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The Role of Vitamin C in Photosensitivity Attenuation of Antimicrobial Quinolones Group

A Thesis Submitted for the degree of Master of science As a partial fulfillment for requirements of the Master of Science in Biophysics.

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DEDICATION

This work is dedicated to my loving family, especially ***my parents, my brothers and my sisters*** *for I would not be who I am today without their love and everlasting support. They always help me and support my ideas and steps in either my social or scientific life. Also, They provide me with special life style full of cooperation, support and fun.*

Summary

Drug-induced photosensitivity refers to a cutaneous adverse event occurring due to the combined interaction of a drug (topically or systemically administered) and radiation from the sun. Phototoxic reactions can manifest within minutes or hours of drug administration and are non-immunological, resulting from direct tissue injury. The ultraviolet radiation that reaches the earth's surface, more than 95% is the longer wavelengths of UVA, with the small amount UVB. UVA effects are dominated by indirect DNA damage.UVA has the longest wavelength with the lowest energy and can penetrate deeply into the dermis and cause aging effects. One of the most obvious acute effects of UV on the skin is the induction of inflammation.

A quinolone antibiotic is a member of a large group of broad-spectrum bactericides. Treatment with both UV radiation and quinolone showed significantly increased back skin swellings, increase sunburn cells and decreased epidermal Langerhans cells. Ciprofloxacin is one of the most widely used antibiotics. The most common side effects of ciprofloxacin are nausea, diarrhea, vomiting, elevating liver enzymes and skin sensitivity to sunlight.

Antioxidants that are reducing agents can also act as pro-oxidants. For example, vitamin C has antioxidant activity when it reduces oxidizing substances such as hydrogen peroxide, however, it will also reduce metal ions that generate free radicals through the Fenton reaction.

2 Fe^{3}+ + Ascorbate (vit C) → 2 Fe^{2+} + Dehydroascorbate

2 Fe^{2+} + 2 H_2O_2 → 2 Fe^{3+} + 2 $OH^{\cdot}$ + 2 OH^{-}

This work aimed to investigate the damaging effect of ultraviolet-A radiation on the antibacterial activity of ciprofloxacin in Esch. Coli infected rats and demonstrate the protective role of Vit. C against UV-A induced oxidative stress.

At the beginning of the experiment Fifty six adult male albino rats weighing approximately150-180g were randomly allocated into seven groups. Each group consisted of 8 rats. The animals were treated according to the following scheme:

Group 1: normal control rats non exposed to UV-A lamp radiation.

Group 2: rats exposed to UV-A lamp radiation for 60 min.

Group 3: rats administered ciprofloxacin (100mg/kg; orally; daily) for 7 consecutive days + E.coli in vivo bacterially infected rats non exposed to UV-A lamp radiation.

Group 4: rats receiving ciprofloxacin (100mg/kg; orally; daily) for 7 consecutive days + E.coli in vivo bacterially infected rats exposed to UV-A lamp radiation for 30 min.

Group 5: rats receiving ciprofloxacin (100mg/kg; orally; daily) for 7 consecutive days + E.coli in vivo bacterially infected rats exposed to UV-A lamp radiation for 45 min.

Group 6: rats receiving ciprofloxacin (100mg/kg; orally; daily) for 7consecutive days+ E.coli in vivo bacterially infected

rats exposed to UV-A lamp radiation for 60 min and served as positive control group.

Group 7: rats receiving ciprofloxacin (100mg/kg; orally; daily) for 7 consecutive days + E.coli in vivo bacterially infected rats exposed to UV-A lamp radiation for 60 min + Vit.C (180mg/kg; orally; daily) for 7 consecutive days.

In all experiments, dorsal side of shaved rats, using sterile surgical blade, is exposed to UV-A lamp radiation at the 5, 6, 7^{th} days from the beginning of the experiment.

Grade of skin reaction (redness, edema, shrinkage, hemorrhage and oozing) at 24, 48 and 72 hr. after UV-A irradiation were conducted once daily,thenmeasured relative weight for organs and oxidative stress (MDA, GSH), inflammatory biomarker tumor necrosis factor-α(TNF-α) in the skin homogenate. Furthermore; total Leukocyte count, liver function parameters such as albumin and total protein levelsin serum by UV spectroscopic analysis.

It is noticeable that skin scores increased were in groups with increasing days of exposure, all the skin scores were increased in comparison with normal control group. The obtained results of MDA, GSH and TNF-α contents indicated the skin MDA and TNF-α contents were increased and decreased GSH content. the obtained results indicated that the total leukocyte count (TLC) in blood was increased and accompanied by a decreased serum total protein (TP) and albumin (ALB) levels, in other hand relative spleen weight were increased and relative thymus weight were decreased, and

the histopathological alterations in skin induced due to photosentivity of ciprofloxacin by UV-A radiation was increased by the time exposure.

Treatment rats by Vit.C showed a significant decrease in all skin score parameters and normalized the skin homogenate contents of lipid peroxide (MDA) ~56%, glutathione (GSH) ~42%, (TNF-α) ~72%and the blood leukocyte count ~31%. Moreover, by testing serum total protein and albumin levels were they increased by ~27% and ~20% respectively, whereas the relative organs weights of spleen and thymus were normalized by Vit.C ~27% and ~49% respectively. Moreover, no histopathological alterations in skin, were noticed.

Treatment rats by Vit.C showed a significant decrease in all skin score parameters and approaches to control values, and skin MDA, TNF-α and TLC decreased accompanied with significant increase in GSH, TP and ALB. Even more normalized relative spleen weight and relative thymus weight. Moreover no histopathological alterations in skin were noticed.

Study concludes that orally administered Vit.C has prophylactic antioxidant and anti-inflammatory effects against ciprofloxacin induced phototoxicity on exposure to UV-A radiation in E.coli systematic infected rats.

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Introduction and Review of Literature

1.1 Introduction

Drug-induced photosensitivity refers to a cutaneous adverse event occurring due to the combined interaction of a drug (topically or systemically administered) and radiation from the sun. The spectrum of cutaneous manifestations varies from trivial pricking and itching on sun-exposed sites to a severe sunburn reaction with blistering **(Seto et al., 2012)**. Phototoxic reactions can manifest within minutes or hours of drug administration and are non-immunological, resulting from direct tissue injury. A potential mechanism for tissue damage involves drug-potentiated oxidation of cellular lipids, proteins and DNA often mediated by reactive oxygen species (ROS) generation; free radicals, superoxide anions, hydroxyl radicals and singlet oxygen **(Bracchitta et al., 2013)**. UV radiation constitutes about 10% of the total light output of the Sun **(Priya et al., 2016).** Many practical applications of UV radiation derive from its biological effects due to interactions with organic molecules **(Valeur et al., 2012).** The ultraviolet radiation that reaches the earth's surface, more than 95% is the longer wavelengths of UVA, with the small amount UVB **(Sage et al., 2012).** UVA effects are dominated by indirect DNA damage caused by reactive oxygen species such as singlet oxygen that induce apoptosis of skin infiltrating T cells, T-cell depletion and induction of collagenase-1expressioni inhuman dermal fibroblast **(Juzeniene et al., 2012).** UVA has the

longest wavelength with the lowest energy and can penetrate deeply into the dermis and cause aging effects **(WHO, 2016).** One of the most obvious acute effects of UV on the skin is the induction of inflammation. If the dose of UV exceeds a threshold damage response, keratinocytes activate apoptotic pathways and die **(Antony et al., 2017).**

A quinolone antibiotic is any member of a large group of broad-spectrum bactericides that share a bicyclic core structure related to the compound 4- quinolone **(Kyriacos and Stephan, 2018).** They are used in human and veterinary medicine to treat bacterial infections, nearly all quinolone antibiotics in modern use are fluoroquinolones, which contain a fluorine atom in their chemical structure and are effective against both Gram-negative and Gram-positive bacteria **(James et al., 2008).**

Quinolone antibiotics are one of the well-known photosensitizers that induce phototoxicity. Treatment with both UV radiation and quinolone showed significantly increased back skin swellings, increase sunburn cells and decreased epidermal Langerhans cells **(Heeb et al., 2011).** Approved fluoroquinolone drugs include levofloxacin (Levaquin), ciprofloxacin (Cipro), moxifloxacin (Avelox), norfloxacin (Noroxin), ofloxacin (Floxin), and gemifloxacin (Factive) **(Jourdan et al., 2017).** Ciprofloxacin is one of the most widely used antibiotics. It is chemically related to nalidixic acid and induces photosensitization of human skin **(Chhorvoin et al., 2017).** The most common side effects of ciprofloxacin are