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Development of photoprotective, antiphototoxic, and antiphotogenotoxic formulations of ocular drugs with fluoroquinolones

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ABSTRACT

The development of innovative solutions in photosafety of photolabile pharmaceutical products may help to reduce the adverse effects of these products, caused by light exposure. Providing new data in this area of study is particularly important in case of drugs applied topically on sensitive organs such as eyes. The main goal of this research is to investigate whether two potential excipients, namely: p-coumaric acid and benzophenone-4, affect the photodegradation, phototoxicity and photogenotoxicity of water solutions of four fluoroquinolones: ciprofloxacin, lomefloxacin, fleroxacin and clinafloxacin. We conducted a set of bioassays combined with the application of high-performance liquid chromatography and mass spectrometry techniques. The significant reduction of phototoxic and photogenotoxic abilities was evaluated in mixtures with ciprofloxacin and p-coumaric acid by using the *umu* test with *Salmonella typhimurium* TA1535/pSK1002, the methylthiazol tetrazolium reduction assay, and the micronucleus assay with the V79 cell line. In the bacterial assay the opposite effect was observed for the formulation with lomefloxacin and p-coumaric acid. This may be explained by the significant differences in the profile of the lomefloxacin photodegradation products. Further, the photoprotective and antiphotomutagenic abilities of ciprofloxacin mixed with benzophenone-4 were assessed. Promising results obtained in compositions with ciprofloxacin may be a basis for further research. Nevertheless, the increase in the DNA damage potential in mixtures with p-coumaric acid and two other antibiotics shows the importance of the safety evaluation of such innovative combinations.

1. Introduction

The development of innovative solutions in photosafety of photolabile pharmaceutical products may help to reduce the adverse effects of these products, caused by light exposure. Providing new data in this area is particularly important in case of drugs applied topically on sensitive organs such as eyes. Eyes are a complex optical system exposed to light from the surrounding environment. They are particularly vulnerable to the ultraviolet (UV) light present in the range of sunlight and generated by some artificial light sources; exposure to UV light may result in corneal damage, cataract formation and the progress of age related macular degeneration. Photosafety testing is particularly relevant for chemicals with a phototoxic and photogenotoxic potential [1], such as fluoroquinolones (FQ). FQ are broad-spectrum antibiotics often used as components of various pharmaceutical preparations, including eye drops and ocular ointments [2,3]. Photolysis of these antibiotics may lead not only to light induced side effects but also to the reduction of their antibacterial activity. The effects may decrease the effectiveness of the treatment and stimulate the development of more resistant bacterial strains. There are many reports about FQ cytotoxicity and genotoxicity in the presence of light in *in vivo* and *in vitro* tests [4–10]. Among the ocular side effects of this group of antibiotics are local irritation and burning, hyperemia, eyelid edema, lid margin crusting, superficial punctate keratitis, corneal precipitation and perforation, blurred vision, and lacrimation [3]. Moreover there are reports about the risk of reduction in the transparency of the eye lenses due to the photopolymerization of α-cristallin and of damage to the lens epithelial cells [11]. An analysis of FQ with respect to their photoreactivity not only as single compounds but also as components of mixtures may provide new information in the photochemistry and new possibilities to increase the photosafety of pharmaceutical formulations. There are reports about the modification of FQ photoreactivity by a combination with various reactive oxygen species scavengers. Umezawa et al. [4] examined the influence of superoxide dismutase,

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catalase, sodium azide and 1,4-diazabicyclo-[2,2,2]-octane on the photodynamic calf thymus DNA strand-breaking activity of several FQ. The obtained results differed depending on the structure of the irradiated antibiotic. Bulera et al. [8] co-incubated the CHO cells with clinafloxacin and several antioxidants which reduced the hydroxyl radical formation but inhibited the photogenotoxicity only to a limited extent. These reports show the variety of results obtained by examining FQ photoreactivity in the presence of antioxidants. The reactive oxygen species scavengers may also be used as ingredients of ocular formulations. There are examples of the photoprotective potential of similar compositions. One of them was eye drops with p-coumaric acid that successfully reduced the photoirritation in rabbit eyes [12]. Moreover, these compounds may exhibit antimutagenic activity, as with bacterial assays [13]. These potential ocular excipients have never been investigated in combination with FQ. We supposed that an analysis of photodegradation, phototoxicity and photogenotoxicity of the above-mentioned composition would provide interesting data because of the previous reports about the photoprotective and antimutagenic potential of p-coumaric acid. Another way to reduce the degradation of photolabile drugs applied topically is to design innovative pharmaceutical formulations with chemical light-absorbers. As reported by Ioele et al. [14], the addition of sunscreens such as octisilate and octyl methoxycinnamate successfully inhibited the diclofenac photodegradation in gel formulations. Similar modifications were considered in some compositions of ocular preparations. A liquid sunscreen applied topically may block the harmful effect of UV irradiation and protect the sensitive surface of an eye. Cejka et al. [15,16] confirmed the photoprotective abilities of actinoquinol as an ocular light absorber through *in vivo* tests. They investigated the changes in corneal optics, hydration, and immunohistochemical conditions. Nowadays, eye drops with this ingredient and hyaluronic acid are available in the market registered as a medical device in Europe. Kek et al. [17] compared the efficacy of seven water- and oil- soluble compounds in eight possible ocular formulations applied directly on the surface of an eye *ex vivo*. They used an ocular spectrometer system to evaluate the changes in the transmission of UV radiation through the anterior eye. Significant increases in the absorption of the UV spectrum were detected in seven of the eight studied formulations, demonstrating their potential as topical ocular sunscreens. One of these compounds was benzophenon-4 (sulisobenzone). Except our latest work about ofloxacin in ointments [18], there are only few reports of a simultaneous evaluation of the photodegradation, photogenotoxicity, and phototoxicity of FQ in various pharmaceutical formulations with different excipients. We investigated four variants of ointments and obtained a significant reduction of ofloxacin photolysis in the ointment with bisoctrizole. Hubicka et al. [19] assessed the photodegradation of ciprofloxacin, moxifloxacin, norfloxacin and ofloxacin in the presence of excipients from tablets; however, they did not perform cytotoxicity or genotoxicity tests.

The main goal of this work was to investigate whether two potential excipients, namely, p-coumaric acid and benzophenone-4, could affect the photodegradation, phototoxicity and photogenotoxicity of water solutions of the four selected FQ. For this purpose, we conducted a set of bioassays combined with the application of high-performance liquid chromatography (HPLC) and mass spectrometry techniques.

2. Materials and Methods

2.1. Chemicals

Ciprofloxacin (CP) (CAS No. 85721-33-1), fleroxacin (FR) (CAS No. 79660-72-3), clinafloxacin (CL) (CAS No. 105956-97-6) and benzophenone-4 (BP-4) (CAS No. 4065-45-6) were purchased from Fluka. Lomefloxacin (LM) (CAS No. 98-79-52-8), p-coumaric acid (p-CA) (CAS No. 501-98-4), ethyl methanosulfonate (EMS) (CAS No. 62-50-0), 4-nitroquinoline N-oxide (4-NQO) (CAS No. 56-57-5) and 2-aminoanthracene (2-AA) (CAS No. 613-13-8) were purchased from Sigma. Acetic acid (CAS No. 64-19-7), neutral red (NR) (CAS No. 553-24-3) and ethanol (CAS No. 64-17-5) were purchased from POCh S.A. Acetonitrile (CAS No. 75-05-8) and methanol (CAS No. 67-56-1) were purchased from Merck. Trifluoroacetic acid (CAS No. 76-05-1) was purchased from J.T. Baker. Vectashield mounting medium with 40,6-diamidino-2-phenylindole (DAPI, CAS No. 28718-90-3) was purchased from Vector Laboratories. Sodium lauryl sulfate (SLS) (CAS No. 151-21-3) was purchased from BDH Chemicals.

2.2. Biological Cultures

Salmonella typhimurium (*S. typhimurium*) TA1535/pSK1002 was purchased from Deutsche Sammlung von Mikroorganismen Und Zellkulturen GmbH in Germany. The Chinese hamster lung fibroblasts cell line V79 (ATCC CCL-93™) was purchased from American Type Culture Collection.

2.3. Preparation and Irradiation of Tested Solutions

The four FQ investigated in this study were CP, LM, FR and CL. They were selected based on the differences in their chemical structure, *i.e.*, the increasing number of halogen atoms in the molecule connected to the increasing photolability of these compounds. All the compounds were water soluble. Two (CP and LM) of them were selected because of their application in ocular formulations. All the solutions were prepared in water or PBS. Solvents and the concentration range differed depending on the bioassay. Because of the very low range of tested concentrations in the bacterial assay, deionised water was chosen as a solvent to avoid the influence of other ions in the solution on the kinetics of the photodegradation process. Further, deionised water is a preferred negative control in the *umu* test according to the ISO protocol. For the neutral red uptake assay (NRU), the micronucleus test, and the methylthiazol tetrazolium reduction assay (MTT), all FQ were dissolved in PBS because of the relatively high sensitivity of mammalian cells to the changes in the medium osmolarity. In the bioassays, we could choose a relatively high range of FQ concentration; therefore, the influence of additional ions on the kinetics of the photodegradation process was acceptable. The concentrations of BP-4 and p-CA used in the mixtures with FQ were selected based on their preliminary toxicity and phototoxicity evaluation. If the range of nontoxic concentrations was wide, we selected the lowest concentration that exhibited a sufficient photoprotective effect in combination with at least one of the drugs. We also considered the available literature data [12,17]. BP-4 had good water solubility, and p-CA was diluted in water with ethanol (1:1). All the solutions were freshly prepared before every bioassay.

The samples were irradiated in the sunlight simulator SUNTEST CPS + (Atlas) with a 1500 W xenon lamp and maximum air cooling. The lamp emitted light in the UV–Vis (300–800 nm) wavelength range, and the radiation intensity was 58 mW/cm^2. The temperature inside the SUNTEST chamber had no effect on the FQ degradation at least during this experiment. The samples were irradiated in plugged quartz tubes just before each assay. The irradiation time differed among the compounds depending on their photolability. For the *umu* test, the solutions were exposed to light for 30 min (CP) or 5 min (LM, FR, and CL), which was sufficient for their total photodegradation without the excipients. For the NRU and MTT assay, because of the relatively high range of the tested concentrations, the photolysis was slower; therefore, we extended the irradiation time to 15 min (FR), 30 min (LM and CL), and 90 min (CP). FQ concentrations before and after light exposure were measured with HPLC.

2.4. Chromatographic Analysis

The drug concentrations during irradiation were monitored using a Shimadzu HPLC system with LC-10AT pumps, CTO-10AS column oven, and SPD-M10Avp photodiode-array detector. The column parameters