Evaluation of biochemical findings in mice exposed to thiamphenicol treatment

Article *in* African journal of pharmacy and pharmacology · April 2011

CITATIONS
4

READS
464

5 authors, including:

Adnan Adil Hismiogullari
Balikesir University
26 PUBLICATIONS **120** CITATIONS
SEE PROFILE

Sahver Ege Hismiogullari
Balikesir University
28 PUBLICATIONS **193** CITATIONS
SEE PROFILE

Ozlem Yavuz
Sağlık Bilimleri Üniversitesi
70 PUBLICATIONS **705** CITATIONS
SEE PROFILE

Khalid Rahman
Liverpool John Moores University
164 PUBLICATIONS **4,941** CITATIONS
SEE PROFILE

African Journal of Pharmacy and Pharmacology Vol. 5(3), pp. 428-431, March 2011
Available online http://www.academicjournals.org/ajpp
ISSN 1996-0816 ©2011 Academic Journals

Full Length Research Paper

Evaluation of biochemical findings in mice exposed to thiamphenicol treatment

Adnan Adil Hismiogullari[1]*, Sahver Ege Hismiogullari[2], Ozlem Yavuz[1], Dinc Essiz[3], and Khalid Rahman[4]

[1]Department of Biochemistry, School of Medicine, Balikesir University, Balikesir, Turkey.
[2]Department of Pharmacology and Toxicology, School of Veterinary Medicine, Balikesir University, Balikesir, Turkey.
[3]Department of Pharmacology and Toxicology, School of Veterinary Medicine, Kafkas University, Kars, Turkey.
[4]School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Liverpool, UK.

Accepted 7 April, 2011

The aim of the study was to investigate the acute and subacute dose and the dose regime of thiamphenicol-induced changes in serum biochemical parameters in mice. Thiamphenicol was given to the trial groups at a dosage of 100 mg/kg bw (group 2) and 200 mg/kg bw (group 3) in drinking water for 7 days. Blood samples were collected from all animals on days 1, 3, 7 and 14 of the study. Serum concentrations of urea, creatinin, triglyceride and total protein as well as serum activities of hepatotoxicity markers as ALT, AST, LDH and ALP were measured spectrophotometrically. The results obtained in this study show that oral administration of thiamphenicol in mice does not show biochemical alterations.

Key words: Antibiotic, biochemical parameters, hepatotoxicity, mice, thiamphenicol.

INTRODUCTION

Thiamphenicol (TAP) is an antibiotic used for the treatment of infectious diseases. The chemical structure of TAP is an analogue of choramphenicol, except for the substituent in the “para” position of the benzene ring. Chloramphenicol has a nitro group at that position, whereas thiamphenicol has a sulfonylmethyl group (Ferrari, 1981). TAP was developed as a replacement for chloramphenicol in the 1960s (Bishop, 1998) and although not available in certain countries such as the UK, USA and Australia, it is widely used in Europe (France, Germany, Italy, Spain) and Japan (Francesehinis, 1981).

The antibacterial mechanism of TAP is through the inhibition of protein synthesis in bacteria and it displays broad-spectrum antibiotic activity (Sutter and Finegold, 1976; Van Beers et al., 1975). It has not got side effect such as aplastic anaemia in spite of extensive use in human. TAP is mostly effective in respiratory infections (Kitamura et al., 1997), refractory psoriasis and pustulose in children, bacterial prostatitis and sexually transmitted diseases (Kucers et al., 1997; Reynold, 1989) and it is also used extensively in veterinary medicine (Bishop, 1998). The reversible bone marrow suppression is seen after TAP treatment in man (Ablini et al., 1999; Ali et al., 2003) and experimental animals (Bishop, 1998). It has been reported that some suppression of erythropoiesis was observed in the highest-dose group along with slightly reduced spermatogenesis in the testes of male rats. A study from Paulo et al. (2000) also revealed that there is no risk of relative overdose in individuals with altered hepatic function.

Thiamphenicol has been previously investigated at a dose of 8 to 9 mg/kg in humans, 30 to 60 mg/kg for calves, 20 to 40 mg/kg for pigs, 15 to 67 mg/kg for poultry and 30 mg/kg in dairy cows (Nau et al., 1981; FAO, 1999). Due to the lack of knowledge reported in the literature concerning the biochemical parameters in serum affected by thiamphenicol, the present study was undertaken.

MATERIALS AND METHODS

Experimental animal

Ninety male Swiss albino white mice, weighting 25 to 30 g, were used in this study. The experimental animal protocol for animal

*Corresponding author. E-mail: ahismiogullari@gmail.com.

studies was approved by the University of Balikesir Institutional Animal Care and Use Committee. The animals were allowed free access to normal cow diet and drinking water for 3 days before starting the study.

Experimental design and treatment of animals

The mice were randomly divided into 3 groups: A control (group 1) and 2 trial groups (groups 2 and 3). While, the control group received normal cow diet and tap water, the trial groups received thiamphenicol in drinking water for 7 days. Five millilitre of thiamphenicol were administrated to the mice in Group 2 daily. The concentration of medicine was 0.6 mg/ml and was prepared as 100 mg/kg dose; therefore, each mouse in group 2, received 3 mg medicine daily. The same procedure was followed for group 3. Five millilitre of thiamphenicol were administrated to the mice in group 3 daily. The concentration of thiamphenicol was 1.2 mg/ml and prepared as 200 mg/kg dose. Meanwhile, each mouse in group 3, received 6 mg medicine daily. Following administration of thiamphenicol, blood samples were taken from all animals on 1, 3, 7 and 14 days of feeding. In every sampling period, 200 µl of blood was taken from each animal by orbital sinus venipucture. Due to difficulties in obtaining blood from mice it was decided to increase the number of mice of similar weight in each group so their blood could be combined in order to have enough volume to perform the analysis.

Biochemical analysis

The blood samples were centrifuged at 825 x g for 10 min to separate the sera in order to avoid haemolysis. The serum levels of urea, creatinine, triglyceride and total protein as well as alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehidrogenase (LDH) and alkaline phosphatase (ALP) activities were measured in auto-analyser (ERBA XL 600, India) using commercial available kits (TECO diagnostic kits, California, U.S.A).

Statistical analysis

The data are expressed as mean ± standard deviation (SD). The significance of differences among all steps of the groups was analyzed through analysis of variance (ANOVA); if the F value was found to be significant, differences between means were then analyzed with the post-hoc (Duncan) test. Differences were considered statistically significant when the p value was < 0.05.

RESULTS

On day 1 of the study, the levels of urea, total protein and LDH were increased in group 3, whilst level of AST was decreased in this group and this continued to day 3. The level of urea returned to its basal level on day 3 of the study. In addition, decreased values of triglyceride were detected in groups 2 and 3, however, the total protein levels showed an increase in all the drug administered groups. Lactate dehydrogenase activity was also decreased on day 3 in groups 2 and 3. There was no significant difference in creatinine and triglyceride levels between the drug administered group and the control group on day 3 (Table 1).

On day 7 of the study no significant differences in creatinine levels were observed, however, the activities of AST, LDH and ALP were decreased in groups 2 and 3, whilst an increase in the activity of ALT was detected in these groups. There was no significant difference in serum creatinine and ALT and ALP activities on day 14 of the study between the control group and the drug administered groups. In contrast, a decrease in the level of urea and the activities of LDH and AST were detected in groups 2 and 3 respectively. A decrease in triglyceride levels was also observed in group 2, on day 14 of the study (Table 1). The changes in serum clinical parameters of mice after the administration of thiamphenicol at acute and sub-acute doses appears to be temporary and does not persist, hence the use of thiamphenicol at the doses and time scales reported here can be recommended.

In this study, concerning the biochemical parameters, dose and time dependent, some significant changes were occurred but they were not continued throughout the study (temporary increases and reductions), however, no statistically significant difference was observed between the groups.

DISCUSSION

The serum biochemistry tests were used to evaluate changes in some tissues, which may have been caused by thiamphenicol. The changes in serum biochemistry could be the reflections of alterations in some tissues, organs and systems (Table 1). These changes in serum biochemistry parameters and the other findings may be a guide to understanding the effects of thiamphenicol. In this study, concerning the biochemical parameters, dose and time dependent, some significant changes were occurred but they were not continued throughout the study (temporary increases and reductions), however, no statistically significant difference was observed between the groups.

The significant changes in triglyceride, total protein, AST and LDH activities were observed on the 1st day of study. The urea levels, total protein concentrations and LDH activity in the highest-dose group were higher than the other groups, however, triglyceride levels in the lowest dose-group together with AST activity were lower compared to control group. In case of deterioration of kidney function, urea and creatinin levels always increases in combination and because of this reason, they were taken into account in order to assess kidney function. Creatinine levels throughout the study did not change, suggesting that kidney function was not effected

Table 1.Biochemical parameters of mice in different treatment groups (means±S.D.)

Parameters	Groups	Days			
		1	3	7	14
Urea (mg dL^{-1})	Group 1	37.22±4.31[a]	60.50±4.43	137.33±6.80[a]	137.30±15.64[a]
	Group 2	36.40±6.87[a]	62.16±7.13	124.00±2.16[a]	114.80±4.91[b]
	Group 3	46.80±4.26[b]	56.00±3.16	114.20±4.54[b]	108.60±2.88[b]
Creatinin (mg dL^{-1})	Group 1	43. 2±3.51	45.40±4.21	45.40±4.21	48.24±4.12
	Group 2	46.30±3.47	48.33±3.7	47.33±2.7	47.33±2.7
	Group 3	49.53±5.3	52.53±5.5	53.53±25	53.53±25
Triglyceride (mg dL^{-1})	Group 1	109.40±6.36[a]	75.04±5.45	101.23±5.12[a]	97.56±14.57[a]
	Group 2	78.20±4.49[c]	76.83±9.08	76.75±2.06[b]	105.33±8.73[b]
	Group 3	90.60±5.59[b]	81.60±18.36	69.60±1.51[c]	89.33±6.80[a]
Total Protein (mg dL^{-1})	Group 1	4.99±0.08[a]	4.2±3.56[a]	4.66±2.14[a]	3.72±1.8[a]
	Group 2	5.69±0.11[a]	6.06±0.12[b]	6.09±0.17[c]	5.97±0.13[b]
	Group 3	6.82±0.22[b]	5.87±0.23[b]	5.43±0.12[b]	5.77±0.05[b]
ALT ($IUdL^{-1}$)	Group 1	73.32±4.12	43.25±1.45[a]	52.27±3.52[a]	75.40±12.46
	Group 2	78.60±13.75	66.16±10.34[b]	80.33±6.80[b]	81.00±1.58
	Group 3	70.40±15.07	59.80±2.38[b]	81.33±7.50[b]	86.00±2.00
AST ($IUdL^{-1}$)	Group 1	110.30±3.32[a]	108.62±4.28[a]	88.00±5.24[a]	80.40±18.91[a]
	Group 2	115.20±5.80[a]	108.50±5.54[a]	77.66±6.80[ab]	74.20±5.54[c]
	Group 3	65.00±49.93[b]	76.20±18.30[b]	49.00±16.09[b]	57.60±6.22[bc]
LDH ($IUdL^{-1}$)	Group 1	1340.00±108.56[a]	2089.25±89.75[a]	1522.37±400.61[a]	1950.40±74.42[a]
	Group 2	1344.20±80.19[a]	1774.6±214.62[b]	1090.50±31.81[b]	1711.6±134.47[b]
	Group 3	1766.00±496.44[b]	1418.60±155.24[c]	1031.00±59.10[b]	1744.40±134.11[b]
ALP ($IUdL^{-1}$)	Group 1	139.70±14.42[ab]	133.00±13.32	148.65±9.17[a]	98.60±27.40
	Group 2	139.80±21.17[ab]	145.83±43.17	123.00±7.07[b]	117.80±26.31
	Group 3	148.25±18.62[a]	122.20±13.29	120.50±9.98[bc]	102.80±7.56

[a,b,c]The difference is significant in the columns between the groups, which carries different superscripts ($P<0.05$).

by administration of the thiamphenicol. However, the increase in urea level in all groups may be due to optimum conditions with regular basis and high nutritional intake. No alteration in creatinine concentrations substantiates this hypothesis. On the other hand, LDH activity increased on 1st day of the study and then its activity decreased on 3rd day which may have occurred due to blood sampling stress. Most of the drugs may cause an increase in LDH activity, therefore, leading to false positive results. It is known that chloramphenicol increases LDH activity, moreover, thiamphenicol may have that kind of effect due to its similar chemical structure with chloramphenicol.

AST activity in group 2 and 3 significantly increased only on 14th day of study. Despite this increase, the value was within normal range (mice normal AST levels range between 54-298 IU/l). The numerous drugs including chloramphenicol may lead to an increase or decrease in AST activity. In the present study, the temporary increase in AST activity may be induced by the drug.

Clinical data on toxicity obtained during 1980 to 1982 suggested that thiamphenicol acts as hematopoietic suppressant in a dose dependent manner which appeared to be alteration only after repeated dosing (Ferrari, 1981). Thiamphenicol was used in the treatment of chancroid with 1.128 cases had a longer post-treatment anemia, was reversible even at high doses and did not alter hepatic function (Paulo, 2000). The ALP levels did not change during the treatment in our study, the temporary increase in ALT activity was observed in the last day of study. These temporary increases in transaminase

activity suggest that there was no hepatotoxicity induced by the drug. In 1997, the study examining thiamphenicol's subchronic toxicity on 344 rats by Ando et al. (1997) reported that a significant decrease in the levels of TP, ALB, and Ca, in contrast at high-dose group of male rats, the A/G ratio and BUN levels were found to be increased. In the same study, ALT was decreased in all treatment groups, on the other hand, at high-dose group of female rats, the significant decreases in the levels of TP, ALP, total cholesterol (CHO), calcium (Ca) were detected, but the albumin / globulin ratio and the levels of BUN, AST, ALP, glucose (GLU) were found to be increased. The observed biochemical changes (such as the decreased levels of TP and ALB) were bound to decrease in food intake due to absence of histological changes related to hepatic and renal damages. In our study, the observed increases in the levels of urea and TP during the treatment could be connected to feeding rats on regular basis. Because during the study, there were no changes in the levels of creatinin and the renal functions was thought not to be affected by treatment. The temporary increases and decreases in the level of LDH activity were observed in our study. These changes could be considered to be associated due to the drug's effect. As a result, the thiamphenicol treatment with the dose of 200 mg/kg for 7 days did not cause any changes in the biochemical parameters measured. In the present study, there was no statistically significant increases in the biochemical parameters, related to dose and time, although, thiamphenicol was given for 7 days. From the result of the present studies we concluded that the administration of thiamphenicol to mice at 100 to 200 mg/kg for 7 days provides a useful information of the doses and the dose regimen of thiamphenicol.

Ali et al. (2003) have reported that the treatment with florfenicol, a structural analogue of thiamphenicol (20 mg/kg by the i.m or i.v routes) caused no clinically adverse effects. Plasma enzyme activities (AST and sorbitol dehydrogenase) and the concentrations of metabolites, indicative of hepatic and renal functions (bilirubin, creatinine and urea) were found to be normal on 1st, 2nd, 4th and 7th days following the drug treatment in camels, sheep and goats. This is in agreement with our results that the side effects were not observed during treatment with thiamphenicol. Another study from Kitamura et al. (1997) indicated that TAP is neither toxic nor carcinogenic, for any organs or tissues of F344 rats when given continuously at the levels of 125 or 250 ppm in drinking water for 2 years. This result and our data suggest that TAP can be used safely for long periods. Consequently, our results suggested that the administration of thiamphenicol at the doses up to 200 mg/kg for 7 days to mice seems not to cause any toxic effects.

REFERENCES

Ablini E, Belluco G, Berton M, Schioppacassi G, Ungheri D (1999). In vitro antibacterial activity of thiamphenicol glycinate acetycysteinate against respiratory pathogens. Arzneimittel-Forschung, 49: 533-537.

Ali BH, Al-Qarawi AA, Hashaad M (2003) Comparative plasma pharmacokinetics and tolerance of florfenicol following intramuscular and intravenous administration to camel, sheep and goats. Vet. Res. Comm., 27: 475-483.

Ando J, Ishihara R, Imai S, Takano S, Kitamura T, Takahashi M, Yoshida M, Maekawa A (1997). Thirteen-week subchronic toxicity study of thiamphenicol in F344 rats. Toxicol Lett., 28: 91(2): 137-46.

Bishop Y (1998). The Veterinary Formulary. The Pharmaceutical Press, London

Ferrari V (1981) Introductory address-salient features of thiamphenicol: Review of clinical pharmacokinetics and toxicity. Sex Trans. Dis., 11: 336-339.

Francesehinis R (1981) Drug utilization data for chloramphenicol and thiamphenicol in Recent years. In: Najen, Y, Tognoni, G, & Yunis, AA, (eds), Safety Problems Related to Chloramphenicol and Thiamphenicol. New York: Raven Press, 81-89.

Kitamura T, Ando J, Ishihara R, Takano S, Iijima T, Nishimura S, Yoshida M, Takahashi M, Maekawa A (1997). Lack of carcinogenicity of thiamphenicol in F344 rats. Food Chem. Toxicol., 35(10-11): 1075-1080.

Kucers A, Crowe SM, Grayson ML, Hoy JF (1997). Chloramphenicol and thiamphenicol. In: The Use of Antibiotics. Butter-worth-Heinemann: Oxford; 548-559.

Nau H, Welsch F, Ulbrich B, Bass R, Lange J (1981). Thiamphenicol during the first trimester of human pregnancy: placental transfer in vivo, placental uptake in vitro, and inhibition of mitochondrial function. Toxicol. Appl. Pharmacol. 60(1): 131-141.

Paulo S (2000). Thiamphenicol in the treatment of chancroid. A study of 14,128 cases. Rev. Inst Med. Trop., 42(3): 133-135.

Reynold JEF (1989). Martindale. The Extra Pharmacopoeia, 29th ed., Pharmaceutical Press, London.

Sutter VL, Finegold SM (1976). Susceptibility of anaerobic bacteria to 23 antimicrobial agents, Antimicrob Agents Chemother, 10: 736-752.

Van Beers D, Schoutens E, Vanderlinden MP, Yourassowsky E (1975). Comparative in vitro activity of chloramphenicol and thiamphenicol on common aerobic and anaerobic Gram-negative basilli (Salmonella and Shigella excluded). Chemotherapy, 21: 73-81.
www.fao.org/docrep/w4601e/w4601eOd.htm.