Metabolic effects as a cause of myotoxic effects of fluoroquinolones

Thomas Metterlein, Frank Schuster1, Martin Hager1, Norbert Roewer1, Martin Anetseder2

Introduction

Fluoroquinolones (FQ) act primarily as specific inhibitors of bacterial DNA gyrase, and second of topoisomerase IV, thereby preventing the transcription of microbial DNA. They are frequently used for the treatment of various infections. Clinical trials have demonstrated that FQs are specifically useful against intracellular pathogens because they can easily enter cells.1

In general, FQs are well-tolerated, however, myotoxicity has been reported.2,3 Affected patients usually report variable myalgia, muscle weakness, and cramps. An increased creatine kinase activity can be measured in the serum. Cases of severe rhabdomyolysis are rare but have been documented.4-6 Interestingly, the myotoxic effects do not seem to be dose dependent. Clinical reports indicate that the combination of known myotoxic substances increases the risk of adverse effects.6

The exact mechanisms of the FQ-induced-myotoxicity are unknown, however, several pathogenic factors are discussed. Histological findings suggest that FQs lead to a diminished aerobic capacity that may account for the myotoxic effects.7 However, direct measurements of the maximum oxidative capacity and ATP synthesis showed no effect.8 P13 magnetic resonance spectroscopy results indicate that a preexisting abnormality in intracellular proton handling might be responsible for the induced muscle injuries. During exercise, a significant reduction in pH was measured in individuals with FQ-induced myotoxicity, compared to individuals who showed no adverse reaction to FQs. Similar results can be seen in patients with mitochondrial myopathies.9

Furthermore, results from magnetic resonance studies indicate that a disruption of the intracellular calcium homeostasis could be a potential mechanism for myotoxicity.2

ABSTRACT

Objectives: To investigate if fluoroquinolones (FQs) influence skeletal muscle metabolism of healthy and malignant hyperthermia susceptible (MHS) pigs.

Materials and Methods: After approval from of the Animal Care Committee, 10 MHS pigs, and 6 MHS pigs were anesthetized with hemodynamic and systemic metabolic monitoring. Microdialysis catheters were placed intramuscularly. After equilibration, levofloxacin and ciprofloxacin were injected as a rapid bolus and continuous infusions. Lactate was measured in the dialysate and statistically analyzed was done (Wilcoxon-test; U-test; P < 0.05).

Results: There were no differences in age, weight, and baseline lactate levels between the groups. Both applications of levofloxacin- and ciprofloxacin-induced an increase of local lactate levels in healthy and MHS pigs. No difference between the two groups was observed.

Conclusion: FQs influence skeletal muscle metabolism. Myotoxic effects of FQs can, therefore, be explained by an influence on the cellular energy balance.

KEY WORDS: Fluoroquinolones, malignant hyperthermia, metabolism, myotoxicity
Elevated intracellular calcium levels after exposure to FQs were indirectly measured in an in vitro study. Calcium, as the second messenger, stimulates cellular energy consumption.

Theoretically, both a diminished energy supply and an increased energy consumption can lead to energy depletion with intracellular acidosis and cell death.

Malignant hyperthermia (MH) patients have a vulnerable intracellular energy homeostasis. Various stimuli induce a cellular hypermetabolism that is caused by increased intracellular calcium levels. A mutated type 1 ryanodine-receptor (RYR 1) causes a calcium leakage from the sarcoplasmic reticulum (SR) into the cytoplasm. Hence, malignant hyperthermia susceptible (MHS) muscle cells are at higher risk of cellular energy depletion.

MHS patients seem to be at higher risk of developing adverse events after treatment with FQs. In a prior study, patients with FQ-induced rhabdomyolysis were tested for their MH status. Thirty-three percent of the examined individuals developed a pathologic reaction after exposure to halothane and caffeine, indicating MH susceptibility. The threshold for an increased muscle cell breakdown is apparently lower with a preexisting muscle cell disorder. The exposure to an otherwise harmless substance can lead to severe complications in these individuals. A fluorine moiety, as an integral part of both FQs and volatile anesthetics, might play an important role, which explains why nonfluorinated quinolones do not have myotoxic properties.

Recently, a novel model to investigate in vivo metabolic processes in muscle tissue has been developed using a microdialysis probe which is placed in the tissue and the specific substance is injected. Microdialysis measurements can serve as an important tool to evaluate the metabolic effects of various substances. This in vivo metabolic measurement seems to be even more sensitive than in vitro models.

Hence, this study was aimed to use this novel model to examine how muscle tissue reacts to exposure to levofloxacin or ciprofloxacin in vivo. Because of the impaired energy balance, we postulate a greater rise of local lactate levels in MHS muscle compared to healthy muscle.

Materials and Methods

Following approval by the Animal Care Committee (University Hospital Wuerzburg, Wuerzburg, Germany), 10 MHS, and 6 MHS pietrain swine, from a special breeding program, were included in the study. The MH genotype of the swine was determined by DNA analyses of skin tissue. Furthermore, MH phenotype was verified by an in vitro contracture test with halothane and caffeine according to the protocol of the European MH Group (EMHG). After sedation with intramuscular ketamine (Ketavet®, Pfizer, Berlin, Germany), and insertion of an intravenous line into an ear vein, anesthesia was started by administration of fentanyl (Fentanyl-Janssen; Janssen-Cilag, Neuss, Germany) and sodium thiopental (Trapanal®; Nycomed, Konstanz, Germany). The trachea was intubated without administration of a muscle relaxant. The swine were mechanically ventilated with 30% oxygen, and anesthesia was maintained by continuous intravenous administration of midazolam (Midazolam-ratiopharm®; Ratiopharm, Ulm, Germany) and fentanyl. Monitoring included electrocardiography, pulse oximetry, and rectal temperature measurement. Radiant heat application and warming blankets were used to maintain a stable body temperature.

Four microdialysis probes, with a cut-off of 20 kD and attached microtube, were placed in the sartorius muscle. Correct intramuscular placement was confirmed with ultrasound. The probes were perfused with Ringer’s solution at a rate of 1 µl min⁻¹. Samples were collected every 15 min.

After 30 min equilibration, 100 µl bolus of levofloxacin (5 mg/ml) (Tavanic TM, Sanofi-Aventis, Vienna, Austria) and ciprofloxacin (2 mg/ml) (Ciprobay, Bayer, Leverkusen, Germany) were injected over 5 min, via the attached microtube. Levofloxacin (5 mg/ml) and ciprofloxacin (2 mg/ml) were also continuously injected at two further microdialysis probes at a flow rate of 10 µl/min. All solutions were preservative free.

Dialysate samples were collected for 60 min after injection of the substances, and spectrometrically analyzed for lactate.

Data were suspected to be nonparametrically distributed and were given as means with interquartile ranges. The Wilcoxon rank sum test was used to test for significant changes in lactate levels after exposure to the test substances. Differences between the groups at the times of measurement were analyzed with the Mann–Whitney-U-test with P < 0.05 was considered significant.

Results

There were no significant differences in the weights (MHN: 32.0 [30.6–33.2] kg and MHS: 28.2 [26.7–30.5] kg) of the pigs between the two groups. Genetic screening revealed that all animals were either homozygous for the wild type (MHN), or the Arg615Cys mutation (MHS). The presumed MH status was confirmed with the standard contracture test according to the EMHG test protocol.

Baseline lactate levels were not different between the two groups and for the individual test substances [Table 1]. Exposure to levofloxacin- and ciprofloxacin-induced a significant increase of lactate levels from baseline in both MHN and MHS pigs. However, at all given times measured lactate concentrations were not different between MHN and MHS pigs [Figures 1 and 2].

Discussion

In this study, levofloxacin and ciprofloxacin induced a significant increase of intramuscular lactate in both healthy and MHS pigs. The in vivo metabolic test was developed to indirectly measure cellular metabolism. Rise of local lactate is an indicator for increased cellular energy consumption or diminished supply.

Table 1:

| Drug          | Lactate level (mmol/l) in MHS (n=6) | Lactate level (mmol/l) in MHN (n=10) |
|---------------|------------------------------------|-------------------------------------|
| Levofloxacin bolus | 0.7 (0.6-0.4)                     | 0.8 (0.7-1.0)                      |
| Levofloxacin continue | 0.8 (0.6-0.9)                     | 0.8 (0.6-0.9)                      |
| Levofloxacin bolus | 0.6 (0.5-1.0)                     | 0.6 (0.5-0.8)                      |
| Levofloxacin continue | 1.1 (0.6-1.5)                     | 0.8 (0.7-1.1)                      |

Mann–Whitney U-test, data as medians and interquartile ranges. MHN=Malignant hyperthermia nonsusceptible, MHS=Malignant hyperthermia susceptible
FQs interfere with mitochondrial ATP production, and can potentially cause intracellular energy depletion. Elevated resting intracellular calcium levels result in a higher cellular energy consumption. Exposure to various substances can lead to a hypermetabolic syndrome via calcium release from the SR.[9] A fluorine moiety is an integral part of both volatile anesthetics and FQ. Covalently bound fluorine interferes with the RYR resulting in an increased calcium release. In addition, a reduced calcium transport back into the SR can be considered as a further potential reason for increased intracellular calcium levels. Prior studies have shown that covalently bound fluorine is capable of inhibiting the sarcoplasmic calcium ATPase (SERCA).[16] An inhibition of SERCA leads to higher intracellular calcium concentrations. This corresponds with recent in vitro results that indirectly measured elevated intracellular calcium levels after exposure to FQs.[17] Calcium, as a second messenger, activates energy consuming processes. Both the direct inhibition of the mitochondrial aerobic capacity, and the influence on the cellular calcium homeostasis, leads to a disruption of the cellular energy balance. A depletion of energy reserves results in muscle cell breakdown and explains myotoxic properties. With elevated intracellular calcium as an important pathway for FQ-induced myotoxicity dantrolene, an intracellular calcium inhibitor might attenuate FQ-induced tissue damage. This behavior could be proven for other substances.[14,18]

In this study, an effect of FQs on the cellular energy turnover was observed in both MHS and healthy animals. Microdialysis is a sensitive tool that measures tissue concentrations of small, unbound molecules. The metabolic test has been proven to be a reliable method to evaluate the intramuscular metabolic effect of various substances.[14-15] Halothane and sevoflurane, proven myotoxic substances, lead to similar lactate levels as seen with FQs indicating a relevant local muscle damage.[13] Intramuscular application leads to a spherical distribution of the substance with a radius of <2 cm from the injection site.[19] Compared to therapeutic tissue levels, the theoretically achieved tissue concentrations in this study were 3–6 fold higher to induce a relevant reaction.[20-22] Levels about twice the average therapeutic tissue concentration can easily result from dosing errors and usually show no adverse reaction in healthy individuals.[23,24] The actual tissue concentrations were not measured in this study, but taking the local reaction into account, sufficient levels were reached. Both methods of antibiotic application led to an increase of local lactate. The measured levels seem to decrease about 45 min after bolus injection while such a decrease seems to be absent with a continuous application. This indicates a temporary metabolic effect of FQs after bolus injection.

Apparently, the myotoxic properties of FQs are related to an acute disruption of the cellular energy homeostasis. In both healthy and MHS muscle tissue, signs of increased anaerobic metabolism were observed. The preexisting impairment of the cellular energy balance in MHS muscle did not lead to an intensified reaction toward FQs. This is in contrast to prior
findings. In vitro FQ led to an intensified reaction in MHS muscle. This discrepancy between in vivo and in vitro results is not fully understood. A higher substance concentration in the in vivo model might be the explanation. A very similar behavior was seen for halothane. The injection of a high concentration induces a hypermetabolism in both MHS and healthy individuals while lower concentrations of the same substance resulted in a significantly different behavior. This could be explained by shifted dose response curves. In a similar study, this has been shown for volatile anesthetics. Further studies with lower FQ concentrations have to investigate whether a similar dose response behavior exists.

The main finding of this study is that increased cellular metabolism seems to play an important role in FQ-induced myotoxicity. The exact mechanisms are subject to further research.

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Conflicts of Interest

There are no conflicts of interest.

References

1. Bolon MK. The newer fluoroquinolones. Infect Dis Clin North Am 2009;23:1027-51.
2. Guis S, Bendahan D, Kozak-Ribbens G, Mattei JP, Le Fur Y, Confort-Gouyon S, et al. Investigation of fluoroquinolone-induced myalgia using (31)P magnetic resonance spectroscopy and in vitro contracture tests. Arthritis Rheum 2002;46:774-8.
3. Guis S, Jouglard J, Kozak-Ribbens G, Figarella-Branger D, Vanuexem D, Pelissier JF, et al. Malignant hyperthermia susceptibility revealed by myalgia and rhabdomyolysis during fluoroquinolone treatment. J Rheumatol 2001;28:1405-6.
4. Petitjeans F, Nadaud J, Perez JP, Debien B, Olive F, Villeveille T, et al. A case of rhabdomyolysis with fatal outcome after a treatment with levofloxacin. Eur J Clin Pharmacol 2003;59:779-80.
5. Hsiao SH, Chang CM, Tsao CJ, Lee YY, Hsu MY, Wu TJ. Acute rhabdomyolysis associated with ofloxacin/levofloxacin therapy. Ann Pharmacother 2005;39:146-9.
6. Blain H, Klein M, Weryha G, Tréchot P, Hanesse B, Leclère J. Rhabdomyolysis and unexplained malaise. Role of combination of ciprofloxin and norfloxacin. Rev Med Interne 1996;17:859-60.
7. Bernard-Beaupois K, Hecquet C, Hayem G, Rat P, Adolphe M. In vitro study of cytotoxicity of quinolones on rabbit tenocytes. Cell Biol Toxicol 1998;14:283-92.
8. Bendahan D, Desnuelle C, Vanuexem D, Confort-Gouyon S, Figarella-Branger D, Pelissier JF, et al. 31P NMR spectroscopy and ergometer exercise test as evidence for muscle oxidative performance improvement with coenzyme Q in mitochondrial myopathies. Neurology 1992;42:1203-8.
9. Steinfaß M, Wappier F, Scholz J. Malignant hyperthermia. General, clinical and experimental aspects. Anaesth Analg 2002;51:328-45.
10. Tong J, McCarthy TV, MacLennan DH. Measurement of resting cytosolic Ca2+ concentrations and Ca2+ store size in HEK-293 cells transfected with malignant hyperthermia or central core disease mutant Ca2+ release channels. J Biol Chem 1999;274:693-702.
11. Anetseder M, Hager M, Müller CR, Roewer N. Diagnosis of susceptibility to malignant hyperthermia by use of a metabolic test. Lancet 2002;359:1579-80.
12. Schuster F, Schöll H, Hager M, Müller R, Roewer N, Anetseder M. The dose-response relationship and regional distribution of lactate after intramuscular injection of halothane and caffeine in malignant hyperthermia-susceptible pigs. Anesth Analg 2006;102:468-72.
13. Schuster F, Metterlein T, Negele S, Kranke P, Muellerbach RM, Schwemmer U, et al. An in-vivo metabolic test for detecting malignant hyperthermia susceptibility in humans: A pilot study. Anesth Analg 2008;107:909-14.
14. Metterlein T, Schuster F, Kranke P, Roewer N, Anetseder M. Intramuscular injection of malignant hyperthermia trigger agents induces hypermetabolism in susceptible and nonsusceptible individuals. Eur J Anaesthesiol 2010;27:77-82.
15. Aprotocol for the investigation of malignant hyperpyrexia (MH) susceptibility. The European Malignant Hyperpyrexia Group. Br J Anaesth 1984;56:1267-9.
16. CoI RJ, Murphy AJ. Fluoro-inhibited calcium ATPase of sarcoplasmic reticulum. Magnesium and fluoride stoichiometry. J Biol Chem 1992;267:21584-7.
17. Metterlein T, Schuster F, Tadda L, Hager M, Mulboon S, Capaccione J, et al. Fluoroquinolones influence the intracellular calcium handling in individuals susceptible to malignant hyperthermia. Muscle Nerve 2011;44:208-12.
18. Schuster F, Metterlein T, Negele S, Gardill A, Schwemmer U, Roewer N, et al. Intramuscular injection of sevoflurane detects malignant hyperthermia predisposition in susceptible pigs. Anesthesiology 2007;107:616-20.
19. Schuster F, Hager M, Metterlein T, Muellerbach RM, Wumb T, Wunder C, et al. In-vivo diagnosis of malignant hyperthermia susceptibility: A microdialysis study. Anaesthesiologie 2008;57:767-74.
20. Von Baum H, Böttcher S, Abel R, Germer HJ, Sonntag HG. Tissue and serum concentrations of levofloxacin and ciprofloxacin in healthy adult subjects. Chest 1991;35:2521-5.
21. Dalhoff A, Eickenberg HU. Tissue distribution of ciprofloxacin following oral and intravenous administration. Infection 1985;13:78-81.
22. Dan M, Golomb J, Gorea A, Braf Z, Berger SA. Concentration of ciprofloxacin in human prostate tissue after oral administration. Antimicrob Agents Chemother 1986;30:88-9.
23. Fabre D, Bressolle F, Gomel I, Arich C, Lemesle F, Bezaiu H, et al. Steady-state pharmacokinetics of ciprofloxacin in plasma from patients with nosocomial pneumonia: Penetration of the bronchial mucosa. Antimicrob Agents Chemother 1991;35:2521-5.
24. Gallotin MH, Danziger LH, Rodvold KA. Steady-state plasma and intrapulmonary concentrations of levofloxacin and ciprofloxacin in healthy adult subjects. Chest 2001;119:1144-22.