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The Effect of Oral Ampicillin Applicaitons on Liver Mineral Status

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Abstract: This study was carried out to determine the changes of Cu, Zn, Fe, Ca, Mg, K and Na levels in liver during treatment of oral ampicillin in mice. The investigation was supported by serum ALT and AST enzyme activities, serum Total Protein (TP), Albumin (ALB), Globulin (GLB) levels determinations. Twenty one male Swiss albino mice (weighing 28.36±2.33 g, aged 4 months) were used for this study. The standard commercial diet was given to all groups. First group (A) were used control and took only tap water. Other groups were received water with ampicillin. Dose of ampicillin for second (B) and third (C) groups were as follows; 8 and 40 mg/100 mL in drinking water. Dry ashing method and Flame Atomic Absorption Spectrometer (FAAS) were used for determination of minerals. Statistically significant increase (minimum $p<0.05$) for Cu, Ca and Na and decrease for K were observed. Significant positive correlation between Cu-Ca, Cu-Na, Zn-Mg, Ca-Na, Mg-K (minimum $r = 0.450$, $p<0.05$) and negative correlation between Cu-K ($r = 0.456$, $p<0.05$) were observed. There was a significant difference in AST levels of groups ($p<0.001$) but not other serum parameters of ALT, TP, ALB and GLB. These results indicate that oral ampicillin treatment affect liver Cu, Ca, Na and K levels. Therefore, this fluctuation must be considered on ampicillin applications during antimicrobial therapy.

Key words: Ampicillin, liver, Cu, Zn, Fe, Ca, Mg, K, Na, level

INTRODUCTION

Mineral elements are not synthesized by living organism thus must be taken externally. Minerals mainly act as structural components of body organs and tissues and catalyses enzyme and hormone system (McDowell, 1992) and the main storage site is liver. Mineral content of liver gives precious overview of body mineral conditions (Radostits *et al*., 2000).

Thiim and Friedman (2003) reported that antimicrobial agents such as amoxicillin-clavulanate, sulfamethoxazole/trimethoprim, minocycline, nitrofurantoin may be associated with liver damage. Investigations has already reported that amoxicillin use caused liver damage (Cundiff and Joe, 2007, 2004; Endo *et al*., 2002; Ersoz *et al*., 2001; Sontag *et al*., 2001). Ampicillin is a aminopenisilin group antibiotic, like amoxicillin and widely used for therapy of bacterial infections (Cai *et al*., 2009; Koluman *et al*., 2009; Sagoo *et al*., 2009). Following oral administration ampicillin is mainly deposited in liver and may cause liver dysfunction (Huber, 1988). However, there is limited information about liver injury and its mineral content. Whereas, it was reported that there were some fluctuation on mineral contents of liver during liver damage induced (Yüksek *et al*., 2005).

It was also reported that diarrhea occurs in patients received ampicillin (Bartlett, 1992, 2002; Beaugerie and Petit, 2004; Moulina *et al*., 1995) and that diarrhea is known to cause malabsorption due to inflammation stress. Malabsorption may give rise to insufficient utilization of minerals (Radostits *et al*., 2000). Similarly, stress associated with inflammation also causes mineral level fluctuations in serum (Chirase *et al*., 1991; Karademir, 2007; Orr *et al*., 1990). But no investigation has yet disclosed the effect of inflammation stress on mineral content of liver.

The aim of this study was to investigate the effect of ampicillin on the status of mineral (Cu, Zn, Fe, Ca, Mg, K, Na) content of liver.

MATERIALS AND METHODS

Animals and procedures: This study involved clinically healthy 21 male Swiss albino mice, aged 4 months and

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Table 1: Food and water Cu, Zn, Fe, Ca, Mg, K and Na levels given to mice

	Mineral content						
Diets	Cu	Zn	Fe	Ca	Mg	K	Na
Food (mg kg^{-1} in DM)	11.700	109.370	452.910	29229.30	2710.20	11328.20	5289.20
Water (mg L^{-1})	0.015	0.048	0.063	98.83	35.80	3.23	21.09

DM: Dry Matter

weighing 26.9±1.04 g. The animals were divided into three equal experimental groups. Administrations of treatment groups were as follows; Group A: received only tap water as control, Group B: received ampicillin 8 mg in 100 mL tap water and Group C: received ampicillin 40 mg in 100 mL tap water for 10 days.

The animals were fed with a commercial animal food. The food and water were given *ad libitum* before and during the experiment. Mineral content of food and water was determined by Flame system Atomic Absorption Spectrometer (FAAS) (Thermo Elemental S4, Thermo Electron Corporation, Cambridge, UK) and the results are shown in Table 1.

The commercial food was purchased from Bayramoglu Yem ve Un San. Tic. A.S. ISO 9001: 2000, ISO 22000: 2005 and the composition is given in Table 2.

Blood and liver samples collections: Blood was collected via cardiac puncture under the ether anesthesia. After blood collections, the mice were scarified by atlanto-occipital dislocation and livers were taken and frozen at -20°C until analyses.

Laboratory analyses: Liver mineral content was measured by FAAS. Serum was separated by centrifugation at 3500 rpm for 15 min and serum ALT and AST enzyme activities, serum Total Protein (TP) and Albumin (ALB) levels were also determined spectrophotometrically using commercial kits. Globulin (GLB) levels were obtained calculations with the formula of TP - ALB = GLB.

In order to determine mineral levels approximately 2 g liver tissues were weight and dried in a drying oven at 135°C for 3 days. The dried liver tissues were again weighted and noted. These samples were made ash with a muffle furnace at 600°C for 6 h. Heating was made gradually as 200-400-600°C. The ashed samples were melted in 20% HCl and complete to 10 mL with deionized water. Liver minerals were measured from this solution using different dilution rates by FAAS. Dilution liquids were deionized water for Cu, Zn, Fe, K and Na and 0.1% lanthanum (as chloride) (w v^{-1}) (Merck, 112219) for Ca and Mg analyses.

Standard solutions for Cu, Zn, Ca, Mg, K and Na supplied from Fluka Chemie GmbH, Switzerland (Fluka 61147, 96457, 21049, 63046, 60032 and 71173, respectively) and for Fe Merck KgaA, Germany (119781).

Table 2: Ingredients of diet given to mice

Diet composition	Units
Dry matter (%)	88
Crude protein (%)	17
Crude cellulose (%)	12
Crude ash (%)	10
Acid insoluble ash (%)	1
Calcium (%)	1.5
Phosphorus (%)	0.75
NaCl (%)	0.6
Vitamin A (IU kg^{-1})	5000
Vitamin D3 (IU kg^{-1})	600
Vitamin E (mg kg^{-1})	25
Metabolic energy (kcal kg^{-1})	2600

Raw-materials for this composition: Barly, corn, corn chaff, corn glutein, wheat, rye, craff, cottonseed meal, sunflower meal, dicalcium phosphate, vitamin, mineral

Control of accuracy of FAAS was performed using a previously prepared known standard solution (Fluka and Merck) for each mineral measurement.

These solutions were aspirated for 6 times per 10 samples during analyses. Coefficients of Variations (CV) of these parameters were calculated from the findings. CV results were as follows; Cu: 4.34%, Zn: 3.49%, Fe: 1.92%, Ca: 2.32%, Mg: 2.07%, K: 2.08% and Na: 1.41%. All lab-ware used were plastic material in the laboratory.

Fecal consistency was scored as between 0-5: hard solid, solid, slight solid, slight watery, moderately watery and watery.

Statistical analysis: A one-way variance analysis was made and Tukey test employed for the comparisons. Pearson's correlation test was used to determine the relationships between mineral levels of the liver, drinking water and feces consistency.

Statistical analyses were performed using Minitab statistical software version 10.0 (Minitab, 1995). Data were presented as means±SEM.

RESULTS

Mean values for serum ALT, AST enzyme activities and Total Protein (TP), Albumin (ALB) and Globulin (GLB) levels are summarized in Table 3.

Mean water consumption of the groups were as follow; Group A: 57.0±2.3 mL day^{-1}, Group B: 58.8±2.25 mL day^{-1} and Group C: 63.3±2.26 mL day^{-1}. This difference among groups was not statistically significant.

Table 3: ALT and AST enzyme activities and serum TP, ALB and GLB levels according to groups with their statistical evaluations (mean±SEM)

Groups	ALT	AST	TP	ALB	GLB
A	59.92±2.31	211.3±19.7^{c}	5.36±0.10	3.49±0.06	1.87±0.08
B	70.59±7.06	454.1±47.3^{b}	5.20±0.14	3.34±0.08	1.86±0.08
C	97.6±18.7	682.2±84.8^{a}	5.41±0.17	3.49±0.11	1.93±0.10
p-value	0.088	0.000	0.549	0.410	0.832

^{abc}: Means with different superscript letters are statistically different in columns

Table 4: Liver mineral content of the experimental groups (mean±SEM). Values are as mg in 1000 g dry matter of liver tissues

Groups	Cu	Zn	Fe	Ca	Mg	K	Na
A	14.59±0.96^{b}	106.04±1.64	496.1±56.2	20.43±1.16^{b}	787.5±22.6	10487±284^{a}	2981±126^{b}
B	18.07±0.84^{a}	114.75±3.2	482.9±47.4	26.33±1.46^{a}	817.3±23	10263±217^{a}	3413±94^{a}
C	20.44±0.43^{a}	98.52±7.4	553.1±50.7	27.16±1.99^{a}	756.3±25.6	9154±345^{b}	3454±125^{a}
p-value	0.000	NS	NS	0.014	NS	0.009	0.018

^{abc}: Means with different superscript letters are statistically different in columns

Table 5: Correlation coefficients (r) between liver minerals and their p-value

Mineral contents	Cu	Zn	Fe	Ca	Mg	K
Zn	0.104^{NS}					
Fe	0.407^{NS}	0.311^{NS}				
Ca	0.537*	0.422^{NS}	0.250^{NS}			
Mg	0.065^{NS}	0.450*	-0.010^{NS}	0.422^{NS}		
K	-0.456*	0.265^{NS}	-0.281^{NS}	0.014^{NS}	0.739***	
Na	0.505*	0.217^{NS}	0.253^{NS}	0.653**	0.322^{NS}	-0.201^{NS}

^{NS}: Not Significant, *: p<0.05, **: p<0.01, ***: p<0.001

Ampicillin consumptions by the groups were as follow: Group A: 0 mg/kg/day, Group B: 23.75±0.91 mg/kg/day and Group C: 127.81±4.57 mg/kg/day.

Results of fecal consistency evaluation were as follow: Group A: 0.0±0.00, Group B: 0.30±0.13 and Group C: 1.35±0.34. The value of Group C was statistically different than other groups ($p<0.05$). There was no correlation between water consumption and faces consistency ($r = 0.244$, $p>0.05$).

Mean values of liver Cu, Zn, Fe, Ca, Mg, K and Na contents are given in Table 4. Correlation between liver mineral contents is given in Table 5.

DISCUSSION

Minerals are essential nutrients and must be taken orally (McDowell, 1992). The liver mineral content gives valuable information for body mineral status as blood serum levels (Radostits *et al*., 2000).

It is known that use of antibacterial agents may cause liver damage (Cundiff and Joe, 2007; Endo *et al*., 2002; Ersoz *et al*., 2001; Cundiff and Joe, 2004; Lamouliatte *et al*., 1998; Sontag *et al*., 2001). It is also known that there is a fluctuation in liver minerals content when liver was injured (Yuksek *et al*., 2005). In this study, serum ALT and AST enzyme activity and serum protein measurements were carried out to determine any liver damage. AST activities showed statistically significant differences between groups ($p = 0.000$). There were increase in AST and ALT activities in groups received different doses of ampicillin but differences in ALT was not statistically significant ($p>0.05$). This may be reflecting no liver injury as ALT enzyme activities levels are more specific for liver injury than other parameters including AST.

This may also, be supported by unchanged serum protein level, which is expected to alter during liver injury. This may indicate that liver damage was not evident or this damage may be disregarded during ampicillin application.

Oral ampicillin treatment has been reported to cause diarrhea (Bartlett, 1992, 2002; Beaugerie and Petit, 2004; Moulina *et al*., 1995) and increase in AST enzyme activities as enteritis cause elevation of AST as in this study. Increase in AST in this study may be explained by enteritis as fecal consistency score was higher in treatment groups.

Although, liver injury was not detected by means of ALT, AST enzyme activities, minerals content of liver showed variation in this investigation. After the oral administration, ampicillin is absorbed and distributed to all part of the body. But, it is more intensive in liver (Huber, 1988). Hence, the mineral fluctuation in liver may not be by means of interference of ampicillin or the inflammation stress of mild enteritis may be the cause (Chirase *et al*., 1991; Karademir, 2007; Orr *et al*., 1990).

In general, mineral levels of the present study and serum mineral content of studies where inflammation induced stress was the cause (Chirase *et al*., 1991; Cundiff and Joe, 2004; Orr *et al*., 1990) were similar, but not differed from those of experimentally, induced hepatitis (Yüksek *et al*., 2005). The mineral fluctuation in this study may be due to either inflammation or interaction between the minerals of concern as they are well known to affect each other.

CONCLUSION

Consequently, after the oral ampicillin application some liver mineral content altered and the level of Cu, Ca and Na increased, but K levels of liver decreased. Fluctuations of these minerals levels may affect health and related functions and these fluctuations must be considered when ampicillin is used for therapy.

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REFERENCES

Bartlett, J.G., 1992. Antibiotic-associated diarrhea. Clin. Infect. Dis., 15: 573-581. PMID: 1420669.

Bartlett, J.G., 2002. Clinical practice. Antibiotic-Associated Diarrhea. N. Engl. J. Med., 346 (5): 334-339. PMID: 11821511.

Beaugerie, L. and J. Petit, 2004. Microbial-gut interactions in health and disease. Antibiotic-associated diarrhoea. Best. Pract. Res. Clin. Gastroenterol., 18 (2): 337-352. PMID: 15123074.

Cai, L., M.Q. Yuan., F. Liu, J. Jian and G. Chen, 2009. Enhanced production of medium-chain-length Polyhydroxyalkanoates (PHA) by PHA depolymerase knockout mutant of Pseudomonas putida KT2442. Bioresour. Technol., 100 (7): 2265-2270. PMID: 19103481.

Chirase, N.K., D.P. Hutcheson and G.B. Thompson, 1991. Feed intake, rectal temperature and serum mineral concentrations of feedlot cattle fed zinc oxide or zinc methionine and challenged with infectious bovine rhinotracheitis virus. J. Anim. Sci., 69: 4137-4145. PMID: 1778828.

Cundiff, J.G. and S. Joe, 2004. Augmentin-induced hepatitis in the treatment of chronic rhinosinusitis. Otolaryngol. Head Neck Surg., 131 (2): 266. http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6WP4-4D466W4-R0&_user=4019113&_rdoc=1&_fmt=&_orig=search&_sort=d&view=c&_acct=C000062080&_version=1&_urlVersion=0&_userid=4019113&md5=d2063fea42891349a777a79285155c68.

Cundiff, J. and S. Joe, 2007. Amoxicillin-clavulanic acid-induced hepatitis. Am. J. Otolaryngol., 28 (1): 28-30. PMID: 17162128.

Endo, H., H. Yoshida, Y. Kohno and T. Sug., 2002. Effects of clarithromycin and amoxicillin on gastric emptying in rats. Antimicrob. Agents. Chemother., 46(10): 3331-3333. http://aac.asm.org/cgi/content/full/46/10/3331.

Ersoz, G., Z. Karasu, C. Yildiz, U.S. Akarca, G. Yuce and Y. Batur, 2001. Severe toxic hepatitis associated with amoxycillin and clavulanic acid. J. Clin. Pharm. Ther., 26 (3): 225-229. PMID: 11422607.

Huber, G.H., 1988. Penicillins. Veterinary Pharmacology and Therapeutics. 6th Edn. In: Booth, N.H. and L.E. McDonald (Eds.). Iowa State University Press/AMES, pp: 796-812. ISBN: 0-8138-1739-0.

Koluman, A., L.S. Akan and F.P. Cakiroglu, 2009. Occurrence and antimicrobial resistance of enterococci in retail foods. Food Control, 20 (3): 281-283. http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6T6S-4SJP7BF5&_user=4019113&_rdoc=1&_fmt=&_orig=search&_sort=d&view=c&_acct=C000062080&_version=1&_urlVersion=0&_userid=4019113&md5=24fe8664e8b26b2c4a33b9f87d9ef01a.

Karademir, B., 2007. Effect of stress induced by vaccination on blood plasma copper, zinc, potassium and magnesium. Kafkas. Univ. Vet. Fak. Derg., 13 (1): 49-54 (in Turkish). http://vetdergi.kafkas.edu.tr/extdocs/13_1/49_54.pdf.

Lamouliatte, H., R. Cayla, F. Zerbib, S. Forestier, A. Mascarel, M. Joubert-Collin and F. Megraud, 1998. Dual therapy using a double dose of lansoprazole with amoxicillin versus triple therapy using a double dose of lansoprazole, amoxicillin and clarithromycin to eradicate *Helicobacter pylori* infection: Results of a prospective randomized open study. Am. J. Gastroenterol., 93 (9): 1531-1534. PMID: 9732938.

McDowell, L.R., 1992. Minerals in animal and human nutrition. Academic Press Inc., London. ISBN: 0-12-483369-1.

Minitab, 1995. Minitab reference manual (Release 10.0) for Windows, Minitab Inc. USA. ISBN: 0-92563628-2.

Moulina, F., J. Raymondb, M. Bergeretb, J.L. Iniguez, F. Habiba, M. Chemillier-Truong, M.A. Legalla, J. Badouala and D. Gendrela, 1995. Quinolones in the treatment of severe salmonella infections in children after failure of conventional therapy. Arch. Pediatr., 2 (4): 317-323. PMID: 7780538.

Orr, C.L., D.P. Hutcheson, R.B. Grainger, J.M. Cummins and Mock, 1990. Serum copper, zinc, calcium and phosphorus concentrations of calves stressed by bovine respiratory disease and infectious bovine rhinotracheitis. J. Anim. Sci., 68: 2893-2900. PMID: 2211419.

Radostits, O.M., C.C. Gay, D.C. Blood and K.W. Hinchcliff, 2000. Veterinary Medicine. 9th Edn. W.B. Saunders Campany Ltd, London. ISBN: 0-7020-26042.

Sagoo, S.K., C.L. Little, M. Greenwood, V. Mithani, K.A. Grant, J. McLauchlin, E. Pinna and E.J. Threlfall, 2009. Assessment of the microbiological safety of dried spices and herbs from production and retail premises in the United Kingdom. Food Microbiol., 26 (1): 39-43. PMID: 19028303.

Sontag, S.J., S. O'Connell, T. Schnell, G. Chejfec, J. Seide and A. Sonnenberg, 2001. Reduced symptoms and need for antisecretory therapy in veterans 3 years after helicobacter pylori eradication with ranitidine bismuth citrate/amoxicillin/clarithromycin. Am. J. Gastroenterol., 96 (5): 1390-1395. PMID: 11374672.

Thiim, M. and L.S. Friedman 2003. Hepatotoxicity of antibiotics and antifungals. Clin. Liver Dis., 7 (2): 381-399. PMID: 12879990.

Yüksek, N., N. Altug, Z. Aoglu and A. Karasu, 2005. Setermination of mineral elements in liver and serum of dogs with carbontetrachlorur intoxication. YYÜ Vet. Fak. Derg., 16 (2): 43-46 (in Turkish). http://www.yyu.edu.tr/calismaayrinti.aspx?sicilno=1206&adsoyad=Yrd.Doc.Dr.%20%20%20Nazmi%20%20%20YÜKSEK.