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Anaesthetic effect of excitatory amino acid receptors antagonist in oral administration as separate and concomitant with Clonazepam in cat

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ABSTRACT

Ketamine is one of agents blockade receptors influenced glutamate (as a potent excitatory amino acid of CNS). This drug has some side effects such as hypertension, histamine releasing effects, hallucination, hyper salivation (especially with oral administration) and etc. Clonazepam is a benzodiazepine that can pass trough blood-brain barrier and causes CNS suppression. Then it seems that co administration of Clonazepam and ketamine cause more effective & deep CNS depression effects and also alleviate some of ketamine adverse effects such as hallucination. The aim of this study was evaluation of ketamine and Clonazepam CNS suppressions effects in the manner of single and together in cat. Ten free roaming male & mature cats received drugs [ketamine (20, 40, 80mg/kg) & Clonazepam (1.5, 3, 4.5 mg/kg)] first in mixture of milk (40ml) or meat (30g) or oral spray rout. In 2nd stage they received concomitant doses of Clonazepam & ketamine by the method mentioned above. Each animal was observed continually by educated observer for CNS depression as graded on the behavioral scales. Almost all of the animals rejected receiving drugs in mixture of milk and meat. So oral rout used for administration of drugs. Clonazepam & ketamine showed dose dependent effects in different administered doses. Concomitant use of Clonazepam with ketamine improved depth and duration of ketamine's CNS depression effects. So results of this study showed this fact that administration of ketamine & Clonazepam in mixture of milk & meat is not a suitable method in cats. Also Clonazepam in mentioned doses caused a weak suppression of CNS. But a strong and long time CNS depression (anaesthesia) is achieved when ketamine sprayed in mouth. These Symptoms are considered significant in co administration of these two drugs.

Key words: ketamine, Clonazepam, anaesthesia, Cat.

INTRODUCTION

Ketamine (KT) is a synthetic available anesthetic that antagonize glutamate affected receptors. This agent blocks NMDA (N-methyl-D-Aspartate) receptors in CNS [5]. It has been used in human & animal operations for almost 35 years. Several studies had showed its wide margin of safety [13, 15]. KT induces one form of anesthesia that called dissociative anesthesia in which there is a marked sensory loss and analgesia, as well as amnesia and paralysis of movement, without actual loss of consciousness [17]. KT has some other effects such as sedation, analgesia, and

immobility. This drug has low intestinal absorption rate. It's bioavailability in human with oral administration is $\%20\pm7$ [21] and with rectal administration in cat is $\%43.5\pm6/1$ (8). KT was stated to be metabolized to at least two major compounds of pharmacological interest: to norketamine (NK) by *N*-demethylation, which then is converted to dehydronorketamine (DHNK) by dehydrogenation [1]. Its major metabolite NK, however, is active with one-third of the potency of its parent drug as an anesthetic. Thus the first-pass effect after oral administration results in an active metabolite that can contribute to the pharmacological effects.

Oral KT has been used sporadically as premedication for anesthesia in children [4, 6]. Also In fractious cats, is administered by squirting the drug into the mouth with a syringe when the animal is hissing [18, 2]. KT in anesthetized cats cause psychotic symptoms, release of histamine and induce cardiovascular system hyper activity such as increase of hart rate and hypertension. Benzodiazepines are one group of sedative-hypnotic drugs that used to reduce hallucination induced by KT. Clonazepam is one of long action benzodiazepines that had been used as anticonvulsant and anxiolytic agent [17].

Because of membership of this drug in benzodiazepines group it can cause a good & strong CNS suppression. Also this drug has little respiratory or cardiovascular depression effects (16). Nevertheless it seems that co administration of Clonazepam with ketamine improves CNS suppressing effect of KT and decreases some side effect of this drug (e.g. hallucination, convulsion). The main and important aim of this study was evaluation of CNS suppressing effect of or ally administrated ketamine & Clonazepam in the manner of single and together.

MATERIALS AND METHODS

Animals: Male, mature, sturdy free roaming and mixed breed cats selected randomly and were maintained as group housing in wide space (in a big room) to exhibit a wide range of complex behaviors. Animals had free access to food and water, and maintained on a 12-hour light-dark cycle. Temperature 25^{oc} with humidity between 45 and 65% provided for them allover the study. Food was withheld for 12 h and water for 2 h prior to the study to minimize the effects of gastric contents. They were kept one week before the examination in their room to achieve maximum adaptation to environmental situations. Animals had not be implicated any healthy problem allover the study. The numbers of cats in all of the treatment groups were ten animals.

The procedure has been approved by research organization Islamic Azad University ethic committee and was conducted in conformity with the NIH guidelines for the care and use of animals.

Drugs: Racemic ketamine (ketamine hydrochloride, Sigma, St. Louis, MO, U.S.A.) was dissolved in normal saline and the ph of each solution was adjusted to 5. Ketamine at a dose of 20, 40, 80 mg/kg [18], was administered orally to cats in mixed with milk (40 cc) or meat (30 g) or sprayed in mouth by a ordinary syringe (when animals were rejecting the drug in mixture with milk or meat). For comparison, a similar study was performed with Clonazepam. Clonazepam (Centaur India) was dissolved in normal saline and different doses of Clonazepam (1.5, 3, 4.5 mg/kg) [20] were administered as a mentioned method. To prevent absorption of the drugs from lower parts of gastrointestinal tract such as stomach or intestine, thus mentioned doses were balanced no exceed of 0.5 ml in treatment groups.

Be remembered because absence of scientific resources about oral doses of this two drugs in cat, therapeutic doses in IV. Administration rout, considered as minimum dose (base dose) for oral route of administration and according to this base, second, third and further doses estimated as two, three and more fold of first dose respectively. In first stage, drugs administered separately. In second step Clonazepam co-administered with Ketamine in treatment groups. In combination regimes high dose of each drug with low dose of other, also middle dose with other's middle dose was used. Hence treatment groups include:

1) ketamine 20 mg/kg, 2) ketamine 40 mg/kg, 3) ketamine 80mg/kg, 4) Clonazepam 1.5 mg/kg, 5) Clonazepam 3 mg/kg, 6) Clonazepam 4.5 mg/kg, 7) ketamine 20 mg/kg plus Clonazepam 4.5 mg/kg, 8) ketamine 40 mg/kg plus Clonazepam 3 mg/kg, 9) ketamine 80 mg/kg plus Clonazepam 1.5 mg/kg.

Each animal was observed continuously by an educated expert for CNS depression as graded on the behavioral scales shown as follow.

Scales for CNS depression were: [3]1) No effect2) impaired gait, prancing gat, some excitement3) Lowered head, braced stance, hindquarter weakness

4) Sternal or lateral recumbency, some responsiveness to repositioning

5) lateral recumbency, no response to movement of limbs and painful excitements

Reflex to pain in cat is evaluated by painful excitation of tail or pads with clamp [9].

Also obtained results in administration of various doses of drugs were evaluated on the base of underneath parameters for each treatment group: [19]

- Onset time of effect: refer to initiation first effect result from drug, which generally reveals by relaxation and mild ataxia.

- Duration of effect: refer to drug effect length of time (from initiation of first drug effect and passing of peak score and then achieving to normal state in animal).

- Peak score for each dose: refer to the highest rate of CNS suppression in administrated dose.

- Percentage of animal reached peak score: lost the reflexes (upon scores) for each dose.

- Onset time of peak score: refer to peak score initiation time of each dose.

- Duration of peak score: refer to time that animal is in highest recordable score in administrated dose.

When ever score 2 recorded we did not recognize any time to Duration of Peak Score & onset time of Peak Score. Also Times more than 6 hours was not recorded in this study.

The results (Onset Time and Duration of CNS depressant effects) are expressed as the Mean \pm SE. Differences between the individual mean values in different groups were analyzed by one-way analysis of variance (ANOVA) and differences with a p<0.05 were considered significant.

RESULTS

CNS suppression effect of orally administered ketamine (20, 40, 80 mg/kg) & Clonazepam (1.5, 3, 4.5 mg/kg) in mixture with milk and meat in cat:

In these routs almost all of cats did not accept drugs in mixture with milk & meat or ice cream or chocolate. Therefore administration by syringe was used over all study time.

Rate of CNS suppression of sublingual administration of ketamine (20, 40, 80 mg/kg):

As shown in Tables 1 & 4, Onset time of effect decrease with increasing dose of ketamine. In dose of 80 mg/kg this time decrease to 1':24" that in comparison with ketamine 20 mg/kg was considered significant (P<0.001).

Also peak score of CNS suppression increased dose dependently so that in dose 80 mg/kg, in 50% of cats analgesia was sow (Score5). Onset time of peak score decreased dose dependently so that in dose of 80 mg/kg this time reached to 2.59 ± 0.5 minute with almost in comparison, is half of 20 mg/kg.

Table 1: Effect of ketamine (20, 40, 80 mg/kg as oral administration)

Onset time and duration of CNS suppression (since onset time of effect to the time of getting normal) were showed. Results are expressed as Mean±SE.

| Dose Of Ketamine (mg/kg) | Onset Time Of Effect (min) | Duration Of Effect (hour) |
|-----------------------------|-------------------------------|------------------------------|
| 20 | 2.31 ± 0.41 | 0.56 ± 0.04 |
| 40 | 1.73 ± 0.23 | 1.87 ± 0.25 * |
| 80 | 1.23 ± 0.16 * | 2.41 ± 0.17 *** |

*** p < 0.001, * p < 0.05 significantly different from the control group (Ketamine 20mg/kg).

Table 2: Effect of ketamine (20, 40, 80 mg/kg as oral administration)

The highest rate of CNS suppression (Peak Score) & percentage of cats reached to seen peak score in each dose, also onset time and duration of peak score were showed. Each group had at least 10 cats. Results are expressed as Mean±SE.

| Dose of Ketamine (mg/kg) | Observed Peak Score | Percentage Of Animals Reached Peak Score | Onset Time Of Peak Score (min) | Duration Of Peak Score (min) |
|-----------------------------|------------------------|---|-----------------------------------|---------------------------------|
| 20 | 3 | 40 % | 4.9±0.7 | 11.15±1.9 |
| | 4 | 60 % | 3.63±0.7 | 23.46±6.9 |
| 40 | 3 | 50 % | 4.27±0.33 | 10.87±2.6 |
| | 4 | 50 % | 3.42±0.13 | 62.33±14.9 |
| 80 | 4 | 40 % | 2.86±0.4 | 86.2±19.8 |
| | 5 | 60 % | 2.32±0.6 | 114.4±24.8 |

Rate of CNS suppression of sublingual administration of Clonazepam (1.5, 3, 4.5 mg/kg):

As regards to tables 3 & 4 Onset time of CNS suppression decrease with increasing dose of Clonazepam. So that in dose 4.5 mg/kg this time is half of 1.5 mg/kg (lowest administered dose) (P<0.001).

Also duration of CNS suppression was lasted with increasing dose of Clonazepam so that this time almost reached to 1:42' so in compare with control group (1.5 mg/kg) this parameter shows a significant elevation (P<0.05). Clonazepam in the highest administered dose only could create score 3 in cats.

Table 3: Effect of Clonazepam (1.5, 3, 4.5 mg/kg as oral administration)

Onset time and duration of CNS suppression (since onset time of effect to the time of getting normal) were showed. Results are expressed as

Mean±SE.

| Dose Of Clonazepam (mg/kg) | Onset Time Of Effect (min) | Duration Of Effect (hour) |
|-------------------------------|-------------------------------|------------------------------|
| 1.5 | 10.71 ± 0.75 | 1.49 ± 0.3 |
| 3 | 6.4 ± 0.56 *** | 1.53 ± 0.5 |
| 4.5 | 5.24 ± 0.3 *** | 1.71 ± 0.5 * |

*** p<0.001, * p<0.05 significantly different from the control group (Clonazepam 1.5mg/kg)

Table 4: Effect of Clonazepam (1.5, 3, 4.5 mg/kg as oral administration)

The highest rate of CNS suppression (Peak Score) & percentage of cats reached to seen peak score in each dose, also onset time and duration of peak score. Results are expressed as Mean±SE. † Whenever score 2 recorded we did not recognize any time to Duration of Peak Score & onset time of Peak Score

| Duration Of Peak Score (min) | Of Onset Time Of Percentage Of Animals Peak Score (min) Percentage Of Animals Reached Peak Score | | Observed Peak Score | Dose Of Clonazepam (mg/kg) |
|------------------------------------|--|-------|------------------------|----------------------------------|
| * | Ť | 100 % | 2 | 1.5 |
| 5.4±0.04 | 10.76±0.52 | 100 % | 3 | 3 |
| 7.9±0.03 | 8.32±0.42 | 100 % | 3 | 4.5 |

Rate of CNS suppression in co-administration (as sublingual method) of ketamine (20, 40, 80 mg/kg) with Clonazepam (1.5, 3, 4.5 mg/kg):

Results of CNS suppression effects of co administration of ketamine 20 mg/kg + Clonazepam 1.5 mg/kg, ketamine 40 mg/kg + Clonazepam 3 mg/kg, ketamine 80 mg/kg + midazolam 1.5 mg/kg has been showed in tables 5 & 6.

So as saw in table 5, onset time of CNS suppression in group ketamine 80 + Clonazepam 1.5 is faster than other two groups in comparison. Also duration of CNS suppression (duration of peak score & duration of effect) in mentioned group is considered significant more long-lasting in compare with other two groups (P<0.001). Also peak score accompanied by further frequency (100%) was saw in this group in group ketamine 80 + midazolam 0.3 was sow in further numbers of cats in comparison with other treatment groups. Besides highest duration of peak score (more than 6 hours) was seen in this treatment group.

Table 5: Effect of ketamine & Clonazepam co administration as oral administration

Onset time and duration of CNS suppression (since onset time of effect to the time of getting normal) were showed. Results are expressed as Mean±SE. †Times more than 6 hours was not recorded.

| Ketamine + Clonazepam | Onset Time Of | Duration Of |
|-----------------------|-----------------------|---------------|
| (mg/kg) (mg/kg) | Effect(min) | Effect (hour) |
| 20 + 4.5 | 2.65 ± 0.23 | 3.54 ±0.16 |
| 40 + 3 | 2.55 ± 0.2 | 4.95 ±0.41 |
| 80 + 1.5 | $****1.06 \pm 0.1***$ | *** |

*** p<0.001 significantly different from the control group (Ketamine 20mg/kg + Clonazepam4.5 mg/kg). **** p<0.001, *** p<0.01 significantly different from the group Ketamine 40mg/kg + Clonazepam 3 mg/kg).

Comparison of onset time & duration of effect among ketamine and ketamine plus Clonazepam with co administration of it & midazolam:

On the base of figures 1 & 2 Clonazepam in higher doses (3, 4.5 mg/kg) shows significant effect in lasting of ketamine's induced onset time of effect. But in lower doses (e.g. 1.5 mg/kg) has not such effect.

Also augmentation of Clonazepam as mentioned doses to ketamine regime can cause a significant long lasting in CNS suppression effect in compare with ketamine a lone.

Table 6: Effect of ketamine (20, 40, 80 mg/kg) & Clonazepam (1.5, 3, 4.5 mg/kg) co administration as oral administration

The highest rate of CNS suppression (Peak Score) & percentage of cats reached to seen peak score in each dose, also onset time and duration of peak score were showed. Results are expressed as Mean±SE. †Times more than 6 hours was not recorded.

| Duration Of Peak Score (hour) | Onset Time Of Peak Score (min) | Percentage Of Animals Reached Peak Score | Observed Peak Score | Ketamine + Clonazepam (mg/kg) (mg/kg) |
|-------------------------------------|--------------------------------------|---|------------------------|--|
| 0.9±0.05 | 4.66±0.54 | 30% | 3 | 20 + 4.5 |
| 0.85±0.22 | 5.67±0.32 | 70% | 4 | |
| 3.2±0.6 | 3.7±0.77 | 30 % | 4 | 40 + 3 |
| 3.85±0.47 | 4.4±0.48 | 70 % | 5 | |
| † | 1.89±0.27 | 100 % | 5 | 80 + 1.5 |



Fig :1. Comparison onset time of CNS suppression between ketamine & Ketamine+Clonazepam Results are expressed as Mean±SE. * p<0.05.



Fig :2. Comparison duration of CNS suppression between ketamine & Ketamine+Clonazepam Results are expressed as Mean±SE. *** p<0.001.

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DISCUSSION

The results of present study show some notable facts:

- 1) Administration of ketamine or Clonazepam as mixed with milk, meat, ice cream or chocolate is not an appropriate rout of administration of these drugs in cat.
- 2) Oral Administration of ketamine & Clonazepam in administrated doses absolutely causes a dose dependently CNS suppression.
- 3) CNS depression in co administration of Clonazepam with ketamine improved in compare with sporadically Ketamine administration.

Ketamine is a drug with high lipid solubility and rapidly leaves plasma to the CNS (brain) and has various properties such as sleep induction, anaesthetic, analgesic and anti depressant [23]. After i.v. administration, maximum within $\underline{1}$ minute it reaches to the highest brain concentration. There for this fact is compatible with its rapid onset time of effect that seems to be some seconds after IV administration [10, 11]. This agent can also induce anaesthesia in I.P. route in addition to its essential routes of drug administration I.V or I.M. by dose of 0.5 ml in rat [22]. But I.v. administration of ketamine has some obvious CNS suppression effects in cat. In the present study oral ketamine administration induced CNS depression effects within 2.5-3 minutes in cats. These effects were dose dependently so that with dose of 80 mg/kg the cats reached to score5 (analgesia). This drug's Rapid onset time of effect with oral (sublingual) administration, indicates its high mucosal absorption from proximal parts of GI (e.g. oral cavity and esophagus).

In other section of this study, orally administration of Clonazepam in different doses induced almost a weak & shortlasting CNS depression effects (maximum scores 3 in highest dose). This effect maybe in result of its mechanism of action, low & slow distribution trough CNS in administered doses or etc. For clarify of its exact mechanism need further studies. But midazolam one ultra short action member of benzodiazepines can induce an intense effect as oral administration in compare with Clonazepam so that midazolam bring about score 3-4 dose dependently in healthy awake cat [12].

But when this drug added to ketamine regime in mentioned doses (oral administration), all of recorded parameters improved in comparison with ketamine and Clonazepam alone. So that it seems due to synergistic effects between them in suppression of CNS.

These effects in group ketmanie80+Clonazepam1.5 were considered significant (p<0.001) in compare with other combination protocol so that we could achieve anaesthesia in this combination protocol.

It seems that improvement of CNS suppression effect in co-administration of Clonazepam & ketamine is due to high mucosal absorption of Clonazepam (as ketamine) and whenever these two drugs reached to CNS, depth and duration of CNS suppression effect be invigorated because of synergism existence between these two drug.

With due attention to that in present study, in group ketamine 80 plus Clonazepam1.5 onset time and duration of CNS suppression was more suitable so that chiefly anesthesia was seen in allover the animals; it seems that this protocol is a appropriate non-invasive method to induce anaesthesia in cat.

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