The effect of chloramphenicol on hepatic biotransformation enzyme activity and on the duration of pentobarbital or ketamine/xylazine anesthesia in guinea pigs (*Cavia porcellus*)

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PREVENDAR CRNIĆ, A., J. POMPE-GOTAL, F. BOŽIĆ, J. ŠURAN, R. VELEV, D. SAKAR: The effect of chloramphenicol on hepatic biotransformation enzyme activity and on the duration of pentobarbital or ketamine/xylazine anesthesia in guinea pigs (*Cavia porcellus*). Vet. arhiv 79, 385-396, 2009.

ABSTRACT

The effect was investigated of chloramphenicol on the activities of hepatic biotransformation enzymes: aniline hydroxylase (Ah) and ethylmorphine N-demethylase (EtND), as well as on the levels of serum alanine aminotransferase (ALT) and glutamate dehydrogenase (GLDH) in guinea pigs (*Cavia porcellus*). Moreover, the influence was established of the pre-medication with chloramphenicol on the duration of anesthesia using pentobarbital and ketamine/xylazine guinea pigs. Male guinea pigs, weighing 269-353 g, were subcutaneously (s.c.) treated with 60- and 100 mg chloramphenicol sodium succinate/kg body mass once a day for three days. Thereafter the anesthetics were injected: 30 mg pentobarbital/kg b.m., i.p. in one group and 60 mg ketamine/kg b.m., s.c. + 4 mg xylazine/kg b.m., s.c. in the other group. Treatment with chloramphenicol did not significantly affect the concentration of serum ALT or GLDH. As expected, 100 mg chloramphenicol/kg b.m. significantly decreased the activity of Ah and EtND for -36.1% and -38.6%, respectively. On the other hand, the duration of pentobarbital anaesthesia and ketamine/xylazine-induced anaesthesia in chloramphenicol pre-treated animals was not significantly prolonged. Our results also indicate that decreased enzyme activity in the presence of chloramphenicol did not influence the hepatic metabolism of two tested anaesthetics in guinea pigs, and in contrast to dogs, cats, rats and mice, did not prolong the duration of anaesthesia.

Key words: chloramphenicol, pentobarbital, ketamine/xylazine, biotransformation, alanine aminotransferase, glutamate dehydrogenase, guinea pig, *Cavia porcellus*

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Introduction

Many chemicals and environmental agents can alter the activity of drug metabolizing enzymes by induction and inhibition. Chloramphenicol has been shown to alter the pharmacokinetics of other drugs by inhibition of hepatic drug metabolizing enzymes (CHRISTENSEN and SKOVSTED, 1969; SMITH and WEBER, 1983). Chloramphenicol, the typical wide-spectrum antibiotic, is prohibited in Croatia in food producing animals. On the other hand, it is one of a few antibiotics available for oral and parenteral application to guinea pigs. The recommended therapeutic doses for guinea pigs are 50 mg/kg p.o. three times daily and 20 mg/kg i.m. twice daily. The inhibition of hepatic cytochrome P450-dependent monooxygenases by choramphenicol is well documented in a number of species, accompanied by altered biotransformation processes of concurrently used drugs (DIXON and FOUTS, 1962; ADAMS and DIXIT, 1970; TESKE and CARTER, 1971; ADAMS et al., 1977; KRANER et al., 1994; FAROMBI et al., 2002; PARK et al., 2003). Modulated levels of aniline hydroxylase (Ah) and ethylmorphine N-demethylase (EtNd) are relevant indicators of changes in hepatic biotransformation capacity.

Xylazine, α_2-adrenergic agonist with sedative, analgesic and muscle relaxant properties, is commonly used for anaesthesia in veterinary medicine. The combination of xylazine and ketamine produces sufficient anaesthesia for most surgical procedures (GREENE and THURMON, 1988). Ketamine is a rapid-acting dissociative anaesthetic, and its pharmacological action is characterized by a good analgesia, normal pharyngeal-laryngeal reflexes, respiratory depression and cardiac stimulation (WRIGHT, 1982).

Pentobarbital sodium, a short-acting barbiturate, was commonly used for anaesthesia in veterinary surgery before introduction of safe inhalation anaesthetics: halothane and methoxyflurane, as well as an injectable anaesthetic ketamine. Though the intraperitoneal application of pentobarbital sodium to guinea pigs may be more appropriate than use of inhalation anaesthetics, the animals in pentobarbital-induced anaesthesia must be under constant supervision because of its possible side effects and relatively narrow therapeutic index of this barbiturate.

Pre-treatment of animals with P-450 inhibitors has been shown to prolong the duration of xylazine-ketamine anaesthesia in rats and chickens, or could have deleterious effects, such as death due to pulmonary oedema (AMOUZADEH et al., 1989; AMOUZADEH et al., 1991). Duration of pentobarbital-induced anaesthesia in dogs, cats and several laboratory animal species may be prolonged by the concurrent administration of chloramphenicol (ADAMS, 1970; ADAMS and DIXIT, 1970, TESKE and CARTER, 1971; FREIRE et al., 1974).

The guinea pig (*Cavia porcellus*) has been used extensively as a research model in many disciplines, although guinea pigs are not a genetically homogenous species as laboratory animal breeds, such as Sprague Dawley rats or $B_6C_3F_1$ mice.

The aims of this study were: 1. to investigate the effect of chloramphenicol on the duration of clinical anaesthesia in guinea pigs induced by either xylazine-ketamine combination or by pentobarbital, and 2. to test the effect of chloramphenicol on hepatic cytochrome P450-dependent monooxygenase activity of aniline hydroxylase (Ah) and ethylmorphine N-demethylase (EtND), as well as on serum alanine aminotransferase (ALT) and glutamat dehydrogenase (GLDH) activities in guinea pigs.

Materials and methods

Experimental design. The experiment was carried out in two parts.

I. Part. Male guinea pigs, of roughly equal body mass (269-353 g), were divided into control C_1 (n=6), and two experimental groups: K60 (n=6) and K100 (n=8). The animals were housed individually in wire-bottomed cages in a controlled environment (temperature, relative humidity and air flow velocity). A commercial diet for guinea pigs (Animal feed factory, Hrvatski Leskovac, Croatia) and water was supplied *ad libitum* throughout the study.

One experimental group (K60) was treated with 60 mg chloramphenicol/kg b.m., whereas the second experimental group (K100) was treated with 100 mg chloramphenicol/kg b.m., once a day for three consecutive days. The doses were chosen with a view to testing the effect of the drug on the biotransformation enzyme activity, but not to test its therapeutic effects. Chloramphenicol sodium succinate (Kloramfenikol, Pliva Ltd.) was administered subcutaneously (s.c.) as a fresh 5% water solution, as described before (SCHUCHMAN, 1989).

Animals were treated humanely, without causing unnecessary pain. They were sacrificed in the most rapid and least painful way, by cervical dislocation.

Activities of Ah and EtND were determined in the livers of all animal groups. The livers were promptly removed, weighed and placed immediately into ice-cold 1.15% KCl solution, thereupon pooled and homogenized with 3 volumes of ice-cold 0.25 M sucrose solution using a Potter Elvehjem-type tissue homogenizer. Homogenates were centrifuged at 4° C at $600 \times g$ for 5 min to remove cell debris. The supernatant was centrifuged at $9000 \times g$ in a refrigerated WKF P-18 centrifuge and the resulting post-mitochondrial supernatant (S9 fraction) was used to measure the rate of hydroxylation of aniline and N-demethylation of ethylmorphine. Ah assay was performed following the procedure described by IMAI et al. (1969). The amount of p-aminophenol formed from aniline was determined colorimetrically. The ethylmorphine N-demethylation rate was assayed by measuring the formaldehyde formed from ethylmorphine chloride as described by NASH (1953) and COCHIN and AXELROD (1959).

Blood samples were collected by heart punction post mortem, and serum ALT and GLDH levels were measured within 24 hours using commercial Boehringer diagnostic kits.

The results of enzyme activities and protein concentration are presented as mean ± SEM. Data were analyzed using Student's *t*-test. The significance of mean differences between controls and two experimental groups was based on a *P* value of <0.01.

II. Part. Male guinea pigs of roughly equal body mass were divided into two control groups: Group 1) control C_2 (n=8) and Group 2) control C_3 (n=6), plus four experimental groups: Group 3) chloramphenicol 60 mg/kg b.m. + pentobarbital (K60p; n=7); Group 4) chloramphenicol 60 mg/kg b.m. + ketamine/xylazine (K60k/x; n= 6); Group 5) chloramphenicol 100 mg/kg b.m. + pentobarbital K100p (n= 7) and Group 6) chloramphenicol 100 mg/kg b.m. + ketamine/xylazine (K100k/x; n=7). The animals were housed in wire-bottomed cages in a controlled environment. A commercial diet for guinea pigs (Animal feed factory, Hrvatski Leskovac, Croatia) and water was supplied *ad libitum*.

Table 1. Treatment design for II part

	Treatment group	Nº of animals	Chloramphenicol dose (mg/kg b.m.)	Anesthetic used/ Dose administered (mg/kg b.m.)
1	C_2 control	8	-	Pentobarbital 30 mg
2	C_3 control	6	-	Ketamine 60/Xylazine 4
3	K60p	7	60	Pentobarbital 30 mg
4	K60k/x	6	60	Ketamine 60/Xylazine 4
5	K100p	7	100	Pentobarbital 30 mg
6	K100k/x	7	100	Ketamine 60/Xylazine 4

Animals from the two experimental groups (Groups 3 and 4) were pre-treated for 3 days with 60 mg chloramphenicol/kg b.m., whereas the guinea pigs from other two experimental groups (Groups 5 and 6) were treated with 100 mg chloramphenicol/kg b.m. In both groups the animals were administered chloramphenicol for three days, once a day. Chloramphenicol sodium succinate was s.c. applied as fresh 5% water solution. After a three-day chloramphenicol treatment, the guinea pigs from one control and two experimental groups (C_2, K60p and K100p) were weighed and anesthetized with 30 mg pentobarbital sodium/kg b.m., i.p. (Vetanarcol, Werfft Chemie, Wels, Austria), according to literature data (RAUB and GILLESPIE, 1976).

Simultaneously, the guinea pigs from the other control (C_3) and other two experimental groups (K60k/x and K100k/x) were weighed and anesthetized with 60 mg ketamine/ kg b.m. (Ketonest 50, Parke-Davis Godecke) and 4 mg xylazine/kg b.m. (Rompun 2%

solution, Bayer plc.). Anaesthetics were applied s.c. in separate syringes. The doses were in accordance with literature data (SCHUCHMAN, 1989; FLECKNELL, 2005).

Duration of anaesthesia was measured in anesthetized animals as the time interval in minutes between the moment when the guinea pigs were laid down and the moment they stood up (immobilization time), and also as the time interval between the loss and recovery of the pedal reflex.

The significance of the difference in mean time of anaesthesia between control and test groups of guinea pigs was calculated by the Student's *t* test.

Results

The results of investigation are presented in 4 tables. The results are expressed as mean ± SEM and min-max values in brackets.

Biotransformation enzymes activity. Treatment with 100 mg chloramphenicol/kg b.m. significantly suppressed the activity of both tested biotransformation enzymes.

Table 2. Effect of *in vivo* treatment with chloramphenicol on activities of ethylmorphine N-demethylase (EtND) and aniline hydroxylase (AnH) in liver of guinea pigs

	EtND [1] x ± SE (min-max)	AnH [2] x ± SE (min-max)
C_1 n = 6	1536 ± 87 (1196-1800)	264 ± 24 (193-368)
K60 n = 6	1633 ± 172 n.s. (1130-2201)	311 ± 27 n.s. (215-403)
K100 n = 6	981 ± 121 ** (599-1317)	162 ± 34 * (85-315)

[1] nmol HCOH/g liver/30 min.; [2] nmol p-OH aniline/g liver/30 min.; C_1-control; K60-chloramphenicol 60 mg/kg b.m. s/c during 3 days; K100 - chloramphenicol 100 mg/kg b.m. s/c during 3 days; n = number of animals in a group; n.s. - not significant ; * P<0.05; ** P<0.01.

Duration of pentobarbital-induced anaesthesia. The results indicate that the 3-day-pre-treatment with chloramphenicol did not significantly prolong duration of pentobarbital-induced anaesthesia although there was a numeric prolongation in both treatment groups. However, the absence of pedal reflex was significantly shorter in the K60p and K100p experimental groups of guinea pigs when compared to the control group.

Table 3. Effect of chloramphenicol on duration of pentobarbital-induced anesthesia (30 mg/kg i.p.) in guinea pigs

Group	Duration of pentobarbital-induced anesthesia [1]	
	Immobilization time $x \pm SE$ (min-max)	Absence of pedal reflex $x \pm SE$ (min-max)
C_2 n = 8	208 ± 12 (165-265)	129 ± 6 (111-141)
K60p n = 7	235 ± 15 n.s. (186-296)	81 ± 10 ** (47-122)
K100p n = 7	220 ± 19 n.s. (129-302)	86 ± 8 ** (59-107)

[1] minutes; C_2-control; K60p-chloramphenicol 60 mg/kg b.m. s/c during 3 days + 30 mg pentobarbital/kg b.m., i.p; K100p - chloramphenicol 100 mg/kg b.m. s/c during 3 days + 30 mg pentobarbital/kg b.m., i.p.; n = number of animals in a group; n.s. = not significant; ** P <0.01.

Duration of ketamine/xylazine induced anesthesia. Similarly to previous results, the 3-day-treatment with chloramphenicol did not significantly prolong the immobilization time of ketamine/xylazine-induced anaesthesia, and the absence of pedal reflex was significantly shorter in the K60k/x experimental group of guinea pigs than in the control group.

Table 4. Effect of chloramphenicol on duration of ketamine/xylazine-induced anesthesia (60 mg/kg + 4 mg/kg b. w., s.c.) in guinea pigs

Group	Duration of ketamine/xylazine-induced anesthesia [1]	
	Immobilization time $x \pm SE$ (min-max)	Absence of pedal reflex $x \pm SE$ (min-max)
C_3 n = 6	138 ± 3 (128-147)	129 ± 5 (116-144)
K60k/x n = 6	152 ± 15 n.s. (122-222)	110 ± 2 ** (104-116)
K100k/x n = 7	132 ± 6 n.s. (115-164)	121 ± 2 n.s. (115-130)

[1] minutes; C_2-control; K60k/x-chloramphenicol 60 mg/kg b.m. s/c during 3 days + 60 mg ketamine/kg and 4 mg xylazine/kg b.m., s.c; K100k/x - chloramphenicol 100 mg/kg b.m. s/c during 3 days + 40 mg ketamine/kg and 4 mg xylazine/kg b.m., s.c.; n = number of animals in a group; n.s. = not significant; ** P<0.01.

ALT and GLDH activity. The serum values of ALT and GLDH activities in plasma of control and guinea pigs treated with 60- or 100 mg chloramphenicol/kg b.m. over 3 days were not significantly different.

Table 5. Effect of chloramphenicol on activity of alanine aminotransferase (ALT) and glutamate dehydrogenase (GLDH) in plasma of guinea pigs

group	ALT U/L	GLDH U/L
C_1 n = 6	25.7 ± 2.6 (20.3-33.0)	6.6 ± 1.5 (1.9-11.9)
K60 n = 6	21.8 ± 2.2 n.s. (16.7-30.0)	4.5 ± 1.3 n.s. (1.5-9.3)
K100 n = 8	23.0 ± 0.9 n.s. (18.2-26.3)	11.5 ± 1.9 n.s. (5.9-18.2)

C_1-control; K60-chloramphenicol 60 mg/kg b.m. s/c during 3 days; K100 - chloramphenicol 100 mg/kg b.m. s/c during 3 days; n = number of animals in a group; n.s.-not significant.

Discussion

Biotransformation enzymes activity. There are relatively quite a large number of papers describing hepatic biotransformation enzyme activity in guinea pigs, with investigations of the main hepatic cytochrome P450 enzymes activity being conducted with different aspects. In most cases they encompassed the response of these enzymes to different metals or other chemicals were tested (KITADA et al., 1977; ISCAN et al., 1993; LEE et al., 2002; KOGA et al., 2007). Also, purification, characterization and molecular cloning of the guinea pig cytochrome P450-dependent enzymes (SHIMADA et al., 1997; SUZUKI-KURASAKI et al., 1997; MORI et al., 1998; YAMAMOTO et al., 2004). However, there are no reports of the influence of chloramphenicol on the hepatic biotransformation system in guinea pigs; the inhibitory effect of chloramphenicol on hepatic cytochrome P450-dependent monooxygenases, which is well documented in a number of species (DIXON and FOUTS, 1962; ADAMS and DIXIT, 1970; TESKE and CARTER, 1971; ADAMS et al., 1977; HALPERT et al., 1988), was confirmed in our study too, after application of 100 mg chloramphenicol/kg b.m. to guinea pigs. The decreased activity of aniline hydroxylase and ethylmorphine N-demethylase appreciably alters the biotransformation processes of drugs which are used simultaneously. Administration of 60 mg chloramphenicol/kg b.m. did not inhibit the activity of the tested enzymes; indeed there was slight induction, but the dose of 100 mg chloramphenicol/kg b.w inhibited these enzymes, suggesting that the inhibition/induction of cytochrome P-450 by chloramphencol is dose-dependent in guinea pigs.

ALT and GLDH activity. GLDH is primarily located in the hepatic mitochondria, and its activity is increased in the presence of a hepatic injury (KELLER, 1979). Our study demonstrates non-significant changes of GLDH activity, due to wide-range variability in the serum GLDH levels. Moreover, the enhanced GLDH activity mean value in the livers of guinea pigs treated with 100 mg chloramphenicol/kg b.m. may be an indicator of hepatic injury. On the other hand, the wide-range GLDH activity values observed in the serum of the guinea pigs may be the consequence of population heterogeneity.

Chloramphenicol did not significantly alter serum ALT activity. Induction of ALT activity in guinea pigs, as well as in other animal species is an indicator of a hepatic

and heart injury. This finding is in accordance with the investigation by HAPKE et al. (1977) who reported unchanged ALT activity in rats after i.p. application of 100 mg chloramphenicol/ kg b.m.

Effect of chloramphenicol on duration of anaesthesia. The chloramphenicol dose which results in a significant prolongation of pentobarbital anesthesia ranges from 1-300 mg/kg in dogs, cats, monkeys, rats, and mice (ADAMS, 1970; ADAMS and DIXIT, 1970; TESKE and CARTER, 1971; ADAMS et al., 1977; HALPERT et al., 1985b). A dose-independent prolongation of pentobarbital anaesthesia was noted in mice and dogs, respectively (ADAMS, 1970; TESKE and CARTER, 1971). In dogs there is a dose-dependent trend in prolongation of duration of anaesthesia and inhibition of cytochrome P-450 (NOSSAMAN et al., 1990). The dosage of 30 mg pentobarbital/kg b.m. administered to guinea pigs was within the therapeutic (anaesthetic) range normally used for anaesthesia in this species. In contrast to results gained by others in numerous other species, our results have shown that chloramphenicol pre-treatment did not prolong the duration of pentobarbital-induced anaesthesia in guinea pigs. The possible reasons could be the dose of chloramphenicol, as well as the duration of its application.

Likewise, the pre-treatment of guinea pigs with chloramphenicol did not significantly prolong the duration of ketamine/xylazine-induced anaesthesia in our study, which is in accordance with the results of NOSSAMAN et al. (1990). Moreover, the i.v. application of a therapeutic dose of chloramphenicol did not alter the duration of ketamine/xylazine anaesthesia in dogs and mice (RODER et al., 1993). This is in contrast with the previous report showing that chloramphenicol might prolong the duration of xylazine/ketamine anaesthesia in rats (AMOUZADEH et al., 1989) and in broiler chickens (RODER et al., 1993). GARCIA-VILLER et al. (1981) suggest that xylazine is metabolized by hepatic cytochrome P450 drug metabolizing enzymes similar to ketamine. The mayor metabolic pathway of ketamine is N-demethylation by cytochrome P-450 enzymes (KAKA and HAYTON, 1980). The significantly shorter absence of pedal reflex in the K60p, K100p and in the K60k/x experimental groups of guinea pigs than in the controls was unexpected. The reason for this is unknown yet. The failure of chloramphenicol to prolong pentobarbital and xylazine/ketamine anaesthesia in guinea pigs could be due to various factors. The hepatic cytochrome P450 enzymes are composed of specific isozymes. Chloramphenicol has been shown to inhibit only a few but not all of the isozymes of the P-450 complex (HALPERT et al., 1985a; HALPERT et al., 1985b). Since only specific cytochrome P-450 enzymes are shown to be inhibited by chloramphenicol, it is also possible that isozymes involved in both pentobarbital and xylazine/ketamine metabolism in guinea pigs might not be affected by chloramphenicol.

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PREVENDAR CRNIĆ, A., J. POMPE-GOTAL, F. BOŽIĆ, J. ŠURAN, R. VELEV, D. SAKAR: Učinak kloramfenikola na aktivnost biotransformacijskih enzima jetre i na trajanje anestezije nakon primjene pentobarbitala te kombinacije ketamina i ksilazina u zamorčića (*Cavia porcellus*). Vet. arhiv 79, 385-396, 2009.

SAŽETAK

U radu je istražen učinak kloramfenikola na aktivnost jetrenih biotransformacijskih enzima anilin hidroksilazu (Ah) i etilmorfin N-demetilazu (EtND) te na razinu serumskih enzima alanin aminotransferaze (ALT) i glutamat dehidrogenaze (GLDH) u zamorčića (*Cavia porcellus*). Osim toga, istražen je utjecaj premedikacije kloramfenikolom na trajanje anestezije pentobarbitalom i kombinacijom ksilazin/ketamin. Zamorčićima muškoga spola, težine 269-353 g, supkutano je apliciran kloramfenikol u obliku natrijeva sukcinata u dozi 60 i 100 mg/kg tjelesne mase tijekom tri uzastopna dana. Nakon toga primijenjeni su anestetici: prvoj skupini 30 mg pentobarbitala/kg t.m., i.p. i drugoj skupini 60 mg ketamina i 4 mg ksilazina/kg t.m., s.c. Rezultati su pokazali

da kloramfenikol nije značajno utjecao na koncentaraciju ALT i GLDH u serumu. Međutim, kloramfenikol u dozi od 100 mg/kg t.m. značajno je umanjio aktivnost Ah i EtND, i to za -36,1% i -38,6%. S druge strane, trajanje anestezije nakon primjene pentobarbitala i kombinacije ketamina i ksilazina u zamorčića prethodno obrađenih kloramfenikolom nije bilo statistički značajno produženo. Naši rezultati upućuju na to da umanjena aktivnost biotransformacijskih enzima nakon primjene kloramfenikola nije utjecala na jetreni metabolizam dvaju testiranih anestetika u zamorčića te da primjena kloramfenikola nije produžila vrijeme trajanja anestezije što je suprotno nalazima kod pasa, mačaka, štakora i miševa.

Ključne riječi: kloramfenikol, pentobarbital, ketamin/ksilazin, biotransformacija, alanin aminotransferaza, glutamat dehidrogenaza, zamorčić (*Cavia porcellus*)