov *et al.,* 2002). So that fungus may be used as model for biodegradation of primaquine in wastewaters.

Materials and Methods

Strain *Beauveria bassiana* ATCC 7159 was obtained from American Typical Cultures Collection.

Stock cultures were maintained on agar slants. The spores were washed from the surface of the agar with 5 mL of sterilized water and transported

***Corresponding author's email :** akadmah@gmail.com

to 500 mL flasks, each with 100 mL of a medium containing (per liter): sucrose, 30.0 g; peptone, 5.0 g; NaCl, 3.0 g; $NaNO_3$, 3.0 g; KH_2PO_4, 5.0 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; KCl, 0.5 g; $FeSO_4$, 0.1 g; and $MnSO_4$, 1 mg in deionized water. The pH was adjusted to 5.0. Cultures were grown for 48 hours on a rotary shaker at 28 °C with shaking at 180 rpm (Parshikov and Khasaeva, 2018b).

Primaquine diphosphate was dissolved in deionized water and filter-sterilized; 1 mL was added to each flask to make a final concentration of 100 mg/mL. After dosing, the cultures were incubated for an additional 10 days at 28 °C with shaking at 180 rpm. Cultures without primaquine and dosed, noninoculated controls were also incubated.

Mycelia were separated from the medium with filter paper on a Büchner funnel. Metabolites were extracted from the culture fluid with three equal volumes of methylene chloride (under pH 10 - 11) in a separatory funnel; the solvent was evaporated *in vacuo* on a rotary evaporator.

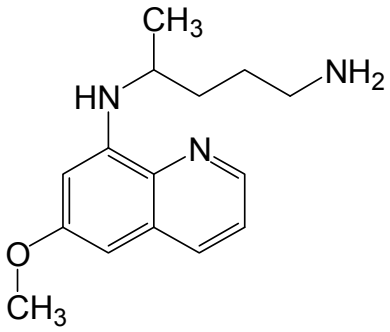
Extracts were analyzed by reversed-phase high-performance liquid chromatography (HPLC). A Waters 2690 separation module chromatograph equipped with the Waters 996 photodiode array detector, monitored at 265 nm, was fitted with a 4.6 × 10 mm Phenomenex Prodigy 5 mm ODS-3 column. The mobile phase components were solvent A (0.7 % triethylamine in water, pH 7.5) and solvent B (methanol). The mobile phase (flow rate = 0.2 ml/min) was a linear 50-min gradient fron 45 % to 80 % solvent B. The resulting biotransformation products were isolated using preparative reversed phase HPLC with using Waters Delta Prep 4000 chromatograph fitted with a semipreparative 10 × 250 mm Phenomenex Prodigy 5 mm ODS-3 column (flow rate = 2.5 ml/min).

Liquid chromatography/electrospray ionization mass spectrometry (LC/ESI MS) was performed using a Hewlett-Packard 1090L/M HPLC system with a 2.0 × 250 mm Prodigy 5-mm ODS-3 column and a Hewlett-Packard 5989B quadrupole mass spectrometer operated in the positive-ion electrospray mode. The mobile phase was a linear 40-min gradient from 95% water/5% acetonitrile to 5% water/95% acetonitrile, with constant 0.1% formic acid, at a flow rate of 0.2 $mlmin^{-1}$.

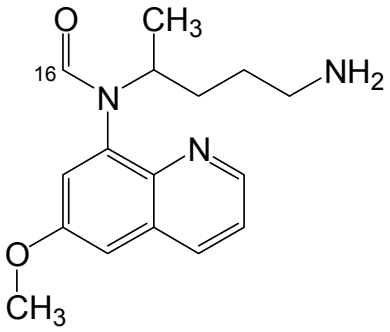
^1H nuclear magnetic resonance (NMR) spectral analyses were performed at 500 MHz on a Bruker AM 500 NMR spectrometer (Bruker Instruments, Billerica, Mass.) at 28 °C. Compounds were dissolved in deuterated dimethyl sulfoxide; chemical shifts are reported on the ppm scale.

Results

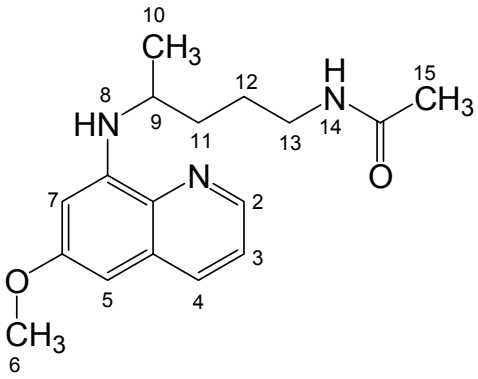
High-performance liquid chromatography (HPLC) analysis of the methylene chloride extracts showed that only *Beauveria bassiana* transformed primaquine



Primaquine



***N*-Formyl primaquine (P-1)**



***N*-Acetyl primaquine (P-2)**

Fig. 1. Structures of primaquine and its metabolites.

to two different metabolites (Fig. 1) showed residual primaquine eluting at 28.4 min and two metabolites (P-1 and P-2) that were not found in controls eluted at at 22.6 and 44.3 min, respectively. Other peaks were found but were also detected in the controls. After 10 days, as shown by the peak areas at 265 nm, 72.7% of the added primaquine remained.

Metabolite P-1, 2.8% of total extract peak area at 265 nm, had a UV absorption spectrum with λ_{max} = 267, 301 and 384 nm. The positive-ion ESI mass spectrum obtained at +200 V included ions at *m/z* 288 (5) $[M+H]^+$, 271 (12) $[M+H\text{-}NH_3]^+$ and 203 (100) $[M+H\text{-}C_5H_{10}NH_2]^+$. The most likely metabolite is *N*-formyl primaquine formylated on the secondary amine (Fig. 1). The ^{1}H NMR spectrum of P-1 showed at Table 1. The basics peaks obtained from *N*-formylprimaquine (m/z 203) and from *N*-acetylprimaquine (m/z 175) were showed by the positive ESI mass spectrometry. Loss of the sidechain from the protonated molecule of *N*-formylprimaquine is reason of formation the ion at *m/z* 203.

Metabolite P-2, 24.5% of total extract peak area at 265 nm, had a UV absorption spectrum with λ_{max} = 265 and 362 nm. The positive-ion ESI mass spectrum obtained at +200 V included ions at *m/z* 302 (5) $[M+H]^+$, 175 (100) $[M+HC_5H_{10}NH_2C_2H_3O]^+$ and 132 (9) $[M+H\text{-}C_2H_3OC_5H_{10}NH_2C_2H_3O]^+$. Loss of the sidechain from the protonated molecule results in a base peak at *m/z* 175 which identical with the base peak for starting material. The fragments are nearly identical with those seen for the starting material, so modifying parts that are lost makes sense. The ^{1}H NMR spectrum of P-2 showed at Table 1. The metabolite was identified as *N*-acetyl primaquine (Fig. 1).

Discussion

Primaquine is an important antimalarial and anticancer (Choi *et al.,* 2016) agent and it is used in medicine for a long time. Unlike other 8-aminoquinolines, primaquine therapy successfully eliminates the sporozoites, merozoites, and gametes residing outside of the infected erythrocytes. The most widespread pathways for the conversions of primaquine by different organisms were studied in different laboratories.

Unfortunately, 8-hydroxylaminoquinolines formed in humans from parent compounds have the hemolytic activity (Bolchoz *et al.,* 2001). Many investigations concerning modification of structure of primaquine by microorganisms were realized (Hufford *et al.,* 1983). However, many studied types of biotransformation reactions on primaquine such as alkylation and acetylation of the primary amine's group, the formation of carboxyprimaquine, hydroxylation of aromatic ring can not help to avoid the problem of 8-hydroxylaminoquinolines formation in human organisms from primaquine and his

Table 1. ^{1}H NMR parameters for primaquine and its metabolites produced by *B. bassiana*

	Chemical shift, ppm[a]			Coupling constants, *J*, in Hz		
Proton	Primaquine	P-1	P-2	Primaquine	P-1	P-2
H2	8.53	8.54	8.52	$J_{2,3}$ = 4.3, $J_{2,4}$ = 1.7	$J_{2,3}$ = 4.3, $J_{2,4}$ = 1.7	$J_{2,3}$ = 4.1, $J_{2,4}$ = 1.7
H3	7.42	7.43	7.41	$J_{3,4}$ = 8.4	$J_{3,4}$ = 8.4	$J_{3,4}$ = 8.4
H4	8.07	8.08	8.06			
H5	6.47	6.47	6.46	$J_{5,7}$ = 2.6	$J_{5,7}$ = 2.6	$J_{5,7}$ = 2.6
H6	3.81	3.81	3.81			
H7	6.28	6.30	6.24			
H8	6.14		6.10	$J_{8,9}$ = 8.6		$J_{8,9}$ = 8.8
H9	3.65	3.71	3.61			
H10	1.21	1.23	1.19	$J_{9,10}$ = 6.5	$J_{9,10}$ = 6.5	$J_{9,10}$ = 6.4
H11	1.58-1.70	1.58-1.70	1.43-1.53,1.64			
H12	1.58-1.70	1.58-1.70	1.43-1.53,1.64			
H13	2.76	2.75	3.03	$J_{12,13}$ = 7.3	$J_{12,13}$ = 7.3	$J_{12,13}$ = 7.3
H14			7.78			
H15			1.76			
H16		8.10				

[a] Dissolved in deuterated dimethyl sulfoxide.

derivatives (Hufford *et al.*, 1983; Bolchoz *et al.*, 2001).

Formation of *N*-formylated or *N*-acetylated derivatives in position 8- of primaquine molecule (Fig. 1) might be able to help to avoid formation 8-hydroxylaminoquinolines in environment and human organisms.

We observed that *B. bassiana* was capable for the formation of the *N*-formylated derivative at secondary amine's group and of the *N*-acetylated derivative at the primary amine's group of the side chaine of primaquine. Of the many organisms present in the environment B. bassiana models only one possibility of changing the structure of primaquine from the variety of other possible ways of its biodegradation.

Acknowledgement

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