In Vivo Pilot Study Regarding the Effects of Amoxicillin on Blood Glucose Levels, Body Weight and Water Intake

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Amoxicillin it is the most commonly prescribed antibiotic agent and the first intention in the short-term treatment of infections in children. The aim of this study was to assess the effects of amoxicillin/clavulanic acid (AMC) chronic administration on the laboratory mice's body weight (BW), water intake and values of non-fasting blood glucose levels (n-FBG). Thus, twenty-eight C57BL/6 male mice, of similar age, randomly divided into a control and 3 treatment groups (n = 7) received subcutaneous injection, once per day, for 60 days. During the experiment the n-FBG, daily water intake, and BW changes were recorded every 10 days. The results of our study revealed that the chronic administration of AMC, at a concentration of 100 and 150 mg/kg BW/day, increased capillary n-FGB, and can be associated with a significant increase in the BW and daily water intake in mice.

Keywords: mice, amoxicillin, clavulanic acid, blood glucose, water intake, body weight

Amoxicillin is a moderate-spectrum, semi-synthetic β-lactam antimicrobial agent used for treatment or prevention of bacterial infections in both, humans and animals. It has a bactericidal action on numerous gram-positive and gram-negative bacteria by inhibiting the peptidoglycan synthesis from the cytoplasmic membrane. It is susceptible to degradation by β-lactamase and therefore is often combined with the clavulanic acid which protects amoxicillin from degradation under bacterial β-lactam enzymes (by their progressive and irreversible inhibition) and thus effectively extends the antibacterial spectrum of amoxicillin [1-3].

Amoxicillin - a critically important antimicrobial agent in human medicine, either as amoxicillin trihydrate or as sodium salt, has been used extensively in humans to treat a variety of infections. Moreover, it is the most commonly prescribed antibiotic agent and the first intention in the short-term treatment of infections in children (in pediatrics and pediatric dentistry). A series of retrospective and experimental studies have suggested that amoxicillin may be involved in the etiology of Molar Incisor Hypomineralisation (MIH) syndrome, but the results were not conclusive, underlining the need for further animal-controlled studies [4-6]. Also, results from our previously published study [7] show that chronic administration of Amoxicillin/Clavulanic Acid (AMC) through subcutaneous injection in C57BL/6 mice leads to disturbances in the formation of lower incisors' enamel, mainly as a dysfunction in the maturation and transitional ameloblasts, resulting in hypomineralised enamel, with quantitative and/or qualitative dose-dependent defects.

The hypothesis that amoxicillin treatment would transiently increase blood glucose levels, and can cause some urine sugar tests to be misinterpreted, has also been raised in the literature. It is assumed that taking amoxicillin does not directly interfere with the level of insulin in human body. According to the University of Iowa Health Care website, a high blood glucose level is a common result of infection, which would in turn affect needs for insulin in patients with diabetes, and potentially affect the rennin-angiotensin secretion [8]. In most cases, blood glucose levels decrease once the infection is cleared [9, 10]. However, based on our knowledge, there are no studies confirming or denying these assumptions.

In the present pilot study were assessed the effects of AMC chronic administration in healthy C57BL/6 strain laboratory mice (on the body weight, on the amount of water intake and on the values of non-fasting blood glucose levels (n-FBG)).

Experimental part

The experimental design was fully approved by the Research Ethics Committee of the Grigore T. Popa University of Medicine and Pharmacy Iasi, Romania. AMC in the form of sterile powder vials for injection or perfusion (Amoxiplus®, batch P26 2251, Antibiotice SA, Romania) and sterile water for injections (10 mL vials, Antibiotice S.A., Romania) were purchased from a reputable pharmaceutical company. Each 1.2 g vial of Amoxiplus® contains 1000 mg amoxicillin, as sodium salt (fig. 1), and 200 mg clavulanic acid (as potassium salt). Hand-held whole-blood glucose monitor (Accu-Chek® Active) and test strips were obtained from Roche Romania S.R.L., diagnostics division. For our study we used twenty-eight C57BL/6 inbred strain (homozygote) adult male mice, purchased from the Baneasa Station, unit of the Cantacuzino National Institute in Research and Development in Microbiology and Immunology (Bucharest, Romania).

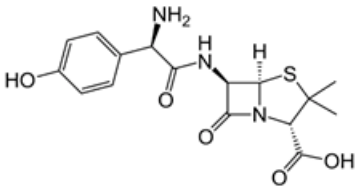

Fig.1. The amoxicillin structure [1]

During acclimatization and experimental study, all mice were kept at 24±1°C in standard plastic caging system for rodents, under a 12-hr light-dark cycle and allowed ad libitum access to food and distilled water. They were fed with a standardized laboratory rodent pellet diet (18.8% proteins, 2.3% fats, and 6.1% fibers; Cantacuzino, Romania). After two weeks of acclimatization, mice were randomly divided into 4 groups, control and 3 treatment groups (experimental) of 7 mice each (n = 7):

- group I: control one, received only solvent (0.1 mL sterile distilled water once per day)

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- group II: received 50 mg/kg BW (body weight) of AMC once per day
- group III: received 100 mg/kg BW of AMC once per day
- group IV: received 150 mg/kg BW of AMC once per day.

Extemporaneously prepared Amoxiplus® was administered as reconstituted solution (sterile water as solvent) through subcutaneous injection (into the loose skin of the posterior neck), in a single dose per day and same time slot for 60 days. In order to use adequate concentration of amoxicillin or to adjust dosage, every 10 days mice were weigh using a precision balance (Precisa XT 2200C, Precisa Ltd., UK), and the mean value of BW was calculated for each study group. The injections were performed using 1 mL sterile syringes (gradations at each 0.02 mL) with 29 gauge needle (0.33 mm diameter, and 13 mm long). Maximum injectable volume was set at 0.1 mL.

The amount of Amoxiplus® powder needed per group to prepare the injectable solution was determined using a Precisa 125A SCS (Precisa Ltd., UK) digital analytical balance using the following formula:

$$G = a \times n \times c \times g,$$

where: a = the ratio of the unit weight of the pharmaceutical preparation to the amount of active substance/interest of that preparation; n = the number of animals to be treated or the batch; c = the administered dose per animal in mg/kg body weight; g = the average weight of the animals to be treated in kg.

Subsequently, the powder obtained by weighing was deposited in 10 mL sterile tapped polypropylene tubes, 0.7 mL of water for injections was added and the mixture thus obtained was homogenized for 30 seconds using a tube shaker (Bio Vortex V1, Biosan Ltd., Latvia).

For each mouse, every 10 days during the study period, capillary blood was collected via tail vessel lancing (standard method), applied to a glucose test strip and immediately analyzed with a blood glucose monitoring device (ACCU-CHEK® Active, Roche Diagnostics GmbH). The obtained non-fasting blood glucose (n-FBG) values were expressed in mg/dL. Prior and during the experiment, daily water intake, and body weight changes were also recorded every 10 days.

All **s**tatistical analysis was done with SPSS software program version 21.0 (IBM Corp., Chicago, IL, USA). Obtained data was represented as Mean ± Standard Deviation (SD). Blood glucose level, water intake, and body weight variations were analyzed using one-way ANOVA test, followed by post hoc Dunnett's t test. Values were considered statistically significant for a value of $p < 0.05$.

Results and discussions

Since AMC is one of the most commonly prescribed antibiotics [11] we chose to test it in our study instead of Amoxicillin. The usual dose of AMC in humans is 50 mg/kg body weight/day, and the maximum dose for adults may be up to 2-3 g per day [12]. Based on these standards, we chose to use a 50 mg/kg body/day dose of AMC, and we also decided to use two experimental doses of 100 and 150 mg/kg body weight/day of AMC. Moreover, the bioavailability of oral amoxicillin was reported to be 80% in humans and only 44% in rats [1]. The low bioavailability of the drug in small rodents (mice, rats) was related to the presystemic degradation of amoxicillin in the intestine due to the lack of the first passage of hepatic metabolism [13]. To avoid these inconveniences, we used the parenteral route of administration for AMC, namely subcutaneous injections. Alternatively, as described in some studies, direct administration on cell lines would also have trigered potentially significant results [14]

n-FBG levels were estimated in control and AMC treatment groups one day before experiment started, and once every 10 days of the treatment period. Post-hoc statistical tests showed significant differences between the control group and all AMC treatment groups ($p < 0.05$). The mean values of n-FBG were 116 in the control group, and 124.80, 132.74, 134.47 mg/dL in AMC treatment groups, respectively. As shown in figure 2, pairwise comparisons of n-FBG mean values between groups depending on estimation number, showed significant differences between control and 100 mg/kg BW of AMC groups at estimation no. 1-6 ($p < 0.05$); between control and 150 mg/kg BW of AMC groups at estimation no. 2-6 ($p < 0.05$); between 50 and 100 mg/kg BW of AMC groups at estimation no. 4 ($p < 0.05$).

According to the boxplot diagram (fig. 3), extreme values of capillary glycaemia varied much intra- and inter-group. Compared to the control group, the trend of n-FBG increase in experimental groups was observed after every 10 days of AMC administration. Also, in the AMC-treated groups an increase in n-FBG values was observed depending on the administered antibiotic doses.

Comparisons at the intra-lot pair level according to number of determination did not revealed significant differences in the control group and in group II. In groups III and IV significant differences have been observed in the case of determination no. 0 compared to the other determinations (1-6 for group III and 2-6 for group IV). In addition, within the group IV, significant differences were found in the case of determination no. 1 in compairason with the determinations 4 to 6 (fig. 3).

Table 1 illustrates the variations in BW and water intake of normal and AMC treated groups. During the study, mice in all groups significantly increased BW ($p < 0.05$); all AMC groups gained more BW than the control one, but statistically significant values were found only between the control and the 100 mg/kg BW of AMC group (p = 0.029); the 150 mg/kg BW of AMC group also gained more weight than the control group, but less than the 100 mg/kg BW of AMC group.

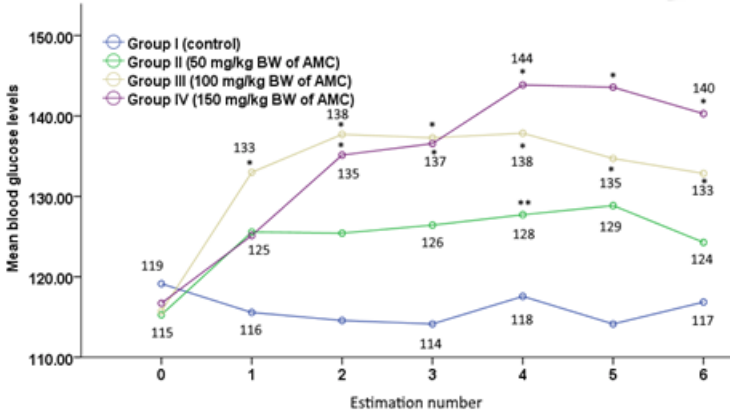

Fig.2. Pairwise comparisons of non-fasting blood glucose levels (n-FBG) between study groups before and throughout treatment period with AMC. n-FBG were expressed in mg/dl, and as mean values of 7 mice per group. Estimation number 0 = n-FBG values one day before treatment. Estimation from 1 to 6: N-FBG every 10 days of the study. *Significantly different from control group ($p < 0.05$), **Significantly different from 150 mg/kg BW of AMC treatment group ($p < 0.005$).

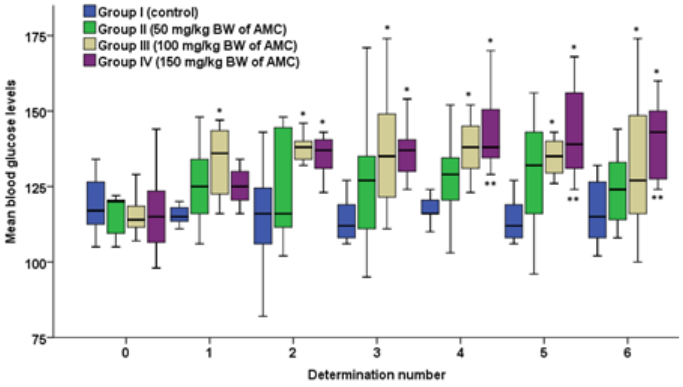

Fig. 3. The aspect of the central tendency and the shape of n-FBG capilary distribution on study batches before administrating AMC (determination 0) and every 10 days of injecting AMC (determination 1-6). *Indicates significant differences inside the batch compared to determination no. 1

Table 1

BODY WEIGHT AND WATER INTAKE CHANGES IN ADMINISTRATION OF AMC AMONG EXPERIMENTAL AND CONTROL GROUPS

Parameters	Control (I)	50 mg/kg BW (II)	100 mg/kg BW (III)	150 mg/kg BW (IV)
Body weight (g)				
Before treatment	22.36±1.18	21.33±1.91	22.07±2.14	23.63±1.30
Last treatment day	24.85±2.67	23.85±2.47	26.57±3.73	26.85±1.06
Body weight gains	2.49±1.68	2.52±0.94	4.5±1.84[a]	3.22±0.50
Water intake (g)				
Before treatment	3.56±0.18	2.98±0.11	4.21±0.20	3.84±0.19[b]
Throughout treatment	3.89±0.42	3.24±0.39[c]	4.41±0.65[d]	4.51±0.71[e]
Water intake changes	0.32	0.26	0.19	0.67

Note. Data represents Mean ±SD values (g) per each mouse at the same group; [a] indicates significant BW gain toward control group; [b] indicates significant increase of water intake before and throughout treatment period at the same group; [c,d] indicates significant decrease of water intake toward control group; [e] indicates significant increase of water intake toward control group; [a-e]$p < 0.05$.

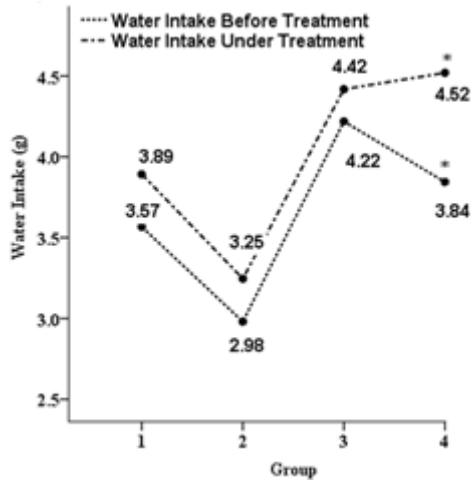

Fig. 4. Pairwise comparison of water intake between study groups before and throughout treatment period with AMC. Water intake was expressed as mean values (g) per each mouse at the same group. Significant differences were in 150 mg/kg BW of AMC treatment group ($p < 0.05$)

Profile plot (fig. 4) for pairwise comparisons of water intake mean values before and throughout treatment period, showed an increase of water intake between all groups, but these were significant only in the 150 mg/kg BW of AMC group ($p = 0.003$). Dunnett t test used for comparing water intake mean values between all AMC treatment groups and the control one, during treatment period only, showed that daily treatment with 50 mg/kg BW AMC and 100 mg/kg BW AMC significantly reduced water intake ($p = 0.011$, $p = 0.045$ respectively), and treatment with 150 mg/kg BW AMC significantly ($p = 0.014$) increased water intake in mice (table 1).

World Health Organization reported that amoxicillin in rats does not have marked effects on feed consumption, body weight gain, and can induce isolated, not treatment related, intergroup differences in hematology [1]. In our study, n-FBG levels were significantly greater in groups treated with 100 and 150 mg/kg BW AMC, and all treatment groups gain more in BW than the control one. Increased n-FBG levels may be associated with increased BW gain due to insulin regulation mechanisms of blood glucose [15]. Even so, n-FBG levels and BW values were significantly lower than the ones previously reported in C57BL/6 healthy mice [16, 17]. Satoh et al. [18] report decreased BW in mice treated with antitumor antibiotics (Adriamycin and Mitomycin) administrated singly via the tail vein on days 8 to 12 of observation. Similar influences may be carried by varying differences of tissue heavy-metal concentrations [19]. Furthermore, based on our knowledge there are no comparative studies between amoxicillin and AMC, or clavulanic acid alone effects on blood glucose level, BW gain and water intake in small mammals.

Conclusions

The results of our study revealed that the chronic administration of AMC by subcutaneous injections (at a concentration of 100 and 150 mg/kg BW/day) in C57BL/6 strain adult male mice increased capillary n-FGB which can be associated with a significant increase in the body weight and daily water consumption. However, in order to confirm this hypothesis, to fully elucidate the involved mechanisms and their interrelations further biochemistry and toxicology studies are necessary.

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