Anaesthetic properties of ketamine in chicks stressed with hydrogen peroxide

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ABSTRACT: The goal of this study was to examine the effect of oxidative stress (OS) induced with hydrogen peroxide (H_2O_2) on the anaesthetic properties of ketamine in seven and 14 day-old broiler chicks. Spectrophotometric analysis revealed that H_2O_2 (0.5%) induced OS through significant inhibition of glutathione (GSH) and elevation of malondialdehyde (MDA) concentrations in the brain of chicks in comparison to control (tap water) group. The hypnotic and analgesic median effective doses (ED_{50s}) decreased by 44% and 19%, respectively, in the stressed group compared to control group of chicks. On the other hand, the acute toxicity of ketamine increased through decreasing the acute median lethal dose (LD_{50}) (22%) in stressed chicks as determined by the up-and-down method. Injection of multiple ketamine doses at 10, 20 and 40 mg/kg, *i.m.* produced hypnotic effects for both groups of chicks depending on the dose, whereas H_2O_2 caused an increase in ketamine hypnotic efficacy in comparison to the control group. In the same manner, the antinociceptive effect of ketamine increased in the stressed chicks that underwent electrostimulation for pain induction. Both AST and ALT concentrations in the plasma were significantly elevated in the stressed group when compared to the control group. The results of this study suggest that H_2O_2-induced OS modifies the anaesthetic properties of ketamine in chicks by increasing its efficacy and acute toxicity probably through its pharmacodynamic and pharmacokinetic interactions; thus, care must be taken when stressed animals are undergoing anaesthesia with ketamine.

Keywords: ketamine; anaesthesia; hypnosis; oxidative stress; H_2O_2

Ketamine is a short acting non-barbiturate anaesthetic agent used widely in human and veterinary medicine to produce anaesthesia that is characterized by good hypnosis and analgesia with weak muscle relaxation (Finkel et al. 2009; White and Trevor 2009). Ketamine produces its hypnotic and analgesic effects through its antagonistic effect on N-methyl-D-aspartate (NMDA) receptors causing a decrease in the calcium conductance in the neurons resulting in central nervous system (CNS) depression (Finkel et al. 2009; White and Trevor 2009). Several previous studies have used the powerful oxidant agent H_2O_2 for induction of OS in laboratory animals such as rabbits, rats and chicks (Wohaieb et al. 1994; Mohammad et al. 1999; Mousa and Mohammad 2012a); further, it is known that OS modulates the pharmacological response to centrally acting sedatives and analgesics in chicks (Mousa and Mohammad 2012a). H_2O_2 induces OS through its ability to elevate the reactive oxygen species and free radical formation in the body tissue especially in the CNS (Pastore et al. 2003; Watt et al. 2004; Sayre et al. 2008) as well as facilitating oxidative damage that affects the blood-brain barrier (Lochhead et al. 2010) which increase the permeability of materials and drugs. The goal of this study was to induce OS in chicks with H_2O_2 and to examine its impact on the anaesthetic properties of ketamine, i.e., its hypnotic and analgesic efficacy in chicks.

MATERIAL AND METHODS

Experimental animals. Broiler chicks of both sexes at one day of age (total number = 117) with average body weights between 50–95 gram were used in all the experiments. They were kept in cages at a temperature of 32–35 °C with continuous lighting and the floor litter consisted of wood shavings.

Table 1. GSH and MDA concentrations in whole brain of H_2O_2-treated chicks

Groups	GSH (µmol/g)		MDA (nmol/g)	
	ages (days)			
	7	14	7	14
Control group (tap water)	0.69 ± 0.07	1.21 ± 0.13	111.2 ± 7.80	133.0 ± 8.30
Stressed group (0.5% H_2O_2 in water)	0.28 ± 0.04*	0.63 ± 0.07*	176.1 ± 5.3*	190.2 ± 6.6*

The values represented mean ± S.E. (n = 6)/day

0.5% H_2O_2 was added to water at Day 1 until the end of the experiment on Day 14

*significantly different from the respective control (tap water) group at $P \leq 0.05$

The chicks had access to drinking water and feed ad libitum. Ketamine (5%, Hameln pharmaceuticals GmbH, Germany) was diluted with physiological saline solution and was injected intramuscularly (*i.m.*) (the volume of injection was 5 ml/kg of body weight). With respect to ethical considerations, the Scientific Committee of the College of Veterinary Medicine, University of Mosul has approved this study, and necessary attention was given regarding the euthanasia of the chicks at the end of all experiments.

Induction of OS with H_2O_2 in chicks. One-day-old chicks (n = 6; as shown in Table 1) were randomly divided into two categories, the first one (control group) supplied with plain tap water while the second one (stressed group) was treated with freshly prepared 0.5% H_2O_2 (Thomas Baker Chemical Ltd., U.K.) v/v in tap water each day. The chicks were euthanised by cutting the jugular vein and the brains were collected on Days 7 and 14 and kept frozen until the entire experiment had ended. GSH (µmol/g) and MDA (nmol/g) concentrations (as biomarkers of OS) were determined according to spectrophotometeric methods described earlier (Ellman 1959; James et al. 1982) and (Ohkawa et al. 1979), respectively.

Determination of the hypnotic or analgesic ED_{50s} for ketamine in the control and stressed chicks according to up-and-down method (Dixon 1980)

A. Hypnotic ED_{50} of ketamine. The initial dose of ketamine injected into the control and stressed group of chicks was 15 mg/kg, *i.m.* (the dose was chosen according to a preliminary study). Every chick was monitored separately for 2 h for the appearance of the hypnotic effects of ketamine (loss of righting reflex) whereupon the following dose of ketamine was decreased (if there was hypnosis) or increased (if there was no hypnosis); doses of

ketamine 3 mg larger or smaller than the initial dosage were used.

B. Analgesic ED_{50} of ketamine. The analgesic ED_{50} of ketamine in the control and stressed chicks was determined as above. The analgesic effect of ketamine was detected by using an electrostimulator (Scientific and Research Ltd., UK) (Mousa and Mohammad 2012b). Electrostimulation can be used for induction of pain sensation in chicks and to examine whether or not ketamine has an analgesic effect after its injection. The apparatus voltage that induces pain sensation (indicated by distress calls) was recorded before and 5 min after ketamine injection for each chick individually. The voltage was increased after injection of ketamine in comparison to voltage before injection in the same chick (marked as X symbol in Table 2).

The effect of OS induced by H_2O_2 on the hypnotic or analgesic ED_{50s} for ketamine can be determined by using the following equation:

The % effect of OS on hypnotic or analgesic ketamine ED_{50} values = ED_{50} value (control group) − ED_{50} value (stressed group)/ED_{50} value (control group) × 100.

The effect of the H_2O_2-induced OS on the acute LD_{50} value of ketamine. This experiment was conducted to evaluate effects of H_2O_2 on the acute LD_{50} value of ketamine according to the up-and-down method (Dixon 1980) described earlier. The initial dose of ketamine used was 250 mg/kg, *i.m.* for both the control and stressed groups of chicks (selected based on a preliminary study). The first experimental chick (injected with initial dose) was monitored for 24 h for the appearance of acute toxicity signs and lethality of ketamine, then the next doses of ketamine were decreased (if there was death) or increased (if the chick was alive); doses 50 mg larger or smaller than the initial dosage were used. The above mentioned equation was used for evaluating the effect of H_2O_2 on the acute ketamine LD_{50} as described in Table 3.

Table 2. Determination of ED_{50} of ketamine analgesia and its relation to OS induced by H_2O_2 in chicks

Parameter	Result	
	control group (tap water)	stressed group (0.5% H_2O_2 in water)
ED_{50} value (mg/kg, *i.m.*)	13.0	10.9
The range of the doses used	12–15	9–15
Initial dose (mg/kg)	15	15
Last dose (mg/kg)	15	12
Increase or decrease in the dose (mg)	3	3
Number of chicks used	5 (XOXOX)	6 (XXOOXX)

% effect of OS on analgesic ED_{50} value of ketamine = 19%

0.5% H_2O_2 was added to water at Day 1 until the end of the experiment on Day 14

Pain induced by electrostimulator was recorded before and 5 min after ketamine injection

X = effect (analgesia), O = no effect (no analgesia)

Dose-response hypnotic effect of ketamine in the control and stressed chicks. The hypnotic effects of ketamine (determined by loss of righting reflex) were monitored in the the control and stressed chicks to evaluate the effect of the OS on the concentration-dependent hypnotic effects of ketamine. Ketamine was injected at 10, 20 and 40 mg/kg, *i.m.* (*n* = 6)/dosing groups of the control and stressed chicks (these doses were chosen depending on the hypnotic ED_{50} value of ketamine). Individual chicks in each dosing group were monitored to record the onset of hypnosis, its duration and recovery (Al-Zubaidy and Mohammad 2005).

Analgesic efficacy of ketamine in the control and stressed chicks. One dose of ketamine (15 mg/kg, *i.m.*) was injected for both the stressed and control chicks (*n* = 6; dose obtained from the analgesic ED_{50} value of ketamine). The voltage of electrostimulation inducing pain sensation was determined before and after 5 min of ketamine injection for each chick individually. The percentage of ketamine analgesia and voltage before and after injection of ketamine in addition to delta voltage (voltage after injection minus voltage before injection) for each group was also recorded in this experiment (Mousa and Mohammad 2012b).

Determination of plasma aspartate transaminase (AST) and alanine transaminase (ALT). Blood samples were collected 1 h after injection of ketamine at 15 mg/kg, *i.m.* and plasma samples were kept frozen. AST (Plummer 1987) and ALT (Reitman and Frankel 1957) levels were measured in plasma using a commercially avialable kit (BIOLABO SA, France), the spectrophotometric method was applied at 505 nm wavelength and enzyme levels were expressed in IU/l.

Statistics. One way analysis of variance was applied for statistical analysis of the parametric data of three groups followed by the least significant difference, while Student's t-test was employed to compare the two groups (Petrie and Watson 1999;

Table 3. Determination of acute LD_{50} of ketamine and its relation to OS induced by H_2O_2 in chicks

Parameter	Result	
	control group (tap water)	stressed group (0.5% H_2O_2 in water)
Acute LD_{50} value (mg/kg, *i.m.*)	254.2	208.5
The range of the doses used	250–300	150–250
Initial dose (mg/kg)	250	250
Last dose (mg/kg)	250	200
Increase or decrease in the dose (mg)	50	50
Number of chicks used	5 (OXOXX)	6 (XXOOXO)
Onset of death (min)	1– 6 min	1– 4 min

% effect of OS on acute LD_{50} value of ketamine = 22%

0.5% H_2O_2 was added to water at Day 1 until the end of the experiment on Day 14

X = effect (death), O = no effect (alive)

Table 4. Determination of ED_{50} value of ketamine hypnosis and its relation to OS induced by H_2O_2 in chicks

Parameter	Result	
	control group (tap water)	stressed group (0.5% H_2O_2 in water)
ED_{50} value (mg/kg, *i.m.*)	14.1	9.8.
The range of the doses used	12–15	9–15
Initial dose (mg/kg)	15	15
Last dose (mg/kg)	15 mg/kg)	12
Increase or decrease in the dose (mg)	3	3
Number of chicks used	5 (OXXOX)	6 (XXOXOX)
Onset of hypnosis (min)	1–2	1–3

% effect of OS on hypnotic ED_{50} value of ketamine = 44 %

0.5% H_2O_2 was added to water at Day 1 until the end of the experiment on Day 14

X = effect (hypnosis), O = no effect (no hypnosis)

Katz 2006). The non-parametric data were analyzed using the Fisher exact probability test and Mann-Whitney U-test (Runyon 1977; Katz 2006). The significance level was set at $P \leq 0.05$.

RESULTS

GSH and MDA biomarkers of OS in H_2O_2-treated chicks

H_2O_2 (0.5% v/v in water) induced OS in both Day 7 and Day 14 chicks through significant inhibition of GSH and elevation of MDA concentrations in the brain of chicks in comparison to the control (tap water) group (Table 1). According to this result, 7 and 14 day-old-chicks subjected to H_2O_2 treatment were used in all following experiments due to the occurrence of OS in both these groups of chicks.

Effect of H_2O_2-induced OS on the hypnotic or analgesic ED_{50s} of ketamine

A. Effect on hypnotic ED_{50} of ketamine

The hypnotic ED_{50} value of ketamine, as determined by loss of righting reflex, was 14.1 mg/kg, *i.m.* in the control group of chicks while this value decreased by 44% in the stressed chicks to 9.8 mg/kg, *i.m.* The signs associated with ketamine hypnosis are struggling, ataxia, distress call, dorsal or lateral recumbency, closed eyelids and loss of righting reflex. The same signs were markedly present in the stressed group of chicks (Table 4).

B. Effect on analgesic ED_{50} of ketamine

The analgesic efficacy of ketamine was increased in the stressed group of chicks. The ED_{50} value was 13.0 mg/kg, *i.m.* in the control chicks while this value decreased to 10.9 mg/kg, *i.m.* in the stressed chicks, a percentage decrease of 19% (Table 2).

Table 5. Dose-dependent hypnotic effects of multiple ketamine doses in the control and stressed chicks

Ketamine (mg/kg, *i.m.*)	Onset of hypnosis (min)	Duration of hypnosis (min)	Recovery from hypnosis (min)
Control group (tap water)			
10	15.50 ± 1.20	5.50 ± 1.00	7.67 ± 1.58
20	2.00 ± 0.45[a]	14.17 ± 1.01[a]	20.83 ± 1.49[a]
40	1.00 ± 0.00[a]	26.83 ± 2.41[a,b]	36.67 ± 1.84[a,b]
Stressed group (0.5% H_2O_2 in water)			
10	2.67 ± 0.61*	16.83 ± 1.22*	23.00 ± 1.37*
20	1.17 ± 0.17[a]	26.50 ± 0.67[*,a]	38.50 ± 2.95[*,a]
40	1.00 ± 0.00[a]	30.50 ± 0.72[a,b]	52.83 ± 4.41[*,a,b]

The values represent mean ± S.E. (*n* = 6)/dosing group

0.5% H_2O_2 was added to water at Day 1 until the end of the experiment on Day 14

*significantly different from the respective control (tap water) group at $P \leq 0.05$

[a]significantly different from ketamine 10 mg/kg, *i.m.* in the same group at $P \leq 0.05$

[b]significantly different from ketamine 20 mg/kg, *i.m.* in the same group at $P \leq 0.05$

Table 6. Analgesic effects of ketamine in the control and stressed chicks

| Groups | Analgesia% | Voltage (volt) | | Delta voltage |
		before injection	after injection	
Control (tap water)	66.67	5.67 ± 0.49	8.83 ± 1.11[a]	3.17 ± 1.05
Stressed (0.5% H_2O_2 in water)	100	4.83 ± 0.65	14.83 ± 1.51[*,a]	10.00 ± 1.81[*]

The values represent Mean ± S.E. (*n* = 6)/group

0.5% H_2O_2 was added to water at one day to the end of the experiment at the day 14th of chick old

Pain-induced by electrostimulator was recorded before and after 5 min of ketamine injection at 15 mg/kg, *i.m.*

[*]significantly different from the respective control (tap water) group at $P \leq 0.05$

[a]significantly different from voltage before injection of ketamine in the same group at $P \leq 0.05$

Effect of H_2O_2-induced OS on the acute LD_{50} value of ketamine

Table 3 shows that H_2O_2-induced OS increased the acute toxicity of ketamine in chicks. The acute LD_{50} value of ketamine in the control group was at 254.2 mg/kg, *i.m.* while this value decreased by 22% in the stressed chicks to 208.5 mg/kg, *i.m.* Signs of ketamine toxicity include paralysis, increased respiratory rate, tremor, jumping, difficulty with breathing and salivation. These same signs were markedly apparent in the stressed chicks.

Concentration-dependent hypnotic effect of ketamine in chicks stressed with H_2O_2

Ketamine injections of 10, 20 and 40 mg/kg, *i.m.* led to a dose-responsive hypnosis in both the control and stressed chicks. However, H_2O_2 was shown to increase the hypnotic efficacy of ketamine; it significantly decreased the onset of hypnosis and increased its duration as well as the time needed for recovery, in comparison to the control group (Table 5).

Table 7. Effect of H_2O_2 on Plasma AST and ALT levels in the chicks treated with ketamine

Groups	AST (IU/l)	ALT (IU/l)
Control (tap water)	74.33 ± 16.78	22.33 ± 2.89
Stressed (0.5% H_2O_2 in water)	131.17 ± 14.95	65.67 ± 17.26[*]

The values represent mean ± S.E. (*n* = 6)/group

0.5% H_2O_2 was added to water at Day 1 until the end of the experiment on Day 14

Ketamine was injected at 15 mg/kg, *i.m.*

[*]significantly different from the respective control (tap water) group at $P \leq 0.05$

Analgesic effect of ketamine in H_2O_2-treated chicks

The antinociceptive efficacy of ketamine was increased in the stressed group of chicks. There was an increase in the percentage of analgesia as well as a significant increase in the delta voltage elicited by electrostimulation for induction of pain sensation in comparison with the control group (Table 6).

Plasma AST and ALT levels in the control and stressed chicks

Both plasma AST and ALT concentrations increased significantly in the stressed chicks when compared to the control chicks as illustrated in Table 7.

DISCUSSION

The goal of the present study was to examine the effect of OS induced by H_2O_2 on the anaesthetic properties of ketamine in seven and 14 day-old broiler chicks. Animals that undergo anaesthesia with ketamine may already suffer from OS and that stress may affect the drug response in animals. This study used H_2O_2 (powerful oxidant for induction of OS experimentally). H_2O_2 (0.5% in water) supplied to chicks for fourteen days induced OS at Days 7 and 14, which was determined in the first experiment, through significant inhibition of GSH and elevation of MDA concentrations (as biomarkers of the OS) in the brain. The levels of GSH and MDA in the brains of chicks were close to what was previously reported for chicks treated with H_2O_2 (Mousa and Mohammad 2012a). The brain was chosen for analysis rather than other tissues due

to the specific central mechanism of anaesthetic action of ketamine and to determine the exact occurrence OS in the brain. In this study, ketamine was selected because it is a well known and popular anaesthetic used in human and veterinary surgical operations. It acts centrally on NMDA receptors causing a decrease in the calcium conductance to the neurons and resultant depression of the CNS (Finkel et al. 2009; White and Trevor 2009). We also selected an anaesthetic dose of ketamine to counteract what was found in a previous study (De Oliveira et al. 2009), namely that a sub-anaesthetic dose of ketamine may cause OS in the rat brain. The hypnotic and analgesic efficacy as well as the lethal effects of ketamine were shown to be augmented in the stressed chicks in comparison to those receiving only tap water which is reported here for the first time by measuring the hypnotic and analgesic ED_{50s} and acute LD_{50} values of ketamine in chicks. These changes can be attributed to the ability of H_2O_2 to elevate reactive oxygen species and free radical formation especially in the CNS (Pastore et al. 2003; Watt et al. 2004; Sayre et al. 2008) as well as oxidative damage that affects the blood-brain barrier (Lochhead et al. 2010). This can increase the permeability of centrally acting drugs such as ketamine, leading to increased ketamine concentrations in the CNS, in turn leading to higher effects on its target receptor. Similar findings on the effects of OS induced by H_2O_2 on the pharmacological response to the centrally acting sedatives and analgesics diazepam and xylazine have been reported in chicks (Mousa and Mohammad 2012a; Mousa and Mohammad 2012b). The increase in ketamine efficacy in stressed chicks could also be attributed to the sensitivity of the brain to inhibition by ketamine which is in accordance with the effect of OS induced by H_2O_2 on the anaesthetic action of pentobarbital in rats (Mohammad et al. 1999). Another possible effect of H_2O_2-induced OS on the anaesthetic properties of ketamine could be mediated by interference with calcium conductance (Akaishi et al. 2004). The liver damage and impaired metabolism caused by H_2O_2 treatment in chicks were clearly highlighted by the significant increase in plasma AST and ALT concentrations which may have a direct action on drug metabolism and delivery to its target receptor. Further studies are also required for detection of possible effects of H_2O_2 on the pharmacokinetics of ketamine that are directly linked to the pharmacological response to ketamine. The results reported here suggest that

H_2O_2-induced OS modifies the anaesthetic properties of ketamine in chicks by increasing its efficacy and lethality, probably through effects on ketamine pharmacodynamics and pharmacokinetics. In conclusion, important care must be taken in stressed animals undergoing anaesthesia with ketamine.

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