**INTRODUCTION**

Pharmaceutical compounds that absorb light in the UV/Vis range and give a positive result in an in vitro assay such as the 3T3-NRU-PT assay must be further evaluated for phototoxicity using an in vivo assay, as specified in the newly published final version of the ICH S10 guidance [1]. For compounds which reach the eye and absorb light above 400 nm, retinal phototoxicity must also be assessed. In addition, ICH S10 guidance suggests the use of pigmented strains, depending on the melanin-binding properties of the active principle to be tested.

**METHODS**

In the present study, pigmented Long Evans (LE) and albino Sprague-Dawley (SD) rats were used to assess the phototoxic potential of sparfloxacin (SPX) and 8-methoxypsoralen (8-MOP), two known melanin-binding phototoxic compounds, using qualitative and quantitative endpoints. The study design was adapted from the UV-LLnA protocol which is in routine use in our facility [2]. Groups of 5 rats received the test items by gavage for 6 consecutive days, accompanied by irradiation at 10 J UVA/Vis on days 4 to 6. Local reactions and clinical signs were noted each day. On day 7, animals were sacrificed, and corneal and auricular lymph nodes were sampled and weighed, and eyes were also sampled for microscopic examination. Before initiation of the study, a preliminary evaluation was performed to check that a 10 J UVA/Vis dose did not lead to any change in the skin or eye, and to ensure that the selected doses of SPX and 8-MOP were well tolerated.

**RESULTS**

Local reactions and post-mortem parameters:

No local reaction or statistically significant increase in lymph node weight (LNW), lymph node cell count (LNCC), and ear weight (EW) was noted in animals not exposed to UVA/Vis whatever the treatment. In animals receiving sparfloxacin and UVA/Vis exposure, local reactions were noted in both pigmented and non-pigmented strains, but at earlier time points and with higher severity in the albino strain. In addition, increases in lymph node weight, lymph node cell count and ear weight were of higher degree in albino rats than in pigmented rats. The sparfloxacin-related parameters were dose-related in LE rats. In the LE rats treated with 8-MOP and UVA/Vis exposure, dose-related local reactions such as erythema/edema were noted. Statistically significant increases in LNW, LNCC and EW were noted from the lowest dose level, and were dose-related.

Eye microscopic examination

We first demonstrated that the amount of UVA/Vis light delivered to the animal did not induce any damage to the retina and cornea structures. Histopathological findings of sparfloxacin-related ocular phototoxicity, consisting in atrophy/degeneration of the retina (loss of outer nuclear layer associated with single cells necrosis/apoptosis), were noted in albino rats only, and demonstrated the photoprotective effect of the melanin pigment in the iris and retinal pigmented epithelium (RPE) of pigmented rats. Ocular phototoxicity of 8-MOP was noted in LE rats in the corneal epithelium namely, with corneal hyperplasia, intercellular edema and single cell necrosis. No phototoxic effect was noted in the retina, highlighting the protective role of melanin contained in the iris and retina at the level of the RPE, since the energy of incoming photons is absorbed and dissipated in these cells.

**CONCLUSION**

The photopotentioal of both chemicals was demonstrated in pigmented LE and albino SD rats. Findings of systemic phototoxicity were noted in both strains, with greater severity in albino rats. Ocular phototoxicity was never noted at the RPE level in LE rats, but only at the corneal level. These results show that an albino strain is more sensitive than a pigmented strain to the phototoxicity of these two drugs. The UV-LLnA protocol in pigmented rats can provide a useful approach to the determination of the phototoxic potential. It may instead provide a more relevant model for humans than albino models as ocular phototoxicity has not been reported in humans with these two chemicals.