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Comparative pharmacokinetics of the amoxicillin-clavulanic acid combination in broiler chickens and turkeys, susceptibility and stability tests of the combination

PhD thesis

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The goal of this dissertation is to supply important scientific data about the amoxicillin-clavulanic acid combination for the poultry industry. At the present time there is no official MRL-value for clavulanic acid in the European Union and in the USA, thus the usage of the combination is not permitted in food producing animals. Widespread resistance against amoxicillin however, might facilitate the introduction of clavulanate potentiated amoxicillin for the treatment of certain bacterial diseases in poultry following the appropriate authorization procedure. Our studies involved the below mentioned three experiments, that can supply information for the future use of this antibiotic combination:

1. Our first goal was to determine basic pharmacokinetic parameters of the amoxicillin-clavulanic acid combination in broiler chickens and turkeys following intravenous and oral administration. Pharmacokinetic data was compared between the two species, and between the two substances in each species. Latter was important to determine whether the two substances behave similarly in the body or not, which phenomenon might confirm their synergistic activity.

2. Susceptibility tests were performed in *E. coli*, *S. enterica* and *P. multocida* strains isolated from chickens and turkey in Hungary. Our goal was to determine actual MIC_{50} and MIC_{90} values for the combination, and to define the incidence of resistance to amoxicillin, and to the clavulanate potentiated amoxicillin among the above mentioned bacteria.

3. In the stability experiments the decomposition rate of the substances were examined at different water hardness levels, at different pH-values, and in metal and plastic troughs. These data supply important information for the poultry and swine industry, where the antibiotics are dissolved in water and their decomposition is highly affected by different environmental conditions.

1. Comparative pharmacokinetics of the amoxicillin-clavulanic acid combination in broiler chickens and turkeys after intravenous and oral administration

There is no published data available about the oral bioavailability of the amoxicillin-clavulanic acid combination in chickens and turkeys, although the absorption and disposition of the antibiotics decisively determine their clinical application, routes of administration, dosage and the dosage interval.

The pharmacokinetic behaviour of the amoxicillin-clavulanate combination was examined in a two-week crossover study. Twelve, Ross-breed, female, six-week-old broiler chickens and twelve, BUT-6 hybrid, six-week-old female turkeys were used in this experiment. Animals were put into two groups, and 6-6 animals were treated with an intravenous and oral dose of 10 mg/kg amoxicillin and 2.5 mg/kg clavulanate. Blood samples were taken (5, 10, 20, 30, 45, 60, 90, 120, 150 minutes and 3, 4, 5, 6, 7 and 8 hours after treatment) into heparinized tubes. Samples were centrifuged, plasma concentrations of the substances were measured simultaneously with a validated RP-HPLC method with UV-detection. In accordance with our results two-compartment open model was used in the case of intravenous and one-compartment open model after oral administration. With the utilization of these models and a computer software (*Kinetica 4.4*) the below-mentioned pharmacokinetic parameters were determined.

The most important pharmacokinetic parameters after 12.5 mg/kg intravenous dose of the combination (10 mg/kg amoxicillin, 2.5 mg/kg clavulanic acid) in **broiler chickens** were the following: distribution half-lives of amoxicillin and clavulanate were 0.11±0.01 h and 0.10±0.01 h, respectively, elimination half-lives 1.28±0.05 h and 1.15±0.06 h, respectively. Volume of distribution values were 1.44±0.06 l/kg for amoxicillin and 1.17±0.08 l/kg for clavulanic acid. Total body clearences were 0.78±0.03 l/h/kg and 0.71 l/h/kg, MRT values were 0.88±0.03 h and 0.78±0.07 h, respectively for amoxicillin and clavulanate. After oral administration of the same 12.5 mg/kg dose of the combination the absorption half-lives were 0.15±0.01 h and 0.16±0.01 h, elimination half-lives 1.28±0.05 h and 1.27±0.07 h, respectively, at the maximal plasma concentrations the ratio of the antibiotics was in the optimal interval (amoxicillin 3.46±0.11 μg/ml, clavulanic acid 1.08±0.05 μg/ml; ~3,2:1). Oral bioavailability of amoxicillin was 63.8±2.6%, the value for clavulanate was 65.7±3.1%.

In **turkeys** after intravenous administration, distribution half-lives were 0.11±0.01 h, and 0.12±0.02 h, respectively, elimination half-lives were 1.28±0.03 h for amoxicillin, 1.18±0.12 h for clavulanate. Clearence values were 0.78±0.12 l/h/kg and 0.61±0.03 l/h/kg, MRT values were 0.89±0.05 h and 0.92±0.05 h, respectively. Volume of distribution proved to be 1.45±0.03 l/kg for amoxicillin and 1.04±0.08 l/kg for clavulanate. After oral administration absorption half-lives were practically the same (0.13±0.01 h), while elimination half-lives were 1.28±0.05 h and 1.27±0.07 h, respectively. Maximal plasma concentrations were 3.20±0.12 μg/ml in the case of amoxicillin and 1.05±0.12 μg/ml in the case of clavulanate. Time to reach C_{max} was 0.48±0.01 h and 0.48±0.02 h, respectively. Oral bioavailability of amoxicillin and clavulanate in turkey were 60.2±2.6% and 60.7±6.3%, respectively.

Statistically significant ($p<0.05$) differences were found between the two species after intravenous administration of clavulanate and after the oral application of both substances. Oral bioavailability of amoxicillin was significantly higher ($p<0,05$) in chickens (F=63.8±2.6%) than in turkeys (F=60.2±2.6%). A similar difference was experienced in the oral bioavailability of clavulanate, it was higher in chickens (F=65.7±3.1%) than in turkeys (F=60,7±6,3%). These deviations proved to be statistically significant but might not biologically of importance. According to our data it can be pronounced that there is no pharmacokinetic difficulty of the concomitant administration of amoxicillin-clavulanate in these species. Similar behaviour (absorption, elimination, distribution volume) of the drugs in the body might preserve the synergistic effect of the beta-lactam+beta-lactamase inhibitor combination in these species.

2. Susceptibility of *E. coli, S. enterica* and *P. multocida* strains isolated from chickens and turkeys to amoxicillin and the amoxicillin-clavulanic acid combination

In the course of our susceptibility tests on *E. coli*, *S. enterica* and *P. multocida* strains, the actual MIC_{50} and MIC_{90} values were determined with agar dilution method to amoxicillin and to clavulanate potentiated amoxicillin. The incidence of resistance was also investigated to amoxicillin and to the clavulanate potentiated amoxicillin among these strains.

As a part of the study, MIC-values were determined for each bacterial strain, in addition to the inhibitory concentrations of 50% and 90% of all strains (MIC_{50} and MIC_{90} values). Our results were set against the break-points described by the CLSI (*Commitee of*

Laboratory Standards Institute), and the proportion of susceptible, moderately susceptible and resistant strains was defined.

The MIC_{50}-value to amoxicillin-clavulanic acid (amoxi-clav) referring to ***E. coli*** proved to be 8 μg/ml, the MIC_{90} proved to be 32 μg/ml. In this bacterium species frequently occuring and high level resistance was experienced. The incidence of susceptible *E. coli* strains to amoxicillin was 41.7%, while being 64.1% to amoxicillin-clavulanic acid. The decrease in resistance can be explained by the broad spectrum lactamase-inhibitory action of clavulanic acid. The remaining resistant strains might overproduce AmpC-type beta-lactamases, and/or underexpress cell wall porin proteins, thus resulting in reduced permeability of the cell to the beta-lactams. In the case of ***S. enterica*** the MIC_{50} for amoxi-clav was 1 μg/ml, the MIC_{90} was 2 μg/ml, 94% of the strains were susceptible to amoxicillin and 97.6% of the strains were susceptible to amoxicillin-clavulanic acid. Minimum inhibitory concentrations of ***P. multocida*** were similar to that were experienced in the case of *S. enterica*. The MIC_{50}-value for amoxi-clav was 1 μg/ml, the MIC_{90} was 4 μg/ml. Our examined strains showed 82.3%, susceptibility to amoxicillin and 98.8% susceptibility to amoxi-clav. Consequently it can be presumed that the resistance of *Pasteurella* spp. to amoxicillin is caused by beta-lactamase production and can be surmounted by the usage of clavulanic acid.

Summarizing our results it can be pronounced, that the amoxicillin-clavulanic acid combination (in a 12.5 mg/kg dose) is moderately effective against diseases caused by *P. multocida*. (MIC_{90}=4 μg/ml) and *S. enterica* (MIC_{90}=2 μg/ml). Without the increase in dosage the combination might not have an ensured activity in these infections. Systemic diseases caused by *E. coli* (MIC_{90}=32 μg/ml) can not be securely treated with the given dose of the amoxicillin-clavulanic acid combination, but the decrease in the number of bacteria in the intestine – where the concentration of the antibiotics are higher - can play an important role in reducing the number of bacteria in the intestine, thus the development of septicaemia. An other solution is to increase the dose, that will eventuate higher C_{max}-values, and longer T>MIC intervals. In this case, more *E. coli* strains would be susceptible to the combination, although MIC_{90}-values of this bacterium (32 μg/ml) could be reached only with extreme high dosages, that was not economical. In diseases caused by pasteurellae and salmonellae the increase in the dose would increase the effectiveness, because higher C_{max} levels rendered the combination effective against most of the strains. Summarizing our recommendations the 12.5 mg/kg dose of the combination could be only moderately effective against such high priority bacterial poultry diseases, like the septicaemia caused by *E. coli*, *S. enterica* and *P. multocida*.

3. Stability of the amoxicillin-trihydrate and potassium-clavulanate in aqueous solutions

In the course of these studies the stability of two water soluble powder products − containing amoxicillin-trihydrate and potassium-clavulanate in a 4:1 ratio − with an 62.5% active substance content was investigated after dissolution. The goal of the study was a.) to determine the stability of the substances after dissolution in water at different water hardness levels; b.) to determine the stability of the substances at different pH values, in acidic, neutral and alkaline environment; c.) to determine the stability of the substances in plastic and metal troughs. According to these goals the decomposition of the substances was measured at different water hardness levels (german water hardness, $GH°$ = 2, 6, 10), at different pH values (pH = 3.0, 7.0, 10.0, molarity of phosphate buffer was 0.2 M), and in plastic and metal troughs (with tap water, pH=7.09).

In each of these experiments the water soluble powders were dissolved to form aqueous solutions according to the user information of the products, and samples were taken directly after the dissolution („0.” hour, 100 % concentration) and in the 2., 6., 12. and 24. hours. The concentrations of the substances were measured simultaneously with a validated RP-HPLC-UV method. Semi-logarithmic plots were created in a concentration-time system, and the plots proved to be linear, following first-order kinetics. The effects of the environmental factors on the decomposition of the substances were analyzed by comparing the slope of the logarithmic plots with a two-sample t-test.

Summarizing our results it can be stated that statistically and clinically important differences were found in the stability of the substances under the above-mentioned experimental conditions. Increasing the water hardness slightly increased the decomposition rate of both substances. Clavulanic acid proved to be less stable at all hardness levels. Maximum stability of amoxicillin was experienced at an acidic pH, increasing the pH decreased the stability of the substance. Degradation rate of clavulanate was significantly higher at each pH value. In acidic pH the stability of the lactamase-inhibitor decreased extremely, thus acidifying the drinking water of the animals should be avoided, when the combination is administered via this route. It can be pronounced that the stability of the combination mainly depends on the more degradable clavulanate. The decomposition rate of the lactamase-inhibitor proved to be higher at all water hardness levels (at neutral pH) and at all pH values (in plastic troughs, under neutral conditions) than that of amoxicillin. In metal troughs however, the stability of amoxicillin decreased significantly. The reference compound proved to be more stable in metal troughs, but application of the amoxicillin-clavulanate

combination in metal troughs is not recommended. In accordance with our data the amoxicillin-clavulanic acid combination should be prepared in plastic troughs and in soft drinking water with neutral pH, where the compound stability is acceptable for approximately 6 hours. Changes in these conditions might facilitate the degradation of both substances resulting in decreased drug uptake and decreased plasma concentrations of the antibiotics. This will eventuate unsuccessful therapy and may contribute to the spreading of antimicrobial resistance.

NEW SCIENTIFIC RESULTS

1. Oral bioavailability and basic pharmacokinetic parameters were determined after intravenous and oral administration of amoxicillin-clavulanic acid to broiler chickens and turkeys. We published the oral bioavailability of amoxicillin-clavulanate in turkeys and the oral bioavailability of amoxicillin in chickens first in the literature.

2. It can be pronounced that the pharmacokinetic behaviour of the antibiotics is very similar in both species, which is an important advantage from a pharmacokinetical aspect. Comparing the pharmacokinetic parameters in the two species statistically significant differences were found – e.g. the oral bioavailability - but these results are not biologically of importance.

3. In the course of our susceptibility tests on *E. coli*, *S. enterica* and *P. multocida* strains, we determined the actual MIC_{50} and MIC_{90} values, and the incidence of resistance to amoxicillin and to the clavulanate potentiated amoxicillin among these strains. There was no published data about the actual MIC-values in Hungary to amoxicillin and the clavulanate potentiated substance. According to our results it can be pronounced that the resistance appearing to amoxicillin in these strains can be usually vanquished by the clavulanate potentiated combination. One important exception is *E. coli*, where MIC_{90} value of the combination was 32 μg/ml. Among *Salmonella* spp. (MIC_{90}=2 μg/ml) and *Pasteurella* spp. (MIC_{90}=4 μg/ml) no resistant strains were found to amoxicillin-clavulanic acid

4. In the stability experiments the decomposition rate of the substances were examined at different water hardness levels, at different pH-values, and in metal and plastic troughs. According to our results it can be pronounced that the degradation rate of clavulanate was higher under all environmental conditions than that of amoxicillin, considering each water hardness level and each pH value. In metal troughs however, the aminopenicillin showed a very rapid decomposition.

5. In accordance with our data water soluble powders containing the amoxicillin-clavulanic acid combination should be prepared in plastic troughs and in soft drinking water

with neutral pH, where the compound stability is acceptable for approximately 6 hours. Acidifying the water and preparing the solution in a metal trough is not recommended.

Publications regarding to the dissertation

A. Jerzsele, G. Semjen: Az amoxicillin-klavulánsav kombináció használata az állatgyógyászatban. Irodalmi összefoglaló. *Magyar Állatorvosok Lapja.* 2006. 7. 419-426.

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A. Jerzsele, G. Nagy: The stability of veterinary amoxicillin trihydrate and potassium clavulanate combinations in aqueous solutions. *Acta Veterinaria Hungarica*. 2009. *Accepted for publication*