Repair of Sparfloxacin-Induced Photochemical DNA Damage *In Vivo*

Melanie Struwe[1,2], Karl-Otto Greulich[2], Elias Perentes[3], Hans-Jörg Martus[1], Willi Suter[1] and Ulla Plappert-Helbig[1]

The induction and subsequent repair of photochemically induced DNA damage by sparfloxacin was assessed in different tissues of juvenile Wistar rats. The animals were treated once orally with 500 mg kg^{-1} of sparfloxacin and irradiated 3 hours later with 7 J cm^{-2} UVA. Induction and repair of DNA damage was studied in the skin, retina and cornea using the alkaline comet assay. After a tissue-specific increase in the initial DNA damage (higher in the cornea than in skin and retina), an exponential decrease was found in the skin and retina, whereas in cornea a further increase of the DNA damage after 1 hour followed by an exponential decrease was observed. The half-lives for DNA repair were approximately 3 hours for skin and retina and 1 hour for cornea. After a recovery time of 6 hours, the majority of the induced DNA damage detectable with the comet assay had been removed. In conclusion, the data indicate that (1) photochemically induced DNA damage by sparfloxacin is efficiently removed in skin, retina and cornea, (2) repair of these DNA lesions follows an exponential decrease, (3) the induction and repair of sparfloxacin-mediated photochemical DNA damage might be tissue specific.

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INTRODUCTION

Sparfloxacin belongs to the class of fluoroquinolone antibiotics, which are used to treat various bacterial infections by inhibiting bacterial DNA synthesis (Hooper, 1999). An undesirable side effect of many fluoroquinolones is their photocytotoxic, -allergic and -genotoxic effects, which has been demonstrated in different *in vitro* (Chetelat *et al.*, 1996; Zhang *et al.*, 2003; Neumann *et al.*, 2005; Struwe *et al.*, 2007) and *in vivo* models (Makinen *et al.*, 1997; Itoh *et al.*, 2002; Zhang *et al.*, 2003, 2004; Neumann *et al.*, 2005; Yabe *et al.*, 2005) as well as in clinical studies (Ferguson, 1995; Arata *et al.*, 1998; Tokura, 1998; Pierfitte *et al.*, 2000; Dawe *et al.*, 2003). Fluoroquinolone administration in combination with the exposure to UVA light was furthermore shown to cause morphological changes in the retina (Shimoda *et al.*, 1993, 2001; Thompson, 2007). In contrast to the aforementioned studies on the induction of photochemical DNA damage, very little is known about DNA repair *in vivo*, particularly in ocular tissues such as cornea and retina. In a previous study, (Struwe *et al.*, 2008), we demonstrated the photogenotoxic effect of sparfloxacin in the skin and eye (retina and cornea) of rats. Furthermore, fluoroquinolones are known to enhance the incidence and shorten the latent period of UVA-induced skin tumors (Johnson *et al.*, 1997; Klecak *et al.*, 1997; Makinen *et al.*, 1997). Thus, the efficient removal of DNA lesions is one important mechanism to prevent tumorigenesis in light-exposed tissues. In general, photochemical DNA modifications are formed in parallel with UV induced DNA lesions, due to the UV-irradiation needed to excite or activate a photosensitizing compound. The induction and repair of UV-induced DNA damage *in vitro* has been extensively studied (Cadet *et al.*, 1997; Griffiths *et al.*, 1998; Ravanat *et al.*, 2001).

The phototoxic potential of fluoroquinolones has been mainly associated with the generation of reactive oxygen species, such as hydroxyl radicals, singlet oxygen, and superoxide that have been directly detected upon irradiation (Umezawa *et al.*, 1997; Araki and Kitaoka, 1998). The phototoxic effect of fluoroquinolones is strongly dependent on the C-8 substituent at the quinolone ring. Although a fluoro-substituent confers a strong phototoxicity, hydrogen- and methoxy substituent have little effect (Yabe *et al.*, 2005). Sparfloxacin has a fluoro-substituent that is eliminated upon exposure to UV light (Engler *et al.*, 1998). In consequence, a highly reactive carbene at the C-8-position is formed that can cause direct DNA single-strand breaks. Furthermore, hydrogen peroxide may also be generated that is converted to hydroxyl radicals by the Fenton reaction (Nakatani *et al.*, 1995; Martinez *et al.*, 1997, 1998). Besides oxidatively generated DNA damage (such as oxidized bases, abasic sites, single-strand breaks), fluoroquinolones have also been demonstrated to photosensitize pyrimidine dimer formation

[1]*Novartis Pharma AG, Preclinical Safety, Genetic Toxicology and Safety Pharmacology, Basel, Switzerland;* [2]*Leibniz Institute for Age Research—Fritz Lipmann Institute, Single cell and single molecule techniques, Jena, Germany and* [3]*Novartis Pharma AG, Preclinical Safety, Toxicologic Pathology, Basel, Switzerland*

Correspondence: Dr Ulla Plappert-Helbig, Novartis Pharma AG, Preclinical Safety, Genetic Toxicology and Safety Pharmacology, MUT-2881.5.14, CH-4002 Basel, Switzerland. E-mail: ulla.plappert_helbig@novartis.com

Abbreviations: CMC, carboxymethylcellulose; $t_{1/2}$, repair half-life

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(Traynor and Gibbs, 1999; Sauvaigo *et al.*, 2001; Lhiaubet-Vallet *et al.*, 2007). As the alkaline comet assay detects a variety of these DNA modifications, either directly or indirectly due to DNA repair processes with high sensitivity, it was considered to be the appropriate system for the present study (Bock *et al.*, 1998; Collins, 2007).

The aim of this study was to investigate DNA repair in skin and two ocular tissues, that is retina and cornea, of Wistar rats after a single oral dose of sparfloxacin and subsequent irradiation with UVA light.

RESULTS

Clinical symptoms and histopathology

During the experiment, animals were monitored for clinical signs of toxicity. Clinically, irradiation (7 J cm^{-2} UVA) of the animals treated with 500 mg kg^{-1} sparfloxacin caused erythema of the skin, ears, and partly the tail, possibly due to a hyperthermic reaction (vasodilatation).

As cytotoxicity may cause DNA fragmentation, for example, due to the activation or release of endonucleases in necrotic or apoptotic cells (Henderson *et al.*, 1998; Quintana *et al.*, 2000), samples from all tissues were examined by histopathology, as recommended in the literature (Hartmann *et al.*, 2003; Burlinson *et al.*, 2007). In the skin of the irradiated vehicle group, the epidermal cells presented a minimal degeneration after 6 hours. In the sparfloxacin-treated groups, increased incidence and/or severity of epidermal cell degeneration/necrosis/apoptosis and koilocytosis were observed in sparfloxacin-treated animals after 3 hours. These findings were more pronounced after 6 hours. In the eyes, no alterations within 6 hours following irradiation were observed. The main histopathological findings are summarized in Table 1.

Previously, we demonstrated that 1% carboxymethylcellulose (CMC) and a higher dose of sparfloxacin (1,000 mg kg^{-1}) without or with irradiation induced no histopathological alterations directly after the exposure to 7 J cm^{-2} UVA (that means 0 hour of recovery time; Struwe *et al.*, 2008). Thus, in this study, histopathological analysis was only done 3 and 6 hours after irradiation. As the identified alterations were neither observed in all animals nor in all parts of the tissues and with only very few or slight/few grading a cytotoxicity impact on the comet assay was excluded.

Table 1. Histopathology analysis of the skin following irradiation after 3 and 6 h recovery time

	1 % CMC		500 mg kg^{-1}	
	3 h	6 h	3 h	6 h
Koilocytosis	—	—	2 (1–2)	2 (1–2)
Degeneration (epidermis)	—	1 (1)	2 (1)	3 (2)
Inflammation	—	—	—	3 (1)
Atrophy	—	—	—	1 (1)

CMC, carboxymethylcellulose.
Numbers given represent number of animals showing the respective finding; numbers in brackets indicate the respective histopathological grading: (1) minimal/very few; (2) slight/few; *n*=3 animals for all other groups.

DNA damage and repair

The irradiation of Wistar rats treated with 500 mg kg^{-1} sparfloxacin induced a significant increase in tail moment in the skin, retina, and cornea as compared to the irradiated vehicle group (0 mg kg^{-1}, that is 1% CMC). The absolute tail moment values as well as the fold-increase between the vehicle-treated and the sparfloxacin-treated irradiated groups were highly tissue specific (Figure 1). In comparison to the vehicle treatment, sparfloxacin induced an increase of the tail moment in the cornea by a factor of 68, followed by the skin (factor 25) and the retina (factor 9).

The induced DNA damage decreased in all tissues within 6 hours after the irradiation (Figure 2). Although in the skin and retina the maximum tail moment was observed directly after the irradiation (0 hour), for the cornea an increase of the tail moment within 1 hour after the irradiation was detected. However, after 6 hours the tail moment for the cornea was at the same level as for the irradiated vehicle-treated animals. For the skin and retina, the tail moments observed were slightly higher than in the respective irradiated vehicle control group. Although the tail moments in the irradiated vehicle control groups remained stable between 0 and 6 hours of recovery time, it was concluded that a dose of 7 J cm^{-2} UVA light alone induced no remarkable DNA damage in the analyzed tissues. Mathematical analysis, that means regression analysis showed an exponential decline of the maximum-induced DNA damage in the comet assay in all three tissues. The DNA repair half-lives were similar in the skin and retina (skin: 187 ± 9 minutes, retina: 187 ± 21 minutes, *n*=3 animals per group), but clearly lower in the cornea. As in the cornea an increase of the tail moment was observed within 1 hour after irradiation, the regression analysis was done from 0 to 6 hours recovery time and from 1 to 6 hours. The repair half-lives observed were 68 ± 9 min-

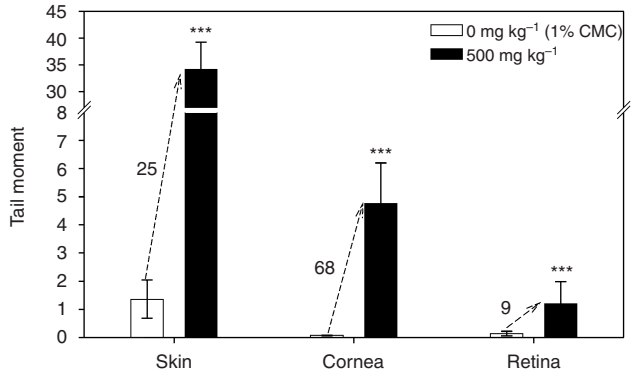

Figure 1. DNA damage in skin, retina and cornea following the irradiation with 7 J cm^{-2} UVA from animals treated with the vehicle (1% CMC, white bars) or with sparfloxacin (500 mg kg^{-1}, black bars). The arrow indicates the factor by which the tail moment increased from the vehicle to the sparfloxacin-treated group (mean ± SD, *n*=3), ***$P<0.001$ *vs* 0 mg kg^{-1} (one-way ANOVA).