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Biological effects of bacterial pigment undecylprodigiosin on human blood cells treated with atmospheric gas plasma *in vitro*

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ABSTRACT

It is known that some bacterial species are more resilient to different kinds of irradiation due to the naturally developed protective mechanisms and compounds such as pigments. On the other hand, reasoned tissue engineering using plasma remains a critical task and requires very precise control of plasma parameters in order to mitigate its potential detrimental effects. Here we isolated a natural protective agent, microbially produced undecylprodigiosin ((5′Z)-4′-methoxy-5′-[(5-undecyl-1H-pyrrol-2-yl)methylene]-1H,5′H-2,2′-bipyrrole), and investigated its effects on human blood cells independently and in combination with plasma. Two approaches were applied; the first, undecylprodigiosin (UP pigment) was added to the blood cultures, which then were exposed to plasma (pre-treatment); and the second- the blood cultures were exposed to plasma and then treated with pigment (post-treatment). The interactions of plasma and UP pigment with blood cells were investigated by conducting a series of biological tests providing the information regarding their genotoxicity, cytotoxicity and redox modulating activities. The exposure of cells to plasma induced oxidative stress as well as certain genotoxic and cytotoxic effects seen as elevated micronuclei incidence, decreased cell proliferation and enhanced apoptosis. In blood cultures treated with UP pigment alone, we found that both cytotoxic and protective effects could be induced depending on the concentration used. The highest UP pigment concentration increased lipid peroxidation and the incidence of micronuclei by more than 70% with maximal suppression of cell proliferation. On the contrary, we found that the lowest UP pigment concentration displayed protective effects. In combined treatments with plasma and UP pigment, we found that UP pigment could provide spatial shielding to plasma exposure. In the pre-treatment approach, the incidence of micronuclei was reduced by 35.52% compared to control while malondialdehyde level decreased by 36% indicating a significant mitigation of membrane damage induced by plasma. These results open perspectives for utilizing UP pigment for protection against overexposures in the field of plasma medicine.

1. Introduction

In everyday life, people are exposed to different agents, which can lead to biological modifications and in some cases irreparable damage. Damage can be characterized at different levels including cellular, molecular, biochemical, and genetic. The effects on human body are mostly cumulative and closely related to production of free radicals. Radicals can be biologically, chemically, and physically created (Valko et al., 2007). Typical physical agents, which create free radicals are different types of irradiation such as

Abbreviations: UP, undecylprodigiosin; CBMN, cytokinesis block micronucleus; BN, binucleated cells; MN, micronuclei; CBPI, cytokinesis-block proliferation index; TBA, thiobarbituric acid; AP, apoptosis; CAT, catalase.

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UV, gamma or plasma irradiation (Halliwell and Gutteridge, 2007). Often irradiation of biological systems results in generation of highly reactive oxygen and nitrogen species (ROS, RNS). Consequently, radicals interact with cellular macromolecules such as membranes, DNA, RNA, and proteins causing their dysfunction, damage, and perturb the integrity and survival of cells (Moller and Wallin, 1998; Undeger et al., 2004).

Gas plasma is an efficient source of radicals. Electrical discharges may be generated at atmospheric pressure and used to treat samples that cannot tolerate vacuum, such as cells. Another important feature is that plasma can be produced in such a way that overheating of sensitive samples is avoided. Charged particles – electrons and ions, photons, metastable species and free radicals – are typical plasma constituents. Numerous chemical reactions may occur both in the gas phase and in interactions with surfaces. The literature refers to this type of plasma as atmospheric non-equilibrium or non-thermal plasma or as cold atmospheric plasma (Becker et al., 2004). Since the 1990s, an abundance of biomedical applications has been developed, utilizing the features mentioned above.

Biomedical applications of non-thermal plasma are gaining significant attention due to increasing number of diseases and problems that can be solved by direct therapies (Kong et al., 2009; Petrovic et al., 2012; von Woedtke et al., 2014). Some of the examples are Hailey-Hailey disease and wound treatments (Heinlin et al., 2011; Isbary et al., 2011; Nosenko et al., 2009). Atmospheric pressure plasma is known to abundantly create radicals while maintaining low temperature and is widely used for biomedical applications, such as sterilization (Hensel et al., 2015; Laroussi et al., 2003), blood coagulation (Kalghatgi et al., 2007), tooth bleaching (Lee et al., 2010), and cancer treatment (Cheng et al., 2014; Utsumi et al., 2013). However, the toxicity of plasma is still controversial, and it depends on dose and exposure time; low dose plasma is relatively non-toxic to the cells, but high dose induces apoptotic cell death (Kalghatgi et al., 2011). Low doses of ROS/RNS have been shown to promote cell survival, proliferation and migration, while excessive ROS levels leading to oxidative stress have been associated with cell senescence and the initiation of apoptosis (Arjunan et al., 2015a). Furthermore, atmospheric pressure plasma can even have selective effects – efficient killing of cancer cells without causing damage to surrounding healthy cells (Iseki et al., 2012; Tanaka et al., 2011).

Cellular antioxidative defense system that includes enzymic and nonenzymic antioxidants keeps the cellular redox homeostasis and reduces the level of damage through reduction of oxidative stress. However, there is a substantial interest in the use of natural products in an effort to prevent or reduce the oxidative damages induced by irradiation (Adaramoye et al., 2010; Sandeep and Nair, 2012). It is well known that some bacteria have developed compounds, usually pigments, for protection against irradiation (Abboud and Arment, 2013; Mohammadi et al., 2012). Undecylprodigiosin ((5′Z)-4′-methoxy-5′-[(5-undecyl-1H-pyrrol-2-yl) methylene]-1H,5′H-2,2′-bipyrrole) (UP) is a dark-red pyrrole-based pigment belonging to prodigiosin family of compounds (Furstner, 2003), which have been characterized to be antimicrobial, antimalarial, immunosuppressive and cytotoxic (Pandey et al.,

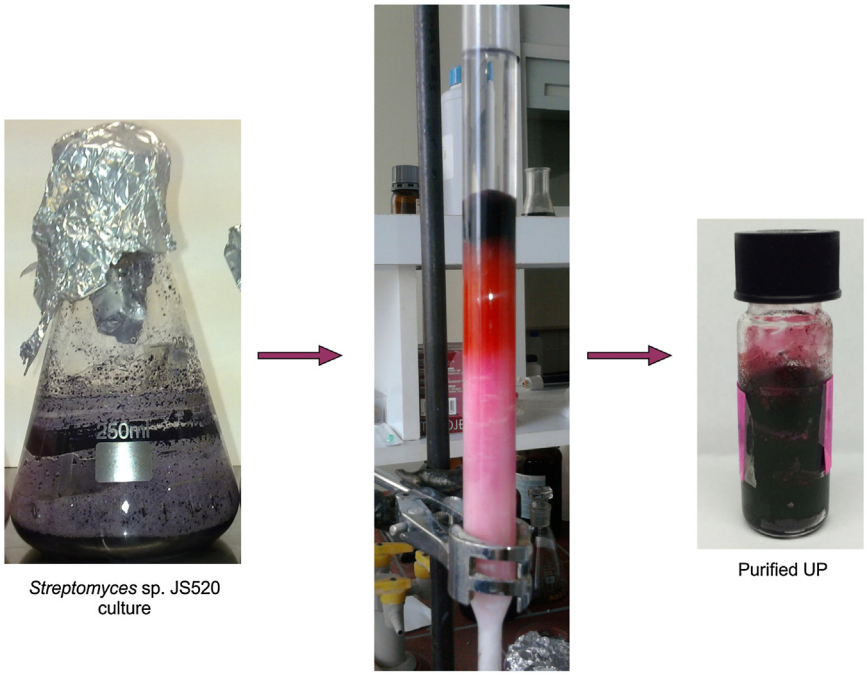

Fig. 1. UP pigment purification process and structural formula.

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