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Letter to Editor Open Access

# Voriconazole does not Potentiate Photo Damage from UVB Exposure

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## Abstract

Voriconazole is an effective anti-fungal triazole commonly used in bone marrow and solid organ transplant recipients. However, reports of accelerated development of aggressive squamous cell carcinomas in immunocompromised patients are documented following voriconazole use. It is hypothesized that voriconazole or its primary N-oxide metabolite, voricinazole-N-oxide increases keratinocyte susceptibility via UV-mediated cell damage. We aimed to investigate whether voriconazole or voriconazole-N-oxide potentiate cell death after UVB irradiation *in vitro*.

Both compounds absorb UVB but voriconazole exhibited weak emission while voriconazole-N-oxide showed no detectable emission in UVA. Exposure of different skin cell lines to these compounds did not show significant reduction in cell survival and regardless whether the cells were exposed to the drugs before or after UVB irradiation. However, in primary human keratinocytes, both drugs caused a small increase in cell survival following drug incubation and UVB irradiation. This is the first report documenting the effect of voriconazole and voriconazole-N-oxide in relation to UVB-associated cell death *in vitro*.

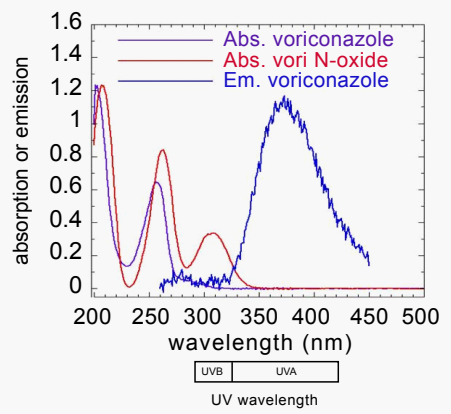
**Keywords:** Voriconazole; Voriconazole-N-oxide; VNO; UV; Phototoxicity; Squamous cell carcinoma

## To the Editor

Voriconazole (Vfend, Pfizer), is a second-generation triazole and is effective for invasive and pulmonary *Aspergillus*, fluconazole-resistant *Candida*, and other fungal pathogens. It is commonly used in bone marrow and solid organ transplant recipients (OTRs) [1-6]. Side effects of voriconazole include premature dermatoheliosis and photosensitivity ranging from sunburn-like erythema to pseudoporphyria with blistering [4,7]. Multiple case studies have reported that voriconazole-associated phototoxicity leads to the accelerated development of aggressive Squamous Cell Carcinomas (SCCs) in immunocompromised patients [4,7,8]. In two large lung transplant studies, voriconazole was associated with an increased risk for SCC after transplantation [9-11]. However, the mechanism of carcinogenesis is unknown. It has been proposed that Voriconazole or its N-oxide metabolite (VNO) increase keratinocyte susceptibility via Ultraviolet (UV)-mediated cell damage, leading to accumulation of mutations. Therefore, we investigated whether these agents potentiate cell death after UVB irradiation *in-vitro*. This is the first report documenting voriconazole and VNO's effect in relation to UVB-associated *in-vitro* cell death. We assume, on the basis of work in UV induced cancers in repair deficient patients, that increased sensitivity would be a predictor of increased mutagenesis and carcinogenesis if increased DNA damage were involved [12].

Spectrophotometric analysis demonstrated that voriconazole only weakly absorbs UVB while VNO has a UVB absorption peak at 308 nm ($\varepsilon$=1.1 × 10^4 $M^{-1}$ $cm^{-1}$). This suggests that the metabolite may be the chromophore associated with solar photosensitivity (Figure 1). Voriconazole exhibited weak emission at a peak at ~370 nm. However, no emission was detectable from VNO, suggesting that the photoexcited states of these molecules decay largely or entirely through non-radiative mechanisms such as chemical reactivity that might be associated phototoxicity [2,7].

Cytotoxicity of voriconazole and VNO (Santa Cruz Biotechnology, Inc) following UV was tested on cultures of GM05659 skin fibroblasts, p53-mutant HaCaT cells, neonatal foreskin primary keratinocytes and SCC12B2squamous carcinoma cells (Figure 2). To determine whether the timing of drug and UV exposure was interdependent, one set of experiments was performed with cells incubated with the drug at a concentration of 13.5 μM for 30 minutes prior to irradiation while a second set of assays was performed with cells grown in the absence of




Wavelengths encompassing UVA and UVB ranges are indicated in boxes. Absorption spectra for the drugs were obtained using a Beckman DU-500 spectrophotometer while the emission spectra were recorded on a SpectraMax Gemini fluorometer with an excitation wavelength of 255 nm and slit widths of 1.5 nm and a scan rate of 1 nm/s. The plotted data is the average of 50 consecutive spectra.

**Figure 1:** Absorbance and emission spectra of voriconazole and its N-oxide metabolite.

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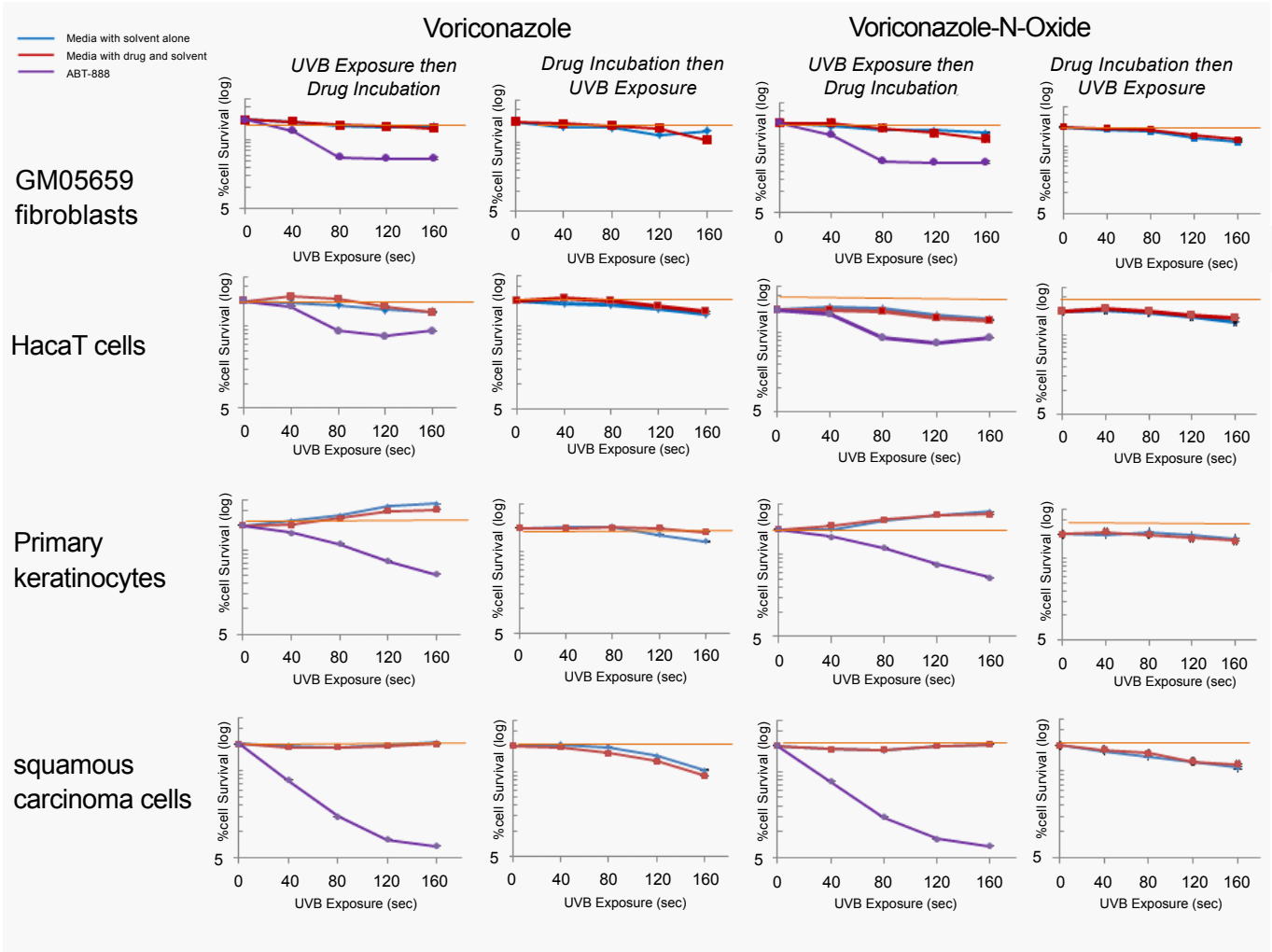


Cell survival measured through MTT assays following incubation of (a) GM05659 fibroblasts; (b) p53-mutant HaCaT keratinocytes, (c) neonatal foreskin primary keratinocytes and, (d) squamous cell carcinoma cell lines with voriconazole or its N-oxide metabolite given UVB pre- or post-incubation (n=6) compared with the methanol and ethanol solvents, respectively (n=6); and, the caffeine and ABT-888 positive controls (n=12). 100% cell survival is indicated by an orange line for each graph. The drug-incubated cells were UVB-irradiated using 6 x BLE8T312 fluorescent lamps. Peak UV emission was seen at 310 nm and 50% intensity of the emitted wavelengths ranged between 295-325 nm as measured via a UVB-Dosimeter (SpectrolineCorporation™). Error bars are calculated based on the standard error of the mean.

**Figure 2:** Voriconazole does not potentiate UVB-mediated cell death.

the drug but incubated with drug for five days after irradiation. Cells were irradiated with UVB (1.0 $J.m^{-2}$.sec) at exposure ranging from 0 to 160 seconds. The growth media was completely aspirated prior to UVB exposure to prevent any filtering effect. Our positive control for potentiation of UV-mediated cytotoxicity was ABT-888, an inhibitor of the PARP-1 polymerase. Cells were irradiated and then cultured in the presence of ABT-888 at a concentration of 50μM [13,14].

Cell survival was assessed five days after UVB irradiation using the (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide MTT assay (Sigma), which measures cellular metabolic activity via NAD(P)H-dependent cellular oxidoreductase enzymes and reflects the number of viable cells in culture [15]. This assay was selected over the commonly used 3T3 neutral red uptake phototoxicity assay, which uses BALB/c murine fibroblasts and thus is not generalizable to human keratinocytes. Neither voriconazole nor VNO exposure were associated with a significant reduction in cell survival. This finding held regardless of whether the cells were exposed to the drug before or after UVB irradiation. In contrast, ABT-888 exposure resulted in a significant UV dose-dependent decrease in cell survival. In primary human keratinocytes, both voriconazole and VNO and their control media (methanol and ethanol, respectively) appeared to cause a small increase in cell survival when the cells were cultured in drug or solvent following UVB irradiation. The most likely explanation for this surprising finding is that the presence of low concentrations of the drug solvent and corresponding control media alter the reduction-oxidation capacity of the cells. The MTT assay system is sensitive to these changes in cellular metabolic activity [15]. In contrast, no such survival benefit was seen in the cultures that were exposed prior to irradiation but not during the five days between irradiation and assay.

We report that neither voriconazole nor its N-oxide metabolite potentiate UVB-associated cell death. We believe that this brief report add significant new information regarding the tumor-enhancing effect

of voriconazole and its primary N-oxide metabolite. Though the result is a negative one, it adds to our understanding of the mechanism by which this drug causes SCC. The prevailing belief is that the drug induces DNA damage; however, another mechanism such as retinoid effect may be in play. Another potential hypothesis is that the effect of voriconazole or VNO requires longer exposure and chronic, intermittent UV radiation, similar to the clinical scenario in which a patient is on medication long-term and has daily sun exposure. Alternately, the effect may be mediated by UVA wavelengths. Though there is a single report of increased erythema to UVA in patients on voriconazole [6], it is not firmly established which specific wavelength of ultraviolet light trigger this response, if it is indeed UV-mediated. The spectrophotometric analysis we present here demonstrate that UVB, rather than UVA, is absorbed by voriconazole N-oxide without emission. Thus, we hypothesize that voriconazole N-oxide potentiates UVB damage and decreases cell survival. Experiments are on-going to understand the role of voriconazole and VNO on mutation rates that would further uncover the potential phototoxic effect of the drugs but are beyond the scope of this report. Future studies may also use solar simulators to test the effect of voriconazole on UVA-mediated cell survival. It is also possible that the association between voriconazole and SCC is not through a photobiologic pathway but through indirect toxicity, or systemic effects. Ruling out a direct phototoxic effect suggests that further experiments to address alternate hypotheses will be needed to elucidate the mechanism. Ruling potential mechanisms out is as important as ruling them in, as this opens additional avenues for research.

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