Ultrastructure of Achilles tendon from rats after treatment with fleroxacin

Abstract Quinolone therapy can be associated with tendon disorders (tendinitis, ruptures), but little is known about possible ultrastructural changes in tendons after exposure to these antimicrobials. We studied the Achilles tendons from fleroxacin-treated adult rats by electron microscopy. Wistar rats were treated orally with single oral doses of 0, 30, 100, 300 or 600 mg fleroxacin/kg body weight \((n = 6 \text{ per group})\). The animals were killed 4 weeks after treatment, Achilles tendon samples were collected and tangential sections were made from the distal part of the tendon. Subsequently, tendons were cut crosswise for preparation of ultrathin sections. Samples were fixed by using glutaraldehyde, osmium tetroxide, tannic acid and finally contrasted with uranyl acetate/lead citrate before they were examined by transmission electron microscopy. The rats did not show any general effects such as behavioural changes or body weight changes which could be attributed to the treatment. However, we were able to detect pathological changes even at the lowest dose level (30 mg/kg), which increased in incidence and severity with increasing doses. Tenocytes exhibited degenerative changes such as multiple vacuoles and large vesicles in the cytoplasm that resulted from swelling and dilatation of cell organelles (mitochondria, endoplasmic reticulum). The nucleus became dense and the chromatin had clumped to form rough plaques. The cells detached from the extracellular matrix. Other important findings were a general decrease of the fibril diameter and an increase in the distance between the collagenous fibrils. The finding that these rather low single doses of a fluoroquinolone induce ultrastructural changes in Achilles tendons from rats, which were not associated with clinical symptoms and which were still present 4 weeks after treatment, is of concern. Further toxicological as well as clinical studies are needed to characterize the conditions under which quinolone-induced tendon lesions develop.

Key words Achilles tendon · Rat · Fleroxacin · Fluroquinolones · Electron microscopy

Introduction

Fluoroquinolone-induced tendon disorders have been reported since 1983 when Bailey and coworkers described the development of tendinitis during treatment with norfloxacin in two patients after renal transplantation (Bailey et al. 1983). Similar case reports have been published since then for all widely used fluoroquinolones (e.g. Carrasco et al. 1997; Lewis et al. 1999). French authors have described several hundred patients who suffered from tendinitis or tendon rupture after treatment with fluoroquinolones; most cases were associated with pefloxacin use, while other cases occurred after treatment with ofloxacin, norfloxacin or ciprofloxacin (Pierfitte and Royer 1996). Although this represents the largest collection of cases, it is not possible to make any valid statement on the incidence of quinolone-induced tendon disorders on the basis of these data because they do not derive from targeted studies.

The epidemiological situation is complicated by two main aspects. Firstly, the potential of an antibacterial drug to induce tendon disorders is very unusual and not generally known, although it is mentioned in the drug datasheets; secondly, it has been reported that a long asymptomatic period of up to 3 or even 5 months can occur after treatment with fluoroquinolones before symptoms of tendopathy arise (Royer et al. 1994; Pier-
fitte and Royer 1996). With this epidemiological background, data from toxicological experiments are of major importance for clarification of the issue.

So far, very few toxicological data are available. In juvenile rats treated with pefloxacin or ofloxacin, Kato and coworkers described oedema with mononuclear cell infiltration mainly in the inner sheath of the inner Achilles tendon (Kato et al. 1995). In a further study, Kashida and Kato (1997) compared the tendon toxicity of ten fluoroquinolones and found that in their model fleroxacin and pefloxacin were the most toxic derivatives.

Using electron microscopy we have recently shown that characteristic changes of the tenocytes as well as the matrix of rat Achilles tendon can be induced by ofloxacin at the rather high dose level of 1200 mg/kg (Shakibaei et al. 2000). Using a modified fixation technique, we improved the level of sensitivity and performed extensive electron microscopy on tendon samples from fleroxacin-treated rats in order to study the quinolone-induced tendon changes in detail at various drug exposure levels.

Materials and methods

Treatment with fleroxacin

Female Wistar rats were kept in Macrolon cages at a temperature of 21 ± 1°C, a relative humidity of 50 ± 5% and a constant light/dark cycle (light from 900 to 2100 hours). Body weight of the rats was 197 ± 12 g (mean ± SD); they received a standard diet (Altromin 1324, Lage, Germany). For treatment with fleroxacin, commercially available tablets (Quinodis, Roche, Basel) containing 200 mg of the drug were suspended in 2% starch solution at a final concentration of 30, 100, 300 or 600 mg fleroxacin/10 ml (n = 6 per group). The freshly prepared suspension was administered by gastric intubation at a volume of 10 ml/kg body weight. Controls received starch solution only.

The rats were killed 4 weeks after treatment and Achilles tendon samples were collected and studied by electron microscopy.

Transmission electron microscopy (TEM)

Achilles tendon samples were prepared from the right foot of animals from each treatment group. Tangential sections were made from the distal part of the tendon by using a razorblade. Subsequently, these tendons were cut crosswise for preparation of ultrathin sections. All samples were fixed in 1% glutaraldehyde with 1% tannic acid in 0.1 M phosphate buffer, pH 7.4, and post-fixed in 1% osmium tetroxide in phosphate buffer. After rinsing and dehydration in ethanol, the preparations were embedded in Epon (Plano, Marburg, Germany), cut with an Ultracut E ultramicrotome (Reichert) and the sections stained with 2% uranyl acetate/lead citrate. The specimens were examined under a transmission electron microscope (Zeiss EM 10).

Results

Our experiments were performed over a wide range of doses (30, 100, 300, and 600 mg fleroxacin/kg body weight). Even at the highest dose used, the rats did not show any general effects such as changes in behaviour or body weight which could be attributed to the treatment. In comparing the doses administered with those used in human therapy, the pharmacokinetic differences in the two species should be considered.

Surprisingly, we were able to detect pathological changes in all samples studied even at the lowest dose level which roughly corresponds to the exposure levels of fleroxacin in humans during therapy. The incidence and severity of changes increased dose-dependently. Figures 1, 2 and 3 show a series of electron micrographs depicting tendon material from controls and fleroxacin-treated rats.

Examples taken from control rats are shown in Fig. 1A, B. The mechanically important structural components of control tendons were strong collagenous fibres that consisted of fibrils of varying diameter. Additionally, elastic structures were observed. Tenocytes were arranged in series and formed plate- and finger-like long processes that were embedded between collagenous

Fig. 1A, B Achilles tendon of control rat (no quinolone treatment). Electron micrographs of tenocytes (T) with plate-like processes, embedded in cross-sectioned fibrils (asterisk). The cells contain a well-developed rough endoplasmic reticulum, a large nucleus (N) with much loosely packed, de-spiralized and functionally active euchromatin and little dense, functionally inactive heterochromatin. The cells produce much pericellular material (arrows). A close contact exists between cell and pericellular matrix. A x10,000, B x20,000