Chondrotoxicity of Quinolone Antimicrobial Agents

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Abstract: Quinolone antimicrobial agents induce two types of histological changes in the articular cartilage of synovial joints in immature, but not mature, laboratory animals. The first is cavity formation in the middle zone of the articular cartilage in animals of all species examined so far, and this can be induced by a single or several daily doses. The second is osteochondrotic lesions in the caudal femoral condyles of rats when subchronic or chronic treatment is started at a juvenile age, but there seems to be only one reference for this effect. The development process of cavity formation is as follows. Degeneration and necrosis of chondrocytes are first observed, and the surrounding matrix becomes edematous with demasked collagen fibrils and decreased safranin O stainability. A flat cleft or cavity is then formed in the edematous cartilage, and erosion is sometimes produced by detachment of the cavity outer wall. The potency of quinolones to form chelate complexes with Mg$^{2+}$ is discussed as causal for cavity formation. This could lead to Mg$^{2+}$ deficiency in the cartilage, resulting in integrin alteration and further radical formation that can finally induce cartilage lesions. On the other hand, an early change of osteochondrosis is a thickened middle zone of the articular cartilage with a thinned deep zone. The thickened cartilage protrudes into the epiphysis and prevents age-dependent thinning of the cartilage. In advanced cases, fissures are formed in the bottom of the thickened cartilage, and extension of the fissures to the cartilage surface induces detachment of the cartilage, subchondral bone necrosis and fibrotic lesions in the marrow space. The lesions are considered to be caused by quinolone-induced inhibition of the differentiation of chondrocytes in the middle zone into those in the deep zone. For chondrotoxicity in juvenile animals, the pediatric use of quinolones is generally contraindicated, but whether or not chondrotoxicity could really occur in pediatric patients is still controversial. (J Toxicol Pathol 2008; 21: 123–131)

Key words: quinolone, chondrotoxicity, cavity formation, osteochondrosis, juvenile animal

Introduction

Fluoroquinolones are widely used antimicrobial agents because of their broad spectrum activity and favorable pharmacokinetics. Adverse drug reactions (ADRs) to quinolones in the human skeletal system include arthralgia, joint swelling, arthropathy and arthritis, but the incidence and degree are low and they are reversible after discontinuation of the treatment. Overall, fluoroquinolones are relatively safe and well-tolerated drugs. In 1962, nalidixic acid was first introduced as a therapy for malaria and was followed by oxolinic acid, pipemidic acid and others as 1st generation drugs. These quinolones have activity on Gram-negative bacteria. Since the late 1970s, the fluorinated quinolones norfloxacin, ofloxacin, ciprofloxacin, pefloxacin and others have been synthesized as 2nd generation drugs. These drugs are characterized by expansion of their spectrum activity to Gram-positive bacteria and by improved tissue distribution. Subsequently, up to the present, many 3rd and 4th generation quinolones have been developed, although several drugs have been withdrawn from the market due to serious ADRs. Ingham et al. first reported gait alterations and chondrotoxic lesions (cavity formation) in juvenile dogs receiving nalidixic acid, oxolinic acid or pipemidic acid. The available information indicates that all quinolones (fluorinated or non-fluorinated) have the potential to induce joint cartilage lesions in immature, but not mature, animals of all of the species examined so far. The doses needed to induce cartilage damage in juvenile dogs fall in the range of the therapeutically used doses, and therefore quinolones are contraindicated for children and adolescents, as well as for women during pregnancy and while breast feeding. Furthermore, cartilage lesions were confirmed to be induced in juvenile rats at doses leading to plasma concentrations in the range of relevant concentrations in humans. However, ciprofloxacin has been approved for use in pediatric patients with cystic fibrosis, and norfloxacin has been approved for pediatric use in Japan after confirmation of their safety, especially in relation to the skeletal system in large scale...
clinical studies. On the basis of the benefit of quinolones, it is still controversial whether pediatric patients are at risk of arthropathy and under what circumstances therapy with quinolones would be appropriate for children.

Quinolones induce two types of histological lesions, cavity formation and osteochondrosis, in the articular cartilage of immature animals. In this review, the author introduces reference information on the general aspects, histological findings, hypotheses for the mechanisms, and a risk assessment for pediatric use in relation to both types of lesion.

**Chondrotoxicity of Quinolones**

**Cavity formation**

Animal species: Cavity formation in the immature articular cartilage has been reported in mice, rats, dogs, rabbits, marmosets, guinea pigs, ferrets, and hamsters. Dogs are generally more sensitive to this than the other species. Although there seem to be no reports focusing on sex differences in susceptibility to quinolone chondrotoxicity, lesions in the articular cartilage have been demonstrated in juvenile dogs and rats of both sexes in some reports.

Ages: The occurrence of cartilage lesions is limited to juvenile animals, but the susceptibility to chondrotoxicity is restricted to a relatively short period of postnatal development in dogs and rats. Pipemidic acid induces histological lesions in 4-week to 9-month-old dogs, but not in dogs at the age of 2 or less weeks or 12 or more months of age. Ofloxacin-induced lesions are observed in juvenile rats between 3 and 6 weeks of age, but not in 8-week-old rats. A high rate of growth during the above-mentioned periods could be a contributing factor. However, the higher and lower limits of age could be somewhat modified by the experimental conditions. Pefloxacin, however, is the reported exception to the "juvenile rule", as histological changes were observed in both juvenile and adult dogs after prolonged administration.

Clinical signs: Severe clinical signs are seen in dogs. Facial and auricular edema are observed shortly after administration of a quinolone, and this is thought to be due to vasodilation caused by histamine release. As the treatment progresses, dogs show joint swelling and pain, a staggering gait, and in severe cases, unwillingness to stand and walk, but these signs gradually decrease in severity and disappear despite continued dosing.

Location of lesions: The weight-bearing regions are suggested to be important in determining the location of lesions in dogs. Cartilage lesions are observed in the articular cartilage of the shoulder, elbow, wrist, hip, knee, and ankle joints, but the predilection sites are the proximal humerus and proximal and distal femur in dogs. In rats, the lesions are seen in the distal humerus and femur, and sometimes in the proximal femur, tibia and tarsal bone. In the femoral condyles, the lesions are observed in a site close to the femoral trochea in rats (Fig. 1). Additionally, the epiphyseal growth plate has also been reported to be damaged in newborn or very young animals. Moxifloxacin induces a change in 10- to 12-week-old dogs but not in 18- to 22-week-old dogs; trovafloxacin does so in rats when the treatment is started at 4 days of age, associated with irreversible effects on postnatal growth. Ofloxacin has also been reported to induce cleft formation in the epiphyseal growth plate in 1 knee joint in 7 rats when orally administered from 32 to 36 days of age. However, no changes were observed in the body weights and clinical appearances of rats used in a 26-week repeated dose toxicity study of the drug. Further studies are required to investigate the details of quinolone toxicity on the epiphyseal growth plate.

Macroscopic findings: In dogs, solitary or multifocal protruding areas, so-called blisters, are seen mostly bilaterally on the articular cartilage of the epiphyses of long bones at necropsy. Severe cases show erosion resulting from desquamation of the outer wall of the blister and an increased amount of slightly turbid synovial fluid. Magnetic resonance imaging (MRI) of the joints of affected immature rabbits revealed thickened articular cartilage, surface irregularities consistent with ruptured vesicles and separation of opposing articular surfaces consistent with synovial effusion. An irregular cartilage surface and dissecant changes are also observed in the distal femoral condyle of immature dogs receiving ofloxacin. Consequently MRI has been recommended for monitoring the joints of juvenile patients receiving ciprofloxacin.

Microscopic findings: The development process and early changes of cavity formation were examined in vivo many years ago. After administration of quinolones, early lesions appear very quickly in the middle zone of the articular cartilage in rats or in the intermediate zone of the articular-epiphyseal-cartilage complex in dogs. Degeneration and necrosis of the chondrocytes with disintegrated cytoplasms are observed in 4-week-old rats 5 h after a single oral administration of a very high dose of ofloxacin. These changes are replaced by an edematous area of the cartilage matrix containing degenerative or necrotic chondrocytes at 8 and 12 h. In this area, the collagen fibrils are demasked, tend to run parallel to the articular surface, accompanied by markedly decreased stainability of the matrix with safranin O. In further progressed lesions at 24 h, a flat cleft or cavity (liquefaction of the cartilage) running parallel to the articular surface is formed in the center of the edematous cartilage (Fig. 2, 1 week treatment with levofloxacin). The cavity occasionally contains tissue debris. The outer wall cartilage of the cavity is sometimes devoid of chondrocytes, protrudes into the joint space and is detached resulting in erosion formation of the cartilage. At 48 h, proliferation (cluster formation) of chondrocytes surrounding the cavity is frequently observed.

As early changes observed under an electron microscope, mitochondrial swelling and vacuolation in the chondrocytes are seen in dogs receiving difloxacin. These changes precede necrosis of chondrocytes, and
disruption of the matrix appears secondarily to the necrosis. Mitochondrial swelling and/or dilated cisternae of the rough endoplasmic reticulum (rER), containing moderately electron-dense substances, are also seen in the chondrocytes of rats receiving ofloxacin.

With regard to in vitro morphological changes, difloxacin damaged the chondrocytes but not the matrix in an organ culture of dog cartilage. The chondrocytes showed cytoplasmic vacuolation and distention of the rER with intracisternal dense substances, which were probably non-secreted protein. On the other hand, a decreased number of collagen fibrils with an increased number of dead chondrocytes were induced by ofloxacin in a cartilage organoid culture of mouse limb-bud mesenchymal cells.

Corresponding to the macroscopic findings, an increased amount of synovial fluid, inflammation, hemorrhagic synovitis, an increased number of chondrocytes were induced by ofloxacin in a cartilage collagen fibrils with an increased number of dead chondrocytes and surface necrosis in the synovial membrane are observed in dogs.

Reversibility: The author has examined the reversibility of cartilage lesions in rats receiving oral ofloxacin for 8 weeks (from 4 to 12 weeks of age). After completion of dosing, eroded defects of the articular cartilage, confirmed on day 8 of dosing, are filled with a matrix containing few or no cells with a slightly irregular cartilage surface. After this kind of long-period treatment, it then becomes difficult or impossible to find lesions at necropsy. Thus, the cartilage lesions reveal a tendency toward recovery even during continued administration of quinolone in rats. However, reversibility of the cartilage lesions has been reported to be incomplete after various recovery periods of up to 17 weeks in rats and up to 87 days in dogs.

Mechanisms: The exact mechanism of quinolone chondrotoxicity is still unknown, although many explanations have been postulated.

Chondrocytes require interaction with their extracellular matrix via integrins for proper function. Integrins connect extracellular matrix proteins with the intracellular cytoskeleton and transmit signals in both directions across the plasma membrane. Extracellular divalent cations (Mg$^{2+}$, Mn$^{2+}$) are important regulators of integrin-ligand-binding activity. Thus, integrins regulate cell adhesion, growth, proliferation, differentiation, migration and proteoglycan synthesis.

Quinolones form chelate complexes with divalent and trivalent cations, and their affinity for magnesium is generally more pronounced than for manganese. Through a magnesium ion, quinolones bind to the DNA-gyrase complex and inhibit DNA synthesis to kill bacteria. For chondrotoxicity, the chelate complex formation of quinolones with magnesium has also been explained as the most plausible of the postulated mechanisms. This could induce a deficit in functionally available extracellular magnesium in the articular cartilage and subsequently changes in integrin receptor functions on the chondrocyte surface, resulting in impaired attachment of the chondrocytes to extracellular matrix proteins. Disruption of cell-matrix contact decreases proteoglycan synthesis and increases fibronectin production in the joint cartilage. The presence of fibronectin fragments upregulates matrix metalloproteinase synthesis via signal transduction through chondrocyte integrin receptors, leading to matrix degradation. These processes may be included in cavity formation in the cartilage. Recently, the development of apoptosis of chondrocytes resulting from integrin disturbance has also been reported.

Another consequence of magnesium depletion seems to be the production of oxygen-derived reactive species. The reactive species are formed as a result of modifications in the mitochondrial and respiratory activities of chondrocytes, and they act as activators of latent metalloproteinases or can be directly toxic against cartilage matrix components for matrix degradation or turnover. However, an excessive amount of reactive species could irreversibly damage the chondrocytes and/or cartilage matrix and lead to cavity formation.

Experimental data supporting the above-mentioned hypotheses, or which have been reported independently of the hypotheses, are described below.

Magnesium deficiency—Juvenile dogs fed a magnesium deficient diet show gait alterations, and a slight increase in fibronectin staining, and swollen mitochondria and an enlarged ER in the chondrocytes in the intermediate cartilage zone. Feeding juvenile rats a magnesium-deficient diet also induces lesions in the articular cartilage that are even more pronounced when combined with quinolone treatment. These changes are similar to those induced by quinolones. Meanwhile, quinolone-induced cartilage lesions can be diminished by feeding rats a magnesium- and/or vitamin E-enriched diet.

Human chondrocytes cultivated in ciprofloxacin-supplemented or Mg$^{2+}$-free (-deficient) medium exhibit decreased ability to adhere to collagen type-II coated coverslips (not by cytotoxicity) with the cells becoming spindle in shape, alterations in f-actin (stress fibers) formation and cytoskeletal fiber (vimentin) organization and reduced cell proliferation; these changes may be interpreted as consequences of disturbed cellular adhesion via integrins. These findings have also been confirmed in the chondrocytes of juvenile rats. On the other hand, supplementation with magnesium can reduce the toxic effects of quinolones on chondrocytes in vitro.

Integrin alteration—The expression of integrins is reduced on chondrocytes adjacent to fissures in the immature joint cartilage of rats after ofloxacin treatment or after being fed a magnesium-deficient diet. Slightly reduced expression of α$V$ integrin on chondrocytes is exhibited in juvenile dogs receiving difloxacin in vivo, and the expression of other integrins are also decreased in ofloxacin-treated mouse chondrocytes in vitro. When chondrocytes from juvenile rabbit joint cartilage are cultured with ofloxacin in alginate microspheres, β$1$-integrin expression is primarily reduced, and this causes subsequent changes in the extracellular signal-regulated kinase 1/2 / mitogen-activated protein kinase signaling pathway,
resulting in caspase-8-dependent apoptosis of chondrocytes; supplementation with Mg^{2+} blocks the changes in integrin and apoptosis^{46}.

Oxidation and mitochondrial change—In an ex vivo study using the chondrocytes of rabbits treated with ofloxacin or pefloxacin, an increase in cellular respiratory burst was observed in young animal cells, but not in adult animal cells, followed by decreased mitochondrial activity (suggestive of an injured membrane) and an increased mitochondrial mass (increased membrane area)^{47}. In vitro, pefloxacin, ofloxacin, nalidixic acid and/or ciprofloxacin also induce an early stimulation of oxidative metabolism in immature, but not mature, chondrocytes^{48,49}. The above

Fig. 1. The ventral aspect of the distal femoral epiphysis of a rat, showing the predilection sites of cavity (blister) formation and the sectioning plane for Fig. 2.

Fig. 2. The articular cartilage of the femoral condyle of a rat receiving oral levofloxacin for 1 week. A flat cleft (arrows) is formed in the middle zone, and the outer wall protrudes partially from the cartilage surface. H-E stain.

Fig. 3. The caudal aspect of the distal femoral epiphysis of a rat, showing the predilection site of the osteochondrotic lesions and the sectioning plane for Figs. 4 and 5.

Fig. 4. The femoral condyle of a rat receiving oral levofloxacin for 4 weeks. The middle zone (M) of the articular cartilage is thickened and the deep zone (D) is thinned; these are accompanied by a small fibrotic lesion in the marrow space (arrow). H-E stain.

Fig. 5. The femoral condyle of a rat receiving oral levofloxacin for 4 weeks. The thickened articular cartilage is detached from the surrounding cartilage (arrow) by an extension of the cleft (C) to the cartilage surface and is accompanied by necrotic areas (N) and extensive fibrotic lesions (F). H-E stain.
results indicate that oxygen-derived reactive species that are highly toxic to cartilage matrix components could be generated. On the other hand, vitamin E supplementation prevents quinolone-induced cartilage lesions in juvenile rats36,37.

Regarding mitochondrial changes, electron microscopy revealed swelling of mitochondria in the chondrocytes of juvenile dogs treated with difloxacin9,25. In an in vitro study using juvenile dog chondrocytes, nalidixic acid, ciprofloxacin, and three other quinolones inhibited mitochondrial dehydrogenase activity with inhibited proteoglycan synthesis50. Compromised mitochondrial integrities have also been shown in other in vitro experiments51,52.

Changes in glycosaminoglycan and DNA synthesis and fibronectin—A single administration of pefloxacin to mice decreases biosynthesis of proteoglycan for the first 24 h, with recovery 48 h later. The decrease is also observed ex vivo, suggesting a direct effect of pefloxacin on this process, and a 10-day treatment induces oxidative damage in collagen, which may be induced by oxygen-derived reactive species in the extracellular matrix53. Moreover, in many in vitro and ex vivo studies, the cartilage of various animal species has exhibited inhibition of synthesis of either collagen or glycosaminoglycans28,29,50–57 or proteoglycan and DNA28,50–52,56. An increase in fibronectin staining is observed in the vicinity of the cavity in the articular cartilage of juvenile dogs and rats receiving difloxacin and ofloxacin, respectively41,42.

Age specific susceptibility to quinolone chondrotoxicity—Feeding a magnesium-deficient diet to rats induces cartilage lesions in animals between 3 and 5 weeks of age, which correlates well with the ages sensitive to quinolone chondrotoxicity, but not of other ages. In 4-week-old rats, the magnesium concentration in the cartilage of the quinolone chondrotoxicity—Feeding a magnesium-deficient diet to juvenile dogs treated with difloxacin9,25. In an in vivo study using juvenile dog chondrocytes, nalidixic acid, ciprofloxacin, and three other quinolones inhibited mitochondrial dehydrogenase activity with inhibited proteoglycan synthesis50. Compromised mitochondrial integrities have also been shown in other in vitro experiments51,52.

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Age specific susceptibility to quinolone chondrotoxicity—Feeding a magnesium-deficient diet to rats induces cartilage lesions in animals between 3 and 5 weeks of age, which correlates well with the ages sensitive to quinolone chondrotoxicity, but not of other ages. In 4-week-old rats, the magnesium concentration in the cartilage of the femoral condyle shows a whitish discoloration, and there is a plaque with a partially detached margin or ulceration of the cartilage. Histologically, during the course of ofloxacin treatment of rats for 8 weeks, the early change is a thickening of the middle zone of the articular cartilage with a thinned deep zone. As administration continues, the columns of chondrocytes in the thickened middle zone become more and more numerous, many degenerated cells are seen and the staining intensity of the cartilage matrix with safranin O decreases slightly. After completion of treatment, the articular cartilage is markedly thickened and made up of mainly middle zone cartilage, protruding into the epiphysis with prevention of age-dependent thinning of the articular cartilage (Fig. 4, 4 weeks treatment with levofloxacin). However, the deep zone sometimes remains thick. In advanced cases, fissures or clefts are formed in the bottom of the thickened cartilage. Extension of the fissure to the cartilage surface causes the thickened cartilage to become detached (Fig. 5, 4 weeks treatment with levofloxacin), resulting in ulceration of the articular cartilage. Beneath the ulceration, there are focal necrosis of the subchondral bone and fibrotic lesions in the marrow space.

These pathologic lesions are considered to be caused by quinolone-induced inhibition of the differentiation of chondrocytes in the middle zone into those in the deep zone, as [1H]thymidine-labeled chondrocytes have been confirmed to remain in the middle zone for at least 2 weeks in drug-treated rats, but they disappear in the controls. However, it is unclear whether or not the mechanisms are related to those of the cavity (blister) formation. Quinolone-induced osteochondrotic lesions are the same as those of spontaneous osteochondrosis in rats49, with the exception of the early changes of the thickened middle or deep zone of the articular cartilage in quinolone-induced or spontaneous osteochondrosis, respectively. This makes it impossible to differentiate between spontaneous and quinolone-induced advanced lesions. Therefore, quinolones are also considered to enhance the onset of spontaneous osteochondrotic lesions with high incidence and high severity.

The author has reported on ofloxacin- and nalidixic acid-induced osteochondrosis in rats49 and has also observed osteochondrosis.

In regard to quinolone chondrotoxicity other than cavity (blister) formation of the articular cartilage, osteochondrosis has been reported to appear in the femoral condyle of juvenile rats receiving ofloxacin or nalidixic acid49.

Animal species, age specificity and predilection site: Quinolone-induced osteochondrotic lesions have not been reported in animal species other than rats. Osteochondrosis is induced when treatment with ofloxacin or nalidixic acid begins at 4 weeks, but not at 8 weeks of age. The predilection site of osteochondrosis is the medial femoral condyle (Fig. 3) based on examinations of the proximal ends of the humerus, femur and tibia, the distal ends of the humerus and femur, the vertebral joint and the costochondral junction. In the femoral condyle, osteochondrotic lesions are noted on the caudal aspect after subchronic or chronic treatment and is different from cavity formation appearing at the site close to the femoral trochlea on the femoral condyle (Fig. 1) after a single or several repeated administrations.

Macroscopic and microscopic findings and mechanisms: An osteochondrotic lesion appears macroscopically as a round white plaque in the articular cartilage. In advanced cases, the entire condyle shows a whitish discoloration, and there is a plaque with a partially detached margin or ulceration of the cartilage. Histologically, during the course of ofloxacin treatment of rats for 8 weeks, the early change is a thickening of the middle zone of the articular cartilage with a thinned deep zone. As administration continues, the columns of chondrocytes in the thickened middle zone become more and more numerous, many degenerated cells are seen and the staining intensity of the cartilage matrix with safranin O decreases slightly. After completion of treatment, the articular cartilage is markedly thickened and made up of mainly middle zone cartilage, protruding into the epiphysis with prevention of age-dependent thinning of the articular cartilage (Fig. 4, 4 weeks treatment with levofloxacin). However, the deep zone sometimes remains thick. In advanced cases, fissures or clefts are formed in the bottom of the thickened cartilage. Extension of the fissure to the cartilage surface causes the thickened cartilage to become detached (Fig. 5, 4 weeks treatment with levofloxacin), resulting in ulceration of the articular cartilage. Beneath the ulceration, there are focal necrosis of the subchondral bone and fibrotic lesions in the marrow space.
lesions induced by levofloxacin and sitafloxacin. Moreover, images of similar lesions are included as figures in an article concerning a norfloxacin chronic dose toxicity study in rats. Therefore, osteochondrosis could be common to all quinolones and may be rat specific among the laboratory animals used in general toxicity studies.

**Risk in pediatric use**

Burkhardt et al. (1997) have assessed the safety of quinolones for children and adolescents based on the data reported in a total of 31 references; the data was collected from 7045 skeletally immature patients receiving ciprofloxacin (5878 patients), ofloxacin (622), norfloxacin (432, including 406 Japanese children), nalidixic acid (97) or pefloxacin (16). Of these patients, 530 received follow-up examinations, including a clinical evaluation, radiography, MRI, ultrasonography and/or histopathology (2 patients only) from 0.6 to 12 years after quinolone treatment. As a result, none of the quinolones induced adverse effect on the skeletal system, and ciprofloxacin, ofloxacin and nalidixic acid had no negative effects on the linear growth of children. It was concluded that according to certain statistical equations, the maximum risk for the occurrence of chondrotoxicity, as seen in juvenile animals, would be not greater than 1 in 2348 patients (3 in 7045) or ~0.04%. With respect to the incidence of adverse reactions, in 1795 case report forms for children receiving ciprofloxacin, which were collected up to the end of 1994 by Bayer Corporation (Germany), arthralgia with and without clinical signs of arthritis occurred in 31 of 2030 treatment courses (1.5%). From this data, quinolones seem safe for children and adolescents. For pefloxacin, however, a relatively higher rate of incidence has been reported as follows: in a review of 63 patients, 9 adolescent patients showed swelling of the large joints, predominantly the knees, in conjunction with treatment, but complete resolution and no long-term sequelae were obtained; 5 of 50 children developed reversible joint manifestations; arthropathy was seen in 14% and 45% of patients aged 11–21 and 15–20 years, respectively; and moreover, 2 patients experienced severe lesions, namely, destructive polyarthropathy and subchondral lesions with unknown details. These high incidence rates may be reflected by a tendency of pefloxacin toward accumulation in the articular cartilage after repeated administration in juvenile rabbits (17.5 µg/g cartilage after 1 day, and 81.5 µg/g after 7 days treatment) and by cartilage lesions observed in adult dogs after prolonged treatment.

For risk assessment of chondrotoxicity, drug concentrations in the articular cartilage must be compared between animals (the lowest C\textsubscript{max} values) receiving the lowest arthropathogenic dose and humans (the highest C\textsubscript{max} values) receiving the usual clinical doses. In juvenile animals, quinolone concentrations are generally higher in the articular cartilage than in plasma. The lowest arthropathogenic concentrations have been reported to be 20.6, 9.4 and 6.9 µg/g for norfloxacin and 34.1, 21.5 and 4.6 µg/g for nalidixic acid in juvenile rats, rabbits and dogs, respectively; 12.2 µg/g for levofloxacin in juvenile rabbits; and 8.7 µg/g for ofloxacin in juvenile dogs. On the other hand, there is only very limited data for humans: the mean cartilage concentrations of levofloxacin are 1.38, 2.19 and 2.18 µg/g (assuming an average bone density of 1.9 kg/l) at 98, 246 and 716 min after completion of 20 min infusion of 200 mg ofloxacin to 23 adult patients who underwent total hip replacement for osteoarthritis. Comparison of the above cartilage ofloxacin concentrations between juvenile dogs and adult humans indicates that ofloxacin seems to be safe in humans in terms of chondrotoxicity, but an exact comparison is impossible. Thus, it is impossible to assert the safety of quinolones in pediatric patients based on cartilage concentrations because almost no data is available for humans. However, cartilage quinolone concentrations are helpful in comparing the chondrotoxic potential between quinolones, without regard for differences in the absorption rates from the intestines or the dosage levels administered.

**Conclusions**

The author introduced reference information about quinolone-induced cavity formation and osteochondrotic lesions in the articular cartilage of juvenile laboratory animals. About 30 years have passed since the first report on cavity formation, and at present, the magnesium deficiency story is as understandable as its mechanisms. However, there are some issues yet to be clearly explained, such as the contribution of physical forces to the development of early changes in relation to magnesium deficiency in the cartilage, etc. For osteochondrosis, quinolone-induced inhibition of chondrocyte differentiation from the middle to deep zone has been shown to be the earliest event, but further studies are necessary to determine more details of the mechanisms. Although the ADRs of quinolones in the skeletal system have been reported in both children and adult humans, the cavity formation and/or osteochondrotic lesions observed in juvenile animals are not thought to occur in humans because the ADRs have been mild and reversible upon withdrawal of the treatment. As one exception, however, special attention should be paid to the ADRs of children and adolescent patients receiving pefloxacin. It is impossible to actively collect cartilage quinolone concentration data for humans, and this obstructs risk assessment for quinolone pediatric use. Therefore, as in the case of some quinolones, repetition of very careful clinical studies in children and investigation of case reports are the only means of assessing the safety of other quinolones for pediatric use. Furthermore, although it is very difficult, toxicologists must find biomarkers that can be easily measured in order to detect joint cartilage lesions using methods other than imaging procedures such as MRI.

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