Journal of Photochemistry & Photobiology, B: Biology 187 (2018) 1–9

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

Photosensitisation by voriconazole-*N*-oxide results from a sequence of solvent and pH-dependent photochemical and thermal reactions

P. Morlière[a,b,c,*], A.M.S. Silva[d], R.S.G.R. Seixas[d], F. Boscá[e], J.-C. Mazière[b,g], J. Ferreira[f], R. Santus[h], P. Filipe[f]

[a] *INSERM, U1088, 1 rue des Louvels, 80037 Amiens, France*
[b] *CHU Amiens-Picardie, Pôle Biologie, Pharmacie et Santé des Populations, Centre de Biologie Humaine, Laboratoire de Biochimie, 80054 Amiens Cedex 1, France*
[c] *Université de Picardie Jules Verne, UFR de Pharmacie, 1 rue des Louvels, 80036 Amiens, France*
[d] *Department of Chemistry & QOPNA, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal*
[e] *Instituto Universitario Mixto de Tecnología Química UPV-CSIC, Universidad Politécnica de Valencia, Avenida de Los Naranjos, s/n, 46022, Valencia, Spain*
[f] *Hospital de Santa Maria, Faculdade de Medicina de Lisboa, Clínica Dermatologica Universitaria and Unidade de Investigação em Dermatologia, Instituto de Medicina Molecular, avenida Prof. Egas Moniz, 1699 Lisboa, Codex, Portugal*
[g] *Université de Picardie Jules Verne, UFR de Médecine, 1 rue des Louvels, 80036 Amiens, France*
[h] *Muséum National d'Histoire Naturelle, Département: Adaptations du Vivant, 43 rue Cuvier, 75231 Paris, France*

ARTICLE INFO

Keywords:
Voriconazole-*N*-oxide
Metabolites
Photochemistry
Photoproducts
Photosensitisation

ABSTRACT

The phototoxicity of voriconazole (VN) prescribed in the treatment of severe fungal infections is frequently reported. Its major metabolite, a *N*-oxide derivative (VNO), was suspected to be the photosensitizer because it shows a maximum absorbance at ~310 nm in aqueous solutions. It was reported that the VNO photoproduct (VNOP) was phototoxic to human keratinocytes. Steady state and laser flash photolyses were performed to shed light on the phototoxic properties of VNO and VNOP. The quantum yield of the VNOP production by UVB-UVA light in buffered or alcoholic solutions is 0.6. VNOP has been identified as (*2R,3S*)-2-(2,4-difluorophenyl)-3-(5-fluoro-7-oxa-1,3-diazabicyclo[4.1.0]hepta-2,4-dien-4-yl)-1-(1*H*-1,2,4-triazol-1-yl)butan-2-ol. VNOP undergoes a marked thermal degradation and an efficient UVA photolysis with well differentiated kinetics and end-products. The temperature-dependent VNOP dark degradation produces a single product VNOPD identified as 6-[(*2S,3R*)-3-(2,4-difluorophenyl)-3-hydroxy-4-(1*H*-1,2,4-triazol-1-yl)butan-2-yl]-5-fluoropyrimidin-4-ol with absorbance maximum at 308 nm and $\varepsilon = 2700\ M^{-1}\ cm^{-1}$. Under UVB-UVA irradiation, VNOPD, the stable end-product, is a remarkable photodynamic photosensitizer towards Trp and His. The Trp photo-oxidation (Φ_{ox}(Trp) = 0.13) mainly involves type I radical reactions whereas His is oxidized by $^1O_2$ (Φ_{ox}(His) = 0.012). These results force us to question the validity of the *in vitro* photosensitization of human keratinocytes by VNO and VNOP previously reported.

1. Introduction

The triazole voriconazole (VN) is a commonly prescribed drug in the treatment and prophylaxis of severe fungal infections such as candidiasis and invasive aspergillosis in immune-depressed patients in paediatric practice and organ transplant recipients [1, 2]. Phototoxicity is a prominent frequently reported adverse effect of the drug. Phototoxicity limited to the sun exposed areas of the skin is demonstrated by actinic keratosis lesion development or by vesicle and bullae formation resembling drug-induced pseudo porphyria [3, 4]. Aggressive squamous cell carcinoma occurrence related to long-term VN treatment has also been reported [4, 5].

The molecular bases of the photosensitizing and photocarcinogenic effects of VN are unknown. It was earlier shown that VN and the *N*-oxide voriconazole (VNO), the major metabolite accounting for ~75% of the VN metabolization do not potentiate photodamage [6]. Recently, it has been suggested that VNO was the putative solar UVA-photosensitizer as – depending on solvent – it shows a prominent absorbance with a maximum in the 310–320 nm region [7] whereas VN does not significantly absorb the UV radiation beyond 290 nm. The same authors suggested that VNO was readily photolysed by UVB light to mainly produced an unidentified photoproduct (VNOP) and that both

* Corresponding author at: CHU Amiens-Picardie Pole Sud, Pôle Biologie, Pharmacie et Santé des Populations, Centre de Biologie Humaine - Biochimie Recherche, INSERM U 1088, 80054 Amiens, Cedex 1, France.
E-mail address: morliere.patrice@chu-amiens.fr (P. Morlière).

https://doi.org/10.1016/j.jphotobiol.2018.07.023
Received 11 April 2018; Received in revised form 15 June 2018; Accepted 24 July 2018
Available online 31 July 2018
1011-1344/ © 2018 Published by Elsevier B.V.

compounds were phototoxic to cultured human keratinocytes [7]. In view of these rather contrasting results and of the scarcity of physical-chemical data on VNO and of the lack of data on VNOP, this study was aimed at unravelling the nature of the electronic excited state(s) of VNO and VNOP that may be involved in their photoactivity. In addition, the subsequent formation of photolysis product(s) of VNOP, their fate and their photosensitizing properties with the possible intervention of type I (radical species formation) or type II (singlet oxygen) photodynamic reactions ([for a review see ref. 8]) have also been investigated. Absorption and fluorescence spectroscopies and laser flash photolysis techniques have been employed to study the interaction of the ground and excited singlet electronic states of VNO with solvents. Purposely, because VNO is a biologically active molecule present in circulating fluids, the role of the aqueous environment on its spectroscopic and photochemical properties has been emphasized. Subsequently, the photosensitizing properties of photo-chemically generated VNO derivatives have been analysed using the amino-acids Trp and His, two convenient and well established probes of type I and II photodynamic reactions of biological interest [8].

2. Materials and Methods

2.1. Chemicals and Routine Equipment

Dimethylsulfoxide (DMSO), Trp, *N*-acetylcysteine and His were supplied by Sigma-Aldrich (St Louis, Mo, USA). Dichloromethane and chloroform, both analytical reagent grade were obtained from Fischer Scientific (Loughborough, UK). 3-Chloroperoxybenzoic acid (MCPBA, 70–75% balance 3-chlorobenzoic acid and water) was purchased from Acros Organics (Geel, Belgium), anhydrous sodium carbonate (for analysis, ACS) from Panreac AppliChem (Barcelona, Spain) and anhydrous sodium sulfate from Labsolve (Odivelas, Portugal). Methanol (MeOH), ethanol (EtOH), chloroform, acetonitrile (ACN) and inorganic chemicals of the purest available grade were provided by Merck (Darmstadt, Germany). Formic acid (98%, mass spectrometry grade) used in mass spectrometry analysis was supplied by Sigma-Aldrich (Steinheim, Germany). Preparative thin-layer chromatography was carried out in glass plates (20 × 20 cm) previously coated with a 0.5 mm thick layer of Silica G/UV_{254} purchased from Macherey-Nagel (Düren, Germany).

Phosphate buffers were prepared in pure water obtained with a reverse osmosis system. DMSO-d_6 and acetone-d_6 with tetramethylsilane (TMS) were obtained from Cortecnet (Voisins le Bretonneux, France). Absorption spectrophotometry was carried out with an Uvikon 922 spectrophotometer. Fluorescence measurements were performed with a RF-5301 PC Shimadzu spectrofluorometer (Shimadzu-France, Marne la Vallée, France). Fluorescence quantum yields have been determined using the Trp fluorescence as a standard value [9]. The fluorescence quantum yield of Trp in pH 7.0, 10 mM phosphate buffer was assumed to be 0.14 upon excitation at 305 nm. All experiments described herein were repeated twice, and no more than a 5% difference was found for the calculations performed with replicates.

2.2. Irradiations

Unless otherwise specified UVB-UVA irradiations were performed with the Vilber-Lourmat (Marne la Vallée, France) TFX-20LM transilluminator (20 × 20 cm) set at 312 nm and equipped with an additional Schott (Mainz, Germany) WG305 long-pass filter. Under these conditions, the UV light output measured as earlier described [10, 11] is maximal at 321 nm (± 19 nm HWHM) with less than 1% of the light arising from wavelengths below ~295 nm and above ~403 nm (procedure A). Solutions to be irradiated (2 mL) were contained in a 1 × 1 × 4 cm optical quartz cuvette lying on the irradiation table. A fluence rate of 6.0×10^{15} quanta $s^{-1} cm^{-2}$, *i.e.* ~3.7 mW cm^{-2} was measured in the quartz cuvette by a chemical actinometry based on the photoreduction of potassium ferrioxalate by the UV radiation [12].

For determination of the VNO photolysis quantum yield, an additional interference optical filter centered at 313 nm (13 nm FWHM) from MTO, (Massy, France), was intercalated between the UVB-UVA irradiation source and the quartz cuvette (procedure B). Under these conditions, the UV light output is maximal at ~314 nm (± 4.5 nm HWHM) with less than 1% of the light arising from wavelengths below ~300 nm and above ~341 nm. A fluence rate of 9.6×10^{13} quanta $s^{-1} cm^{-2}$, *i.e.* ~61 μW cm^{-2} was measured in the quartz cuvette placed on the irradiation table as described above.

UVA irradiations were performed with a Vilber-Lourmat TFP-35 L transilluminator (35 × 20 cm) equipped with an additional Schott WG305 long-pass filter. Under these conditions, the UV light output is maximal at ~355 nm (± 19 nm HWHM) with less than 1% of the light arising from wavelengths below ~320 nm and above ~402 nm. A fluence rate of 8.4×10^{15} quanta $s^{-1} cm^{-2}$, *i.e.* ~4.7 mW cm^{-2} was measured in the quartz cuvette placed on the UVA table as described above. Because of the use of polychromatic lights, for the quantum yield calculations, the absorbed light I_{Abs} was calculated as $I_{Abs} = \int I_{Abs_\lambda} d\lambda = \int I_\lambda \times (1 - 10^{-OD_\lambda})\, d\lambda$, where I_λ and OD_λ are the fluence rate and the absorbance at wavelength λ respectively [11, 13].

For VNOP identification, 2 mL of a 50 μM VNO solution in pH 8.0, 1 mM phosphate buffer were irradiated with UVB for 2 min in the quartz cuvette set as described above. This procedure was repeated 4 times and the pooled irradiated solutions were then lyophilized.

2.3. Synthesis of VNO

The VN [(2*R*,3*S*)-2-(2,4-difluorophenyl)-3-(5-fluoropyrimidin-4-yl)-1-(1*H*-1,2,4-triazol-1-yl)butan-2-ol)] used for the synthesis of VNO [4-((2*S*,3*R*)-3-(2,4-difluorophenyl)-3-hydroxy-4-(1*H*-1,2,4-triazol-1-yl) butan-2-yl)-5-fluoropyrimidine 1-oxide] (Fig. 1A) was either purchased from Sigma-Aldrich or was isolated by liquid-liquid extraction of the lyophilized powder of Vfend® for intravenous infusion provided by the Central Pharmacy of the CHU Amiens-Picardie hospital. The liquid-liquid extraction was performed as follows: 20 mL of H_2O were added to a 200 mg of the lyophilized powder of Vfend®. The aqueous solution was extracted with CH_2Cl_2 (2 × 20 mL) leading to VN in quantitative yield without contamination with the excipient sulfobutyl ether β-cyclodextrin sodium.

VNO was synthesized by reacting VN with 3-chloro-peroxybenzoic acid (MCPBA) following a modified procedure described for the synthesis of pyridine *N*-oxide [14]. To a solution of VN (25.0 mg, 0.072 mmol) in CH_2Cl_2 (1 mL) at 0 °C, MCPBA (70%, 177.5 mg, 0.72 mmol) was slowly added by portions. Then the mixture was ultrasonicated for 1 min and stirred for 24 h at room temperature. After addition of CH_2Cl_2 (10 mL) the mixture was washed with an aqueous solution of sodium carbonate (1%, 2 × 15 mL) and water (1 × 15 mL). The organic layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure. The reaction crude was purified by silica preparative thin-layer chromatography (eluent: CH_2Cl_2/MeOH (92.5/7.5)) leading to VNO (6.8 mg, 26%) as a white solid. The identity of VNO was confirmed by ^{1}H NMR and mass spectrometry (see Fig. S1 in supplementary material file) and is in agreement with the literature [15]. The 10 mM VNO stock solutions were prepared in DMSO and kept frozen at − 20 °C for further studies.

^{1}H NMR (300.13 MHz, acetone-d_6) δ 1.19 (d, *J* 7.1 Hz, 3H, CH_3), 4.00 (dq, *J* 1.0, 7.1 Hz, 1H), 4.52 (d, *J* 14.2 Hz, 1H of CH_2), 4.97 (d, *J* 14.2 Hz, 1H of CH_2), 5.42 (s, 1H, O*H*), 6.89 (dddd, *J* 0.9, 2.6, 8.2, 8.9 Hz, 1H, Ar), 7.02 (ddd, *J* 2.6, 9.0, 12.2 Hz, 1H, Ar), 7.48 (dt, *J* 6.7, 9.1 Hz, 1H, Ar), 7.57 (s, 1H, triazole), 8.17 (s, 1H, triazole), 8.68 (dd, *J* 1.6, 5.5 Hz, 1H, pyrimidine), 8.85 (d, *J* 1.6 Hz, 1H, pyrimidine) ppm; ESI$^+$-MS: *m/z* (%) = 388 ([M + Na]$^+$, 24), 366 ([M + H]$^+$, 73), 224 ([M-142]$^+$, 100).

Download English Version:

https://daneshyari.com/en/article/6493161

Download Persian Version:

https://daneshyari.com/article/6493161

Daneshyari.com