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The effect of milk on plasmatic and tissue levels of macrolides: *in vivo* study in rats

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ABSTRACT

The ingestion of milk with drugs, particularly some antibiotics, is frequently recommended in order to decrease possible gastrointestinal discomfort. The objective of this study was to assess the interference of milk in the absorption and tissue levels of macrolide antibiotics (erythromycin, clarithromycin, roxithromycin and azithromycin). Forty female rats received surgically-implanted PVC sponges on their backs. One week later, granulomatous tissue was observed and the animals were divided into eight groups, which received erythromycin, clarithromycin, roxithromycin and azithromycin with and without milk. One hour after administration of antibiotic, the animals were sacrificed. The serum and tissue samples were submitted to microbiological assay with *Micrococcus luteus* ATCC 9341, in order to determine drug concentration. Milk did not cause any reduction in the serum and tissue levels of azithromycin and clarithromycin ($p>0.05$, *t*-test). However, ingestion of milk reduced by approximately 28.7% the roxithromycin ($p<0.0001$, *t*-test) and by 34.1% the erythromycin ($p<0.0001$, *t* test) serum concentrations. Similar effects were observed on tissue levels. Milk ingestion caused a reduction of approximately 20.8% in the roxithromycin ($p<0.0001$, *t*-test) and 40% in the erythromycin ($p<0.0001$, *t*-test) tissue levels. We concluded that erythromycin and roxithromycin should be not administered with milk.

Keywords: Pharmacokinetics, macrolides, milk, serum concentration.

INTRODUCTION

Macrolides were first isolated by McGuire and collaborators in the 1950s, from *Streptomyces erythreus*, a bacterial strain found in the soil of the Philippine archipelago (Charles & Segreti, 1997).

The antibiotics of this group have a complex molecular structure and a lactone ring of 14 to 16 carbonatons atoms characterizes the pharmocophoric group. Erythromycin (the oldest representative), roxithromycin and clarithromycin possess a 14-carbonning. Azithromycin, a new semi-synthetic derivative (azalide), has 15 carbons in the ring, due to the insertion of an amino-group in the erythromycin ring (Salvador & Enzler, 1999).

In normal doses, macrolides show bacteriostatic activity due to reversible binding to the 23S subunit of the 50S ribosomal structure in susceptible bacteria. The blockage of the translocation of the ribosome along the mRNA during polypeptide elongation and the premature dissociation of the peptidyl-tRNA interferes in RNA-dependent protein synthesis (Vester & Douthwaite, 2001).

Macrolides are active against aerobic and anaerobic Gram-positive cocci. Only in very high concentrations are these antibiotics active against Gram-negative microorganisms, owing to the difficulty of penetrating the outer membrane of these bacteria (Hamilton-Miller, 1992). Some protozoa, such as *Toxoplasma gondii*, *Cryptosporidium* and *Plasmodium* are also sensitive (Saba et al., 1993).

All macrolides can be administered orally. Absorption occurs mainly in the upper-small intestine (Wise et al., 1987). The bioavailability is approximately 50% for erythromycin, 37% for azithromycin, 55% for clarithromycin and 80% for roxithromycin (Mensa et al,. 2003). Macrolides are metabolized in the liver, through the CYP3A4 enzyme (P450 cytochrome), and their excretion is both biliary and renal (Namour et al., 2001; Guay et al., 2001).

The group are extensively used since they are the first choice for patients who are allergic to pencillins/ cephalosporins and they are also very active against most of the upper-air passage pathogens (Wise et al., 1987). Macrolides are indicated for the treatment of sinusitis, pharyngitis, acute bronchitis, acute infectious exacerbations of chronic bronchitis and community-acquired pneumonia caused by typical and atypical bacterial pathogens (Anderson et al., 1991; Levenstein, 1991).

The eradication of *Helicobacter pylori* is another important indication, especially regarding clarithromycin, which, used alone or in association with proton-pump inhibitors, success rates of approximately 78% (Markham & McTavish, 1996).

Due to distinct pharmacokinetic properties, different macrolides are used in different doses. Erythromycin, less soluble, is usually administered at 500 mg Q.I.D.; clarithromycin at 500 or 1000 mg T.I.D. or B.I.D.; roxithromycin at 300 or 600 mg B.I.D. and, finally, azithromycin in a 500 mg single dose (Markham & McTavish, 1996).

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The bioavailability of roxithromycin is usually not affected by milk (Lassman et al., 1988) or food (Puri & Lassman, 1987) ingestion. The serum or salivary concentration of erythromycin in estolate or ethylsuccinate forms can be enhanced when associated with milk (McCracken et al., 1978). However, in healthy foals the plasmatic concentrations of erythromycin were lower after feeding (Lakritz et al., 2000). In dogs, the pharmacokinetic parameters of clarithromycin were not significantly influenced by feeding (Vilmanyi et al., 1996).

The most often related side-effect of these antibiotics, which limits adhesion of patients to the treatment, is gastrointestinal discomfort (nausea, vomiting and abdominal pain), possibly due to the prokinetic activity (Bowler et al, 1992; Ruuskanen, 2004; Maganti, 2003). It is estimated that up to 35% of patients do not complete the treatment due to these side-effects (Tarlow, 1997).

Many professionals recommend the concomitant administration of milk in order to decrease the gastrointestinal discomfort (Welling, 1982; Latare & Setness, 1989). There is little information on the interaction of milk ingestion with the body levels of macrolides. The objective of this study was to observe the interference of milk in serum and tissue levels of the following oral-administered macrolides: erythromycin, azithromycin, clarithromycin and roxithromycin.

MATERIALS AND METHODS

Pharmacological agents: Azithromycin dehydrate, erythromycin estolate, roxithromycin and clarithromycin were obtained from Pfizer Inc. Laboratories, Eli-Lilly Laboratories, Shering-Plough Corporation and Abbott Laboratories, respectively.

Animals: Forty adult female rats (*Rattus norvegicus* - albinus, Wistar), aged 60 days, weighing 282 ± 19g, were obtained from CEMIB/UNICAMP (Centro de Bioterismo – ICLAS Monitoring/Reference Center, Campinas, Brazil) and maintained under aseptic conditions. Ethical guidelines issued in the Helsinki Declaration were applied.

Bacterial strain: *Micrococcus luteus* (ATCC 9341) was used in the macrolide susceptibility tests (Yamasaki et al., 2001).

Granulomatous tissue and groups: two sterilized polyurethane sponge discs (density=35Kg/m^3) were surgically implanted subcutaneously into the back of the each rat. These sponge discs (PRO301 – Proespuma Com. & Ind. Ltd., Sao Paulo, Brazil) were 6.2 mm in diameter and 2 mm thick, weighing 2.1± 0.1 mg. They were positioned 30 mm away from each other.

After 7 days with the spanges in position, the animals were randomly distributed into eight groups of five animals. The groups received the following treatments (po) after overnight fasting:

Group 1: Clarithromycin (28.5mg/Kg)
Group 2: Clarithromycin (28.5mg/Kg) plus milk (3.5mL/kg)
Group 3: Roxithromycin (8.5mg/Kg)
Group 4: Roxithromycin (8.5mg/Kg) plus milk (3.5mL/kg)
Group 5: Erythromycin (28.5mg/Kg)
Group 6: Erythromycin (28.5mg/Kg) plus milk (3.5mL/kg)
Group 7: Azithromycin (14.25mg/Kg)
Group 8: Azithromycin (14.25mg/Kg) plus milk (3.5mL/Kg)

The milk had a calcium ion concentration of 1.1mg/mL and total fat of 32mg/mL.

Surgical procedures and samples: One hour after drug administration and a rapid anesthetic induction with ethyl-ether all rats were killed by cutting the carotid plexus. After centrifugation, 20 µL of serum was placed on two dry discs of esterile filter paper (6.25 mm), which were placed on Mueller-Hinton agar plates previously inoculated with 10^6 cfu/mL of *Micrococcus luteus* ATCC 9341.

After 18h of incubation at 37°C, the inhibition zones were measured and correlated with regression lines in order to determine antibiotic levels.

Regression lines: In order to correlate inhibition zones and serum or tissue concentration of the macrolides, a regression line was assayed for each antibiotic. A series of known concentrations (0.25; 0.5; 1.0; 2.0; 5.0; 10.0; 20.0 and 30.0 µg/mL) of the each antimicrobial agent were diluted in blank serum and 20 µL was placed on two 6.25 mm sterilized paper discs

The discs were placed on Mueller-Hinton agar plates previously inoculated with 10^6 cfu/mL of *Micrococcus luteus* ATCC 9341. After 18h of incubation at 37°C, the inhibition zones were measured and plotted (Excel XP for Windows) against the concentration that generated the zone. The resulting regression line provided a formula to calculate the concentration (in µg/mL) of each macrolide as a function of the inhibition zone (in mm). The following formulas were obtained:

Concentration of azithromycin = **(0.2359 x inhibition zone) – 0.7686**, R^2=0.97;
Concentration of erythromycin = **(0.5415 x inhibition zone) + 0.2058**, R^2=0.99;
Concentration of roxithromycin = **(0.4859 x inhibition zone) + 0.6227**, R^2=0.93;
Concentration of clarithromycin = **(0.5699 x inhibition zone) + 0.0049**, R^2=0.96.

Granulomatous tissues: the two granulomatous tissues were surgically delimited, removed, and placed on to other Muller-Hinton agar plates inoculated with 10^6 cfu/mL of *Micrococcus luteus* ATCC 9341. After 18h of incubation at 37°C, the inhibition zones formed were measured (Figure 1).

The diameters of inhibition zones were submitted to analysis of variance (ANOVA) and student's *t-test* (significance level of 5%).

All procedures were carried out in accordance with the technique for antibiotic determination in previously presented animal models. This rat-based experimental model, has been tested and results can be extrapoled to human antibiotic pharmacokinetics studies (Groppo et al., 2000; Groppo et al., 2004; Fiol et al., 2000).

RESULTS

Figure 1 shows an example of the inhibition halo

produced by the granulomatous tissue. The serum concentration of each antimicrobial agent with or without concomitant milk administration can be observed in Figure 2. There are no statistically significant differences (p>0.05, *t-test*) between clarithromycin groups (1 and 2) or between azithromycin groups (7 and 8).

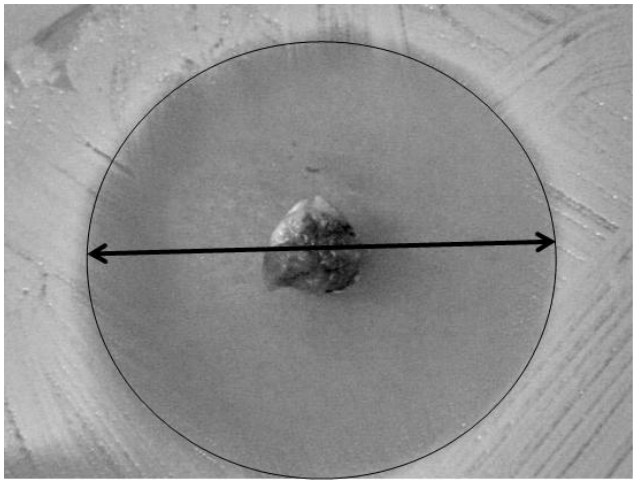

Figure 1 - Granuloma tissue formed in the presence of antibiotic, promoting an inhibition zone against *Micrococcus luteus* ATCC 9341.

However, the serum concentration of roxithromycin (23.09µg/mL) decreased to 16.47µg/mL with the concomitant administration of milk. This difference of approximately 28,7% was statistically significant (p<0.0001, *t-test*). Erythromycin also showed a reduction in serum concentration from 18.73µg/mL to 12.34µg/mL after milk consumption. This difference (approximately 34,1%) was statistically significant (p<0.0001, *t-test*).

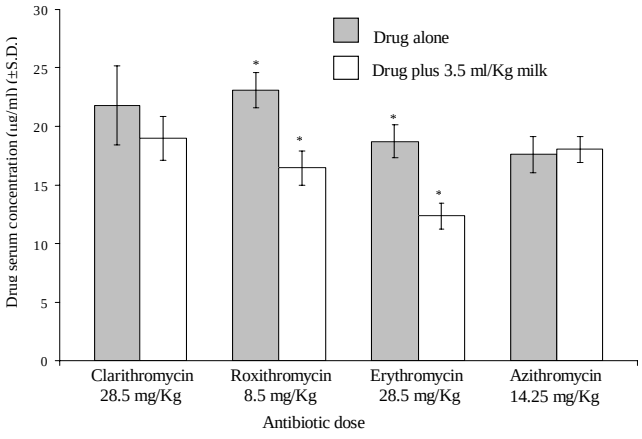

Figure 2 - Effect of milk on macrolide antibiotic absorption. The dark bars show the mean (± SD) plasmatic concentration of the antibiotic administered alone and the white bars, administered with milk (3,5m/Kg). *Indicates statistically significant difference between the forms of administration of each antibiotic (p<0.0001, *t- test*).

The inhibition zones (Figure 3) representing the tissue concentration exhibited results similar to those observed for serum concentration. There are no statistically significant differences between the inhibition zones observed for clarithromycin or azithromycin with and without milk (p>0.05, *t-test*). However, a decrease of approximately 40% in the halo diameter of erythromycin it was observed when it was associated with milk. This difference was statistically significant (p<0.0001, *t-test*). A statistically significant reduction (20.8%) in roxithromycin halos was also observed (p<0.001, *t-test*).

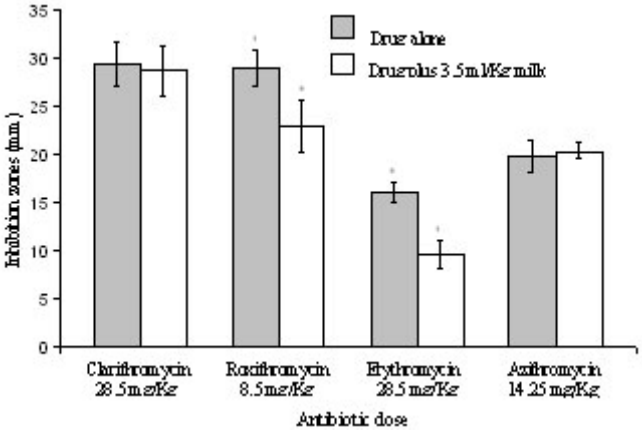

Figure 3 - Effect of milk on macrolide antibiotics tissue penetration (granulomatous tissue). The dark bars show the mean (± SD) of the inhibition zones diameters formed by the granulomatous tissue containing the antibiotic administered alone and the white bars, administered with milk (3.5m/Kg). *Indicates statistically significant difference between the forms of administration of each antibiotic. (p<0.0001, *t-test*).

DISCUSSION

Liquid chromatographic methods for the determination of erythromycin residues in animal tissues showed quantification limit (QL) of 0.25µg/mL and 0.125µg/g in tissue (Dreassi et al., 2000). The microbiological assay antibiotics, is usually as sensitive as HPLC quantification (Hsu & Hsu, 1992; Moore et al., 1996). Previous studies have used the same *Micrococcus luteus* strain to determine erythromycin availability in plasma (Goudah et al., 2004) and milk (Harpster & Katz, 1980), showing QL of 0.03µg/mL and 0.05µg/mL, respectively.

The same microbiological assay was also used to determine azithromycin concentrations in plasma, saliva, normal gingiva, pathological periodontal tissues (Blandizzi et al., 1999), lung tissue and bronchial washings (Danesi et al., 2003). In addition, blood, saliva, gingiva, and alveolar bone collected during third molar removal were submitted to the same assay (Malizia et al., 1997). The limits of detection verified in the present study were considered low and precise enough to determine the macrolides concentration after 1 hour of administration. These limits were similar to those previously described.

The granulomatous tissue model has been used to observe the concentration of antibiotics and the interaction of these agents with other agents, especially in tissue samples (Groppo et al., 2000, Fiol et al., 2000, Groppo et al., 2004).

One of the most common side - effects of macrolides is gastrointestinal intolerance, which probably occurs due to the disruption of intestinal motility. The concomitant use of milk or food is often recommended to improve compliance and efficacy (Latare & Setness, 1989), to increase the absorption of a specific drug, to reduce the gastric irritation induced by the drug or when the nutrient is used to help the proposed therapy (Fleisher et al., 1999).

However, some nutrients can alter significantly the bioavailability, the mode of action, or toxicity of pharmacological agents (Lewis et al., 1995). Thus, the expectation of few side effects with concomitant food or milk ingestion can be false.

The macrolide antibiotics generally do not form chelates with food (Salvador & Enzler, 1999; Mensa et al., 2003; Puri & Lassman, 1987). Thus, the interference of milk in the erythromycin and roxithromycin kinetics revealed in the present study was not caused by chelate formation. These two antibiotics are the least soluble and the most acid susceptible antibiotics of the macrolides group (Salvador & Enzler, 1999).

The ingestion of whole milk, with a fat concentration of approximately 32mg/mL could be responsible for a gastric-emptying delay, which causes longer exposure to the gastric acid (Charman et al., 1997). Therefore, the reduction of both erythromycin and roxithromycin plasmatic and tissue concentrations could be induced by a longer time of exposure to gastric acid, according to previous studies (Welling, 1984; Welling et al, 1978).

McCracken et al., (1978) observed a significant reduction (40% to 60%) in the serum and salivary peak concentrations of some penicillins in children after milk ingestion. However, the same authors showed enhanced absorption of erythromycin ethylsuccinate with milk. In the present study, milk significantly reduced the plasmatic and tissue concentration of erythromycin, probably because the species, the pharmacological formulation and even the milk were different. In healthy foals, the plasmatic concentrations of erythromycin were lower after feeding (Lakritz et al., 2000), which suggests that the species could be an important factor influencing the erythromycin serum concentration.

Welling et al. (1978) observed that some meals (including milk) and coadministered water caused a uniform reduction (47 to 60%) in the erythromycin-stearate serum levels in human subjects. Higher and more uniform serum levels were obtained with an empty stomach together with an adequate volume of water.

The absence of interference in clarithromycin serum and tissue concentrations by milk observed in the present study was also observed by Vilmanyi et al. (1996), who showed that the pharmacokinetic parameters of clarithromycin were not significantly influenced by feeding, in dogs. However, Guay et al. (2001) showed that the bioavailability of clarithromycin in human beings was 30% lower when administered under fasting than under nonfasting conditions. These findings reinforce the influence of the species in the serum concentration of the macrolides.

In addition, the present study showed a reduction in the roxithromycin peak concentration induced by milk ingestion, which is not supported by other studies (Lassman et al., 1988; Puri & Lassman, 1987). Those authors observed that roxithromycin bioavailability in human beings was not affected to a clinical extent when associated with milk or food ingestion. Li et al. (2001) observed differences in the demethylation metabolism of roxithromycin between humans and rats. Different metabolic pathways could be responsible for differences between these two species,with respect to all macrolides.

Further studies are necessary to understand the mechanism of interaction between milk, food and several other clinically important agents.

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RESUMO

*Efeito do leite nos níveis teciduais e plasmáticos de macrolídeos: estudo **in vivo** em ratos*

A ingestão de fármacos com leite, particularmente antibióticos é recomendada com freqüência, com o objetivo de reduzir possíveis desconfortos gastrintestinais. O objetivo deste trabalho foi verificar a interferência do leite na absorção e nos níveis teciduais de antibióticos macrolídeos (eritromicina, claritromicina, roxitromicina e azitromicina). Quarenta ratas receberam cirurgicamente implantes de esponjas de PVC na região dorsal. Uma semana depois se observou-se a formação de tecido granulomatoso e os animais foram divididos em oito grupos que receberam: eritromicina, claritromicina, roxitromicina e azitromicina com e sem leite. Uma hora pós a administração dos antibióticos, os animais foram sacrificados. Amostras de soro e tecido foram retiradas e testadas microbiologicamente com *Micrococcus* luteus ATCC 9341, com o objetivo de se determinar a concentração de antibiótico presente nesses tecidos. O leite não causou qualquer redução nos níveis séricos e teciduais de azitromicina e claritromicina (p>0.05, *t-test*). Por outro lado, a ingestão de leite reduziu em torno de 28,7% a concentração sérica de roxitromicina (p<0.0001, *t-test*) e em torno de 34,1% a de eritromicina (p<0.0001, *t test*). Os mesmos resultados foram observados para os níveis teciduais. A ingestão de leite causou uma redução nos níveis teciduais de antibiótico em torno de 20,8% para a roxitromicina (p<0.0001, *t-test*) e de 40% para a eritromicina (p<0.0001, *t-test*). Concluímos que a ritromicina e a roxitromicina não devem ser administradas com leite.

Palavras-chave: Farmacocinética, macrolídeos, leite, concentração sérica.

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