Effects of intravenous administration of propofol and midazolam on pentylenetetrazole kindled seizures in rats

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ABSTRACT
To date, studies examining the proconvulsants or anticonvulsants in chronic epileptic models in rat are rare. Propofol has conflicting reports regarding the pro- or anti-convulsant effects on chronic seizures. Midazolam is considered another anesthetic option that used in combination with propofol and has documented anticonvulsant properties. The present study was undertaken to examine the effects of intravenous administration of various doses of propofol, in comparison to midazolam, on seizures activity in chronically epileptic rats, using PTZ seizures kindled model. Kindling in control and experimental rats was induced by intraperitoneal sub - convulsive dose of PTZ (35 mg/kg) every alternate day. After each injection of PTZ, rats were monitored for 30 minutes for behavioral seizures occurrence. Seizure scores were rated according to Racín scale. Once kindled, animals were treated with different doses of midazolam or propofol prior to PTZ challenge and were subsequently observed. The present study showed that, propofol or midazolam intravenous administration suppressed PTZ seizures in kindled rats and led to a significant, dose dependent decrease of behavioral seizures score and increased the percent inhibition of PTZ kindled seizures stage. The ED50 of propofol for percent inhibition of PTZ kindled seizures stage was 5.36 mg/kg, whereas the ED50 of midazolam was 1.85 mg/kg, suggesting that the anticonvulsant effects of midazolam against PTZ kindled seizures are more profound. In conclusion, the current study showed that propofol protected against PTZ kindled seizures and provided further evidence for its anticonvulsant effects reported in other chronic animal seizures, namely amygdala-kindled convulsions in rats.

1. Introduction
Propofol is used for anesthesia maintenance and induction because it has a rapid onset of action. One of the undesirable side effects associated with propofol anesthesia, is the induction of seizures or seizure like phenomena during or following recovery from anesthesia (Carvalho et al., 2017; Fernando et al., 2017; Pantelakis et al., 2021). This phenomenon was also reported in mice (Dolin et al., 1992) and rats (Hasan & Woolley, 1999) during recovery from propofol anesthesia. Additionally, propofol was reported to induce ictal-like epileptic activity in hippocampal mouse brain slices (Hannan et al., 2020; Voss et al., 2015). These clinical and experimental findings may suggest that propofol has proconvulsant effects. By contrast, clinical reports indicated the successful use of propofol in the management of status epilepticus (Lee, 2020; Prabhakar & Kalaivani, 2017; Shamlool et al., 2021). The anticonvulsant properties of propofol have been also demonstrated in hippocampal slices preparation (Hasan et al., 1991; Riu et al., 1992) and in animal models of kainic acid induced status epilepticus (Hasan et al., 2014). However, to date, studies describing the anticonvulsant effects of propofol in animal models of chronic seizures are limited.

Midazolam, on the other hand, is an anesthetic option that used in combination with propofol and has documented anticonvulsant properties (Conway et al., 2021). Midazolam shares a number of pharmacological properties with propofol (Wafae et al., 2019). In fact, both agents were used in the management of status epilepticus (Lee, 2020). They showed anticonvulsant activity against PTZ-induced seizures (Dhir & Rogawski, 2012), and picrotoxin-induced seizures (Maciejak et al., 2002). In addition, enhancement of GABA at the GABAA receptor has been suggested.
as the mechanism for the anticonvulsant actions of the two agents (Holtkamp, 2018; Singh et al., 2014). Thus, midazolam was used for comparison purposes, which could ultimately reflect on the choice of best agent anticonvulsant activity.

In light of conflicting reports regarding the pro or anticonvulsant effects of propofol, the present study was undertaken to examine the effects of intravenous administration of propofol, in comparison to midazolam, on PTZ kindled seizure in rat, which is widely accepted as an experimental model of chronic seizures and has been extensively used to examine the anticonvulsant effects of antiepileptic drugs.

2. Materials and methods

2.1. Animals
Adult male Sprague–Dawley rats, weighting 250–300 gm, were used in the study experiments. The animals for this study were provided by the animal facility unit at the Arabian Gulf University/College of Medicine and Medical Sciences. Stainless steel hanging cages were used to house the animals of the study. Housing conditions included 12 hours lights: dark cycle, room temperature (24°C/14°C) and rodent chow and water ad libitum. Animals were treated in accordance with “Guide for the Care and Use of Laboratory Animals” at Arabian Gulf University. The experiments of the current study were performed under a protocol approved by the “Research and Ethics Committee”, College of Medicine and Medical Sciences at the Arabian Gulf University.

2.2. Drugs
Pentylenetetrazole (PTZ) obtained from Sigma-Aldrich, MO, USA. It was administered via IP route 0.5 ml/kg bw dissolved in normal saline. Midazolam from Hoffman-Laroche Ltd., Basel Switzerland was obtained as injectable solution. Propofol as injectable emulsion was obtained as 1% Diprivan from Astra-Zeneca LP, Wilmington, DE. Various doses of either propofol or midazolam were administered intravenously via 25G butterfly cannula inserted into dorsal tail vein in a volume of 1 ml/kg.

2.3. Induction of PTZ kindled seizures
Individual animals from four different groups were injected intraperitoneally (IP) with a subconvulsive dose of PTZ (35 mg/kg) every alternate day. After each injection of PTZ, the rat was placed in Plexiglas cage and was monitored for 30 minutes for the occurrence of behavioral seizures. Severity and seizure score was rated according to Racine scale (Racine, 1972). An animal was considered fully kindled when it had a seizure score of 5 on two consecutive trials. In the present study, 12-15 injections of PTZ were required to reach full kindling stage.

2.4. Evaluation of the anticonvulsant effects of various doses of propofol and midazolam on PTZ kindled seizures
Fully kindled rats (three experimental animal groups, 8 animals per group) were injected with 2.5, 5, 10 mg/kg of propofol. Similarly, three animal groups were treated with similar doses of midazolam. The drugs were administered via the dorsal tail vein as a single IV dose. Control animal groups (8 animals per group) were administered an equal volume of saline. Three minutes following drugs or saline administration, fully kindled rats were challenged with subconvulsant dose of PTZ (35 mg/kg) and closely monitored for the occurrence of behavioral seizures and seizure scores as described in the above section.

2.5. Data analysis
Data was presented as mean ± SEM. Differences in mean seizure score and percent inhibition of kindled seizures stage among groups were determined using ANOVA followed by the least significant difference (LSD) test as a post-hoc analysis. The significance level was at a 0.05. For evaluation effectiveness of propofol and midazolam against PTZ kindled seizures, the ED50 (estimated dose resulting in 50% of inhibition of seizure) and its associated 95% confidence limits, were calculated using Graph Pad Prism version 7 (Graph Pad software, La Jolla, CA, USA).

3. Results

3.1. Evaluation of hypnotic effects of propofol and midazolam
Before testing propofol and midazolam effects on PTZ kindled seizures, a preliminary study was conducted to demine the degree of hypnosis of various doses (2.5, 5, and 10 mg/kg) of propofol and midazolam as previously described (Jembrek & Vlainic, 2015). Loss of righting reflex was used as an index of hypnotic effects of various doses of the two anesthetics. Among the tested doses, only propofol 10 mg/kg, the highest dose used, caused loss of righting reflex. By contrast, all doses of midazolam (2.5, 5, and 10 mg/kg) did not cause loss of righting reflex and a higher dose (20 mg/kg) of midazolam was needed to cause loss of the righting reflex. Therefore, in this study, we examined the effects of two subanesthetic doses (2.5 and 5 mg/kg) and one anesthetic dose of propofol (10 mg/kg) on PTZ
kindled seizures in rats. The effects of subanesthetic doses (2.5, 5, and 10 mg/kg) of midazolam on PTZ kindled seizures were also examined to compare the potency of both anesthetic agents in reducing the intensity and severity of PTZ kindled seizures.

3.2. Propofol effects on PTZ-kindled seizures

The intravenous administration of various doses of propofol significantly reduced the severity of PTZ kindled seizures and caused a significant and dose dependent decreases of PTZ kindled seizure score. The lowest does of propofol (2.5 mg/kg) did not induce significant decrease in PTZ seizure score in fully kindled rats. However, higher doses of propofol (5 and 10 mg/kg) resulted in a significant reduction in PTZ seizure score. The effects of various doses of propofol on PTZ kindled seizures are shown in Figure 1. The effects of various doses of propofol on percent inhibition of PTZ kindled seizures stage are shown in Figure 2. The lowest dose of propofol (2.5 mg/kg) did not produce a significant effect on percent inhibition of seizures stage, however, higher doses (5, and 10 mg/kg) significantly increased the percent inhibition of PTZ seizure stage and at the highest dose tested, and behavioral seizures were almost completely suppressed. The estimated ED50 of propofol (dose which caused 50 percent inhibition of PTZ kindled seizures stage) was 5.36 mg/kg with 95% CI of (4.85-5.96).

3.3. Midazolam anticonvulsant effects against PTZ kindled seizures

For comparison, the effects of intravenous administration of various doses of midazolam on kindled seizures were evaluated. As shown in Figure 3, all sub anesthetic doses of midazolam (2.5, 5, and 10 mg/kg), produced a dose-dependent suppression of seizure intensity and markedly reduced the PTZ seizure score. These doses of midazolam also resulted in a significant increase in percent inhibition of kindling seizures stage in a dose dependent manner and suppression of kindled seizures stage was complete at the highest dose. The percentage inhibition of PTZ kindled seizure stage at the various doses of midazolam is shown in Figure 2. The estimated ED50 of midazolam (dose which caused 50 percent inhibition of PTZ kindled seizures) was 1.85 mg/kg with 95% CI of (1.71–2.05).
4. Discussion

This study, to our knowledge, is the first to examine the anticonvulsant effects of intravenous administration of propofol in chronically epileptic rats using the PTZ kindled seizure model. The PTZ kindling seizure model is widely accepted as an experimental model of chronic seizures and has been extensively used to examine the anticonvulsant effects of antiepileptic drugs. The findings of the present study show that intravenous administration of propofol or midazolam prior to PTZ challenge in fully kindled rat, reduced the intensity and severity of behavioral seizures in a dose dependent manner. Both propofol and midazolam induced marked decrease in seizures score and a significant inhibition of seizure stage. While the anticonvulsant effect of propofol was observed at the higher two doses (5 and 10 mg/kg), the effect of midazolam was observed at sub anesthetic doses (2.5, 5, and 10 mg/kg). These findings suggest that both agents have marked anticonvulsant effects against PTZ kindled seizures and indicate that the effects of midazolam were more profound. The higher potency of midazolam in suppressing PTZ kindled seizures is further indicated by current results where the ED50 of midazolam (1.85 mg/kg) was lower than that of propofol (5.36 mg/kg). The higher potency of midazolam compared to that of propofol against PTZ kindled seizures were consistent with previous findings in other seizure models, which showed that midazolam has a higher potency than propofol in blocking PTX-induced acute convulsions in rats (Borowicz & Czuczwar, 2003).

It is known that PTZ exerts its seizure activity via inhibiting gamma aminobutyric acid (GABA) activated channels and it has been suggested that repetitive single subconvulsant dose administration of PTZ resulted in a decrease in GABAergic activity (Hara et al., 1994). Since these structurally different anesthetics were shown to enhance GABA actions at the GABAA receptors (Dhir, 2012; Kotani et al., 2008; Martinez et al., 2009), the anticonvulsant effects of the two anesthetic agents – propofol and midazolam- that is reported in this study may be accounted for through the GABAA receptors action of the two drugs leading to increased GABAergic inhibition (Holtkamp, 2018; Singh et al., 2014).

The higher efficacy of midazolam, in protecting against PTZ kindled seizures compared to that of propofol is more difficult to explain. However, since the score of PTZ kindled seizures in both propofol and midazolam treated rats were monitored for up to 30 minutes after treatment with either agents, it is possible that the relative potency of the two drugs could be related to differences in their pharmacokinetic properties and the duration of action. It has been reported that propofol has a rapid on- rapid-off action with a short half-life of 20 minutes (Kotegawa et al., 2002). By contrast, midazolam have a longer duration of action compared to that of propofol, and a longer half-life of 49 min (Greenblatt et al., 2000; Hari Keerthy et al., 2015). In addition, the lower potency of propofol against PTZ kindled seizures could have been related to the rapid-on rapid-off action at GABAA receptors. Thus, explaining the lower efficacy of propofol against PTZ kindled seizures compared to midazolam. This phenomenon was previously proposed as an explanation for the observed depression and rebound excitation of limbic evoked potential in rats receiving propofol (Voss et al., 2015).

Our previously published work covered picrotoxin (PTX) induced seizures as an acute seizure model, where it was shown that midazolam was more effective than propofol against acute PTX-induced tonic seizures (Hasan et al., 2014). The current work deals with chronic seizure type which is PTZ kindled seizures. Therefore, the two studies are fundamentally different as they represent how acute versus chronic seizure models are influenced by propofol versus midazolam. Still, in chronic seizures, such as PTZ kindled seizures, midazolam showed more effectiveness compared with propofol.

5. Conclusion

The current study showed that the intravenous midazolam or propofol administration induced marked anticonvulsant effects against PTZ kindled seizures in rats, where the effects of midazolam are more profound than those of propofol. These findings provide further evidence for the anticonvulsant effects of propofol.

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