Influence of hydrogen peroxide in drinking water on diazepam pharmacokinetics in chicks

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Received: 01-04-2012, Accepted: 10-05-2012, Published Online: 16-09-2012
doi: 10.5455/vetworld.2012.658-662

Abstract

Aim: Stressful conditions affect drug pharmacokinetics and pharmacodynamics. This study examines the effect of hydrogen peroxide (H_2O_2) in drinking water on the pharmacokinetics of diazepam in a chick model of oxidative stress.

Materials and Methods: Day old chicks were either provided with plane tap water (control group) or H_2O_2 in tap water as 0.5% v/v drinking solution for two weeks in order to produce oxidative stress. On treatment days 7–14, the chicks were treated with a sedative dose of diazepam at 10 mg/kg, intramuscularly. Blood samples were obtained from chicks (5/each sampling time) at times of between 0.17 to 4 h. The concentrations of diazepam in the plasma were determined by an HPLC method with UV-detector. Pharmacokinetic parameters of diazepam were calculated from the mean drug concentrations in the plasma by a non-compartmental analysis using a Windows-based computer program.

Results: Injection of diazepam resulted in the appearance of the drug in the plasma of control and H_2O_2-treated chicks at mean concentrations ranging between 0.11 to 0.444 and 0.131 to 0.535 µg/ml, respectively when measured between 0.17 to 4 h after administration. Diazepam concentrations of the H_2O_2-treated chicks were significantly higher than those of the control group at the sampling times 0.5, 0.75, 1 and 4 h. The highest concentration of diazepam in the plasma of both the control and H_2O_2-treated chicks occurred one h after the injection. The elimination half-life, mean residence time, maximum plasma concentration, area under the moment curve and area under plasma concentration-time curve in the H_2O_2-treated chicks were higher than those of the control group by 35, 28, 23, 91 and 49%, respectively. Correspondingly, the steady state volume of distribution, elimination rate constant and total body clearance in the H_2O_2-treated chicks decreased from those of the respective control values by 15, 24 and 33%.

Conclusion: The data suggest that oral exposure of chicks to H_2O_2 influences the pharmacokinetics of diazepam by decreasing its elimination from the body.

Key words: chick, diazepam, half-life, H_2O_2, oxidative stress, pharmacokinetics

To cite this article:
Mousa YJ, Mohammad FK (2012) Influence of hydrogen peroxide in drinking water on diazepam pharmacokinetics in chicks, *Vet World*, 5(11): 658-662, doi: 10.5455/vetworld.2012.658-662

Introduction

Many physically-induced stressful conditions adversely affect pharmacokinetic and pharmaco-dynamic aspects of drugs [1-4]. Reactive oxygen species are reported to be associated with various neuronal dysfunctions [5,6]. Oxidative stress (OS) produced by ferric-nitrilotriacetate was found to alter the pharmacokinetics of clomipramine in rats [7]. The brain is highly vulnerable to OS which modifies the systemic response to central nervous system (CNS) active agents [6,8-10]. Hydrogen peroxide (H_2O_2) is used to induce experimental OS in laboratory animals [11-14] and in vitro for experimental models of cellular OS [6,15,16].

H_2O_2-induced OS modifies the response of rabbits and rats to general anesthetics [17,18]. It also potentiates the anticholinesterase poisoning induced by the organophosphate insecticides dichlorvos and diazinon in chicks [12]. More recently we found that OS induced by H_2O_2 sensitizes young chicks to the sedative actions of diazepam and xylazine [19]. As stressful conditions might affect drug metabolism and kinetics [1-3], the purpose of the present study was to examine the effects of H_2O_2 in drinking water on the pharmacokinetics of diazepam in a chick model of OS.

Materials and Methods

Animals: Day old broiler chicks of both sexes were raised in a room with a temperature of 32–35^{0}C, constant lighting and wood shavings as floor litter. The chicks had free access to drinking water and feed throughout the experiment. The Scientific Committee

Table-1. Temporal diazepam concentrations (µg/ml) in the plasma of hydrogen peroxide exposed chicks after a single intramuscular administration at a dose of 10 mg/kg body weight.

Times (h)	Control (tap water)	Hydrogen peroxide
0.17	0.110 ± 0.004	0.131 ± 0.007
0.33	0.141 ± 0.012	0.164 ± 0.030
0.5	0.151 ± 0.012	0.198 ± 0.042 *
0.75	0.173 ± 0.018	0.282 ± 0.050 *
1.0	0.444 ± 0.127	0.535 ± 0.150 *
1.5	0.182 ± 0.045	0.195 ± 0.141
2.0	0.140 ± 0.013	0.167 ± 0.030
4.0	0.121 ± 0.011	0.178 ± 0.027 *

Values are mean ± SE of 5 chicks/each sampling time. Hydrogen peroxide was added to drinking water (0.5%, v/v) of the chicks at one day old and continued for 14 days. *Significantly different from the respective control value, $p < 0.05$.

of the College of Veterinary Medicine at the University of Mosul has reviewed and approved the protocol of the study in chicks. All the experiments complied with institutional regulations addressing animal use, and proper attention and care were given to the chicks used in this study.

Induction of OS and diazepam treatment: Day old chicks were either provided with plane tap water (control group) or H_2O_2 (Thomas Baker Chemical Ltd., U.K.) in tap water as 0.5% v/v drinking solution for two weeks in order to produce OS reported earlier [12, 19]. Each day we supplied the chicks with fresh drinking water. On treatment days 7–14, the chicks were treated with a sedative dose of diazepam (donated by the State Company for Drugs and Medical Appliances-Ninevah, Iraq) given intramuscularly (i.m.) at 10 mg/kg [19]. We dissolved diazepam in warm propylene glycol, and the volume of administration was at 5 ml/kg, i.m. Blood samples (1–2 ml) were collected from chicks (5/each sampling time) by bleeding the jugular vein into heparinized test tubes [20] times of 0.17, 0.33, 0.50, 0.75, 1, 1.50, 2 and 4 h after the diazepam injection. Plasma was obtained by centrifugation of blood samples at 3000 rpm (Centurion, U.K.) for 15 minutes. Plasma samples were stored at –18ºC pending diazepam determination within one week. We extracted diazepam from the plasma by liquid-liquid extraction method using diethylether [21]. Diazepam concentration in the plasma samples was determined by an HPLC method using a C_{18} column and UV detector with the HPLC pump of Cecil, U.K. [22,23]. The mobile phase consisted of acetonitrile: potassium dihydrogen phosphate pH 2.1 buffer (70:30); the flow rate was at 1 ml/min and the UV detector wave length was set at 245 nm.

To reduce the effect of individual variation in plasma diazepam concentrations, means of drug concentrations in the plasma at each sampling time (0.17–4 h) were used to calculate the pharmacokinetic parameters by a non-compartmental analysis [24, 25] using a Windows-based computer program [26]. These were area under plasma concentration-time curve (AUC_{0-}), area under the moment curve ($AUMC_{0-}$) from time zero to infinity, elimination half-life($t_{1/2\beta}$), elimination rate constant (k_{el}=0.693/ $t_{1/2\beta}$), steady state volume of distribution [V_{ss}=Dose.AUMC/ $(AUC)^2$], maximum diazepam concentration (Cmax), time to maximum diazepam concentration (Tmax), mean residence time (MRT=AUMC/AUC) and total clearance (CL=Dose/AUC). Unpaired Student's-t-test was used to compare plasma diazepam concentrations between the control and H_2O_2-exposed chicks [27]. The level of statistical significance was at $p < 0.05$.

Results

Intramuscular injection of diazepam at the dose rate of 10 mg/kg, resulted in the appearance of the drug in the plasma of control and H_2O_2-treated chicks at mean concentrations ranging between 0.11 to 0.444 and 0.131 to 0.535 µg/ml, respectively when measured between 0.17 to 4 h after the injection (Table 1). Diazepam concentrations of the H_2O_2-treated chicks were significantly higher than those of the control group at the sampling times 0.5, 0.75, 1 and 4 h (Table 1). The highest concentration of diazepam in the plasma of both the control and H_2O_2-treated chicks occurred one h after the injection (Table 1).

The pharmacokinetic parameters of diazepam calculated from the means of drug concentrations in the plasma at times 0.17 to 4 h in chicks are shown in Table 2. The elimination half-life, mean residence time, maximum plasma concentration, area under the moment curve and area under plasma concentration-time curve in the H_2O_2-treated chicks were higher than those of the control group by 35, 28, 23, 91 and 49%, respectively (Table 2). Correspondingly, the steady

Table-2. Pharmacokinetic parameters of diazepam in hydrogen peroxide exposed chicks after a single intramuscular administration at a dose of 10 mg/kg body weight

Variable*	Unit	Control (tap water)	Hydrogen peroxide
Mean residence time (MRT=AUMC/AUC)	h	3.48	4.44
Steady state volume of distribution [Vss=Dose.AUMC/ $(AUC)^2$]	L/kg	36.52	31.17
Elimination rate constant (kel=0.693/ $t_{1/2\beta}$)	h^{-1}	0.33	0.25
Elimination half-life ($t_{1/2\beta}$)	h	2.07	2.79
Tmax	h	1	1
Cmax	µg/ml	0.44	0.54
Total clearance (CL=Dose/AUC)	L/h/kg	10.51	7.03
Area under plasma concentration-time curve (AUC0-∞)	µg.h/ml	0.95	1.42
Area under the moment curve (AUMC0-∞)	$\mu g.h^2/ml$	3.31	6.32

* The means of plasma concentrations of diazepam at each sampling time (0.17–4 h) were used to calculate the Pharmacokinetic parameters by a non-compartmental analysis using a Windows-based computer program [26]. n=5 chicks/each sampling time. Hydrogen peroxide was added to drinking water (0.5%, v/v) of the chicks at one day old and continued for 14 days.

state volume of distribution, elimination rate constant and total body clearance in the H_2O_2-treated chicks decreased from those of the respective control values by 15, 24 and 33% (Table 2).

Discussion

H_2O_2 induces OS by a biochemical process involving the release of free radicals that in turn causes lipid peroxidation and increases intracellular calcium [28, 29]. The toxic effects of H_2O_2, through production of free radicals, might be related to lipid peroxidation in the *CNS* and other tissues [16,28,29]. A recent report also indicates that H_2O_2 stimulates G protein alpha 12 to activate cellular injury [30]. The model of H_2O_2-induced OS in the present study appeared to alter the pharmacokinetics of diazepam in chicks as manifested by higher drug concentration in the plasma compared to the control group as well by the differences in the pharmacokinetics parameters. Oxidative stress induced by ferric-nitrilotriacetate also changed the pharmacokinetics of clomipramine in rats [7]. Other forms of stress such as those induced by immobilization [4], heat and exercise [2], conditioned fear [3], foot shock [31] and isolated physical activities [32] were reported to alter drug pharmacokinetics. Stressful conditions may alter drug disposition and kinetics through changes of plasma protein or tissue binding, alteration of blood flow rates and cardiovascular functions, which might be accompanied with serious clinical outcome [1, 2, 32].

In our earlier study, H_2O_2 sensitized chicks to the sedative effects of diazepam and xylazine by decreasing the median effective doses of both drugs for the induction of sedation [19]. The CNS is susceptible to OS-induced neurotoxicity [9]. H_2O_2 is variably accumulated in different brain regions [28] and frees oxygen radicals that cause cellular and DNA damage [5,8,33–35]. It is therefore possible that altered tissue distribution and elimination of the drug might have resulted from the changes in the pharmacokinetics of diazepam. In this context the volume of distribution, elimination rate constant and total body clearance were decreased, whereas the half-life, area under the plasma concentration curve and area under the moment curve increased in the H_2O_2-exposed chicks.

Although we did not measure tissue diazepam concentration or its urinary excretion, it is also possible that H_2O_2 might have decreased diazepam excretion rate since the total body clearance and elimination rate constant of the drug were reduced considerably (Table 2). We avoided any stress-induced changes on the gastric emptying and intestinal movement [4] by injecting diazepam systemically (i.m.). Increased sedative response to diazepam in the H_2O_2-treated chicks [19] could be an outcome of these pharmacokinetics changes. Other clinical changes in response to diazepam and its own metabolism in animals undergoing OS are not yet clear.

Conclusion

In conclusion, the data of the present study suggest that oral exposure of chicks to H_2O_2 influences the pharmacokinetics of diazepam by decreasing its elimination from the body.

Author's contribution

YJM executed the experiments, conducted statistical analyses and shared in drafting the manuscript. FKM conceptualized the aim of the study, designed the experiment, supervised dosing regimen and statistical analyses, and drafted the manuscript in English. Both

author read and approved the final manuscript.

Acknowledgements

This report represents a portion of a dissertation submitted by the first author to the University of Mosul, Iraq as partial fulfillment of the requirements of PhD degree in Veterinary Pharmacology. The study was supported by the College of Veterinary Medicine, University of Mosul, Iraq.

Competing interests

The authors declare that they have no competing interests.

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