RESEARCH ARTICLE

Halothane Increases Myocardial Sensitivity to Epinephrine-Induced Arrhythmias in Cirrhotic Rats

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Background: Cirrhotic patients are at a greater risk of different kinds of arrhythmias. Despite the well-known arrhythmogenic effects of halothane, developing countries continue to use halothane due to its low cost. This study was designed to investigate the pathophysiology of halothane untoward effects on heart rhythm moreover was aimed to explore the effect of halothane anesthesia on the threshold of epinephrine-induced arrhythmia in cirrhotic rats.

Methods: Bile duct ligation was used to induce cirrhosis. The subjects were anesthetized with halothane or pentobarbital. Arrhythmia was induced by intravenous injections of increasing doses of epinephrine. Blood pressure and the electrocardiogram were monitored. The threshold doses of epinephrine for induction of premature ventricular contraction (PVC), ventricular tachycardia (VT) and ventricular fibrillation (VF) were determined.

Results: Cirrhotic rats had longer QTc intervals in comparison with sham-operated animals. Halothane-anesthetized rats had significantly shorter QTc intervals than pentobarbital-anesthetized rats in both cirrhotic and sham-operated groups. Halothane significantly decreased the threshold dose of epinephrine for induction of PVC, VT and VF in cirrhotic rats. The BP of different experimental groups did not differ with each other.

Conclusion: Cirrhosis intensifies halothane induced ventricular arrhythmia and our results raise concerns regarding the use of halothane in cirrhotic patients.

Keywords: cirrhosis; anesthesia; halothane; epinephrine-induced arrhythmias; rats

Cirrhosis is a severe hepatic disease histopathologically characterized by regenerative nodules surrounded by fibrotic bands [1]. This condition is accompanied by a number of cardiovascular abnormalities [2]. Corrected QT (QTc) interval prolongation is the most widely known electrocardiographic finding in cirrhosis [3]. It is now firmly established that QTc prolongation is associated with torsades de pointes arrhythmia [4]. There is evidence indicating that cirrhotic patients are at an increased risk of developing cardiac arrhythmias [4-6].

Halothane is a halogenated anesthetic which was initially used in 1956. Its use became rapidly popular due to its fast anesthetic induction and recovery and inflammability [7]. Although halothane is no longer used in developed countries due to lethal hepatic failure, developing countries continue to use this agent because of its low cost [8-9]. Halothane has substantial cardiovascular effects such as bradycardia and hypotension. Additionally, it is believed that halothane sensitizes the myocardium to adrenergic stimulation, since cardiac arrhythmias occur with low doses of adrenergic agents during halothane anesthesia [10-11].

Epinephrine is an adrenergic agent used during surgeries for hemostasis, and although strict guidelines and preventive measures have been applied to limit its side effects, arrhythmia and other side effects of epinephrine can still occur in the operation room [12]. As mentioned earlier, halothane is still being used as a general anesthetic in developing countries [8-9]. Considering the high rate of arrhythmias in cirrhosis [4-6] and myocardial sensitizing effects of halothane [10-11], in this study, we attempted to assess the sensitivity of halothane-anesthetized, bile duct ligated (BDL), cirrhotic rats to epinephrine-induced ventricular arrhythmias.

Methods

Animals were handled according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" (NIH US publication 85-23 revised 1996) and in conformity with our institution's "Guidelines for the Care and Use of Animals" (Department of Pharmacology, Tehran University

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of Medical Sciences). Male Sprague–Dawley rats, weighing 200–250 g, were housed in groups of 3–4 in a room with 25 ±1°C temperature. A 12 hour light/dark cycle was provided, and animals had free access to food and tap water. Bile duct ligation and sham operations were performed under general anesthesia induced by intraperitoneal (i.p.) injections of ketamine HCl (50 mg/kg; Gedoon Richter, Budapest, Hungary) and xylazine (30 mg/kg; Daroupakhsh, Tehran, Iran) as previously described [13].

Determination of susceptibility to epinephrine-induced arrhythmias was determined 28 days after BDL or sham operations under general anesthesia induced by halothane (1.5 minimum alveolar concentration, inhalational, Nicholas Piramal India Limited Ennore, Chennai – 600 057) or sodium pentobarbital (50 mg/kg, intraperitoneal (i.p.), Merck, Darmstadt, Germany). Four groups of animals were experimented in this study. Each group consisted of 12 animals. The experimental groups were as follows: sham operated pentobarbital anesthetized rats, BDL pentobarbital anesthetized rats, sham operated halothane anesthetized rats, BDL halothane anesthetized rats. Pentobarbital anesthetized rats served as controls for halothane anesthetized, since pentobarbital has the least cardiovascular effects among general anesthetics [14-15].

General anesthesia with halothane was performed by ventilating animals through a face mask with the following gases: 1.5 minimum alveolar concentration (MAC) of halothane, O2 (800- 1000 ml/kg) and N2O (1250 ml/kg). Pentobarbital-induced general anesthesia was performed by injecting 50 mg/kg of sodium pentobarbital (i.p.) to BDL or sham-operated rats. Blood pressure (BP) of the tails of rats was measured with a pneumatic pulse sensor coupled to a PowerLab/8SP data acquisition system (ADInstruments, Australia). Rats were placed in plastic restrainers during BP assessment. Lead II of the electrocardiogram was recorded using subcutaneous needle electrodes and a cardiac coupler connected to a DMP-4B physiograph (Narco Biosystems, Houston, TX, USA). After the rats were completely anesthetized, the left jugular vein was cannulated for intravenous (i.v.) epinephrine administration. Epinephrine administrations started 10 minutes after left jugular vein cannulation, when the rats' BPs and heart rates were steady. Initially, 0.02 mg/kg of epinephrine (Fluka, Buchs, Switzerland) was administered. After the initial 0.02 mg/kg dose of epinephrine, 0.005 mg/kg of epinephrine was administered every 4 minutes, and the threshold doses which induced primary ventricular contractions (PVC), ventricular tachycardia (VT) and ventricular fibrillation (VF) in rats were determined.

The QT interval (the interval between the beginning of the Q wave and the end of the T wave) was determined for each rat in the sham-operated and BDL groups before left jugular vein cannulation. QT intervals were corrected for RR intervals (average interval between the peaks of consecutive QRS complexes in the electrocardiogram) according to Bazett's formula: $QTc = QT/\sqrt{RR}$ [16].

Epinephrine injection induced a range of arrhythmias, including 1st, 2nd, and 3rd degree heart block, premature atrial contraction (PAC), PVC, VT (defined as three or more consecutive PVCs), and VF. The most frequent ventricular arrhythmias were PVC, VT and VF which were used to compare the different groups in this study.

Blood pressures, QTc intervals and epinephrine doses which induced PVC, VT and VF were expressed as mean ± S.E.M. Data was analyzed by two-way analysis of variance (ANOVA). The SPSS statistical software package (Version 13, Chicago, IL, USA) was used for the analyses. Differences with P values less than 0.05 were considered as statistically significant.

Results

(Figure 1) depicts QTc interval durations in different groups. Bile duct ligated rats had longer QTc intervals in comparison with sham-operated animals. In addition, treatment with halothane reduced QTc interval durations in BDL and sham-operated rats (two way ANOVA: effect of bile duct ligation: $F_{(BDL\ vs\ sham)}= 6.509$, P value < 0.05; effect of halothane in sham groups: $F_{(halothane\ vs\ pentobarbital)} = 27.087$, P value < 0.001; effect of halothane in BDLgroups: $F_{(halothane\ vs\ pentobarbital)}= 0.973$, P value < 0.05).

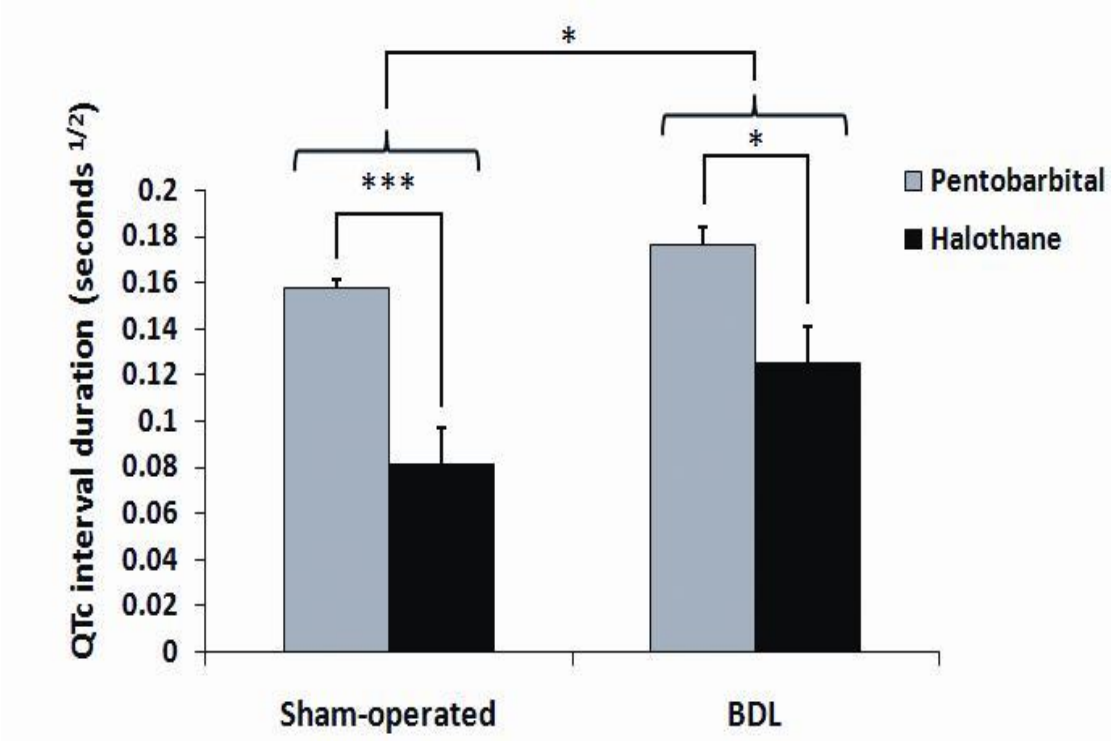
(Figure 2) shows BPs in different experimental groups. There was no significant difference between blood pressures of rats anesthetized with different drugs. However, BDL rats had significantly lower BPs in comparison to sham-operated animals (two way ANOVA: effect of bile duct ligation: $F_{(BDL\ vs\ sham)}= 12.118$, P value < 0.001; effect of halothane in sham groups: $F_{(halothane\ vs\ pentobarbital)}= 3.187$, P value = 0.081; effect of halothane in BDLgroups: $F_{(halothane\ vs\ pentobarbital)}= 0.002$, P value = 0.965).

As depicted in (Figure 3), bile duct ligation significantly reduced the threshold dose of epinephrine for induction of PVC. Moreover, halothane-anesthetized rats were more sensitive to epinephrine-induced PVC in comparison with pentobarbital-anesthetized animals (two way ANOVA: effect of bile duct ligation: $F_{(BDL\ vs\ sham)}= 4.507$, P value < 0.05; effect of halothane in BDL groups: $F_{(halothane\ vs\ pentobarbital)}= 16.427$, P value < 0.001; effect of halothane in sham groups: $F_{(halothane\ vs\ pentobarbital)}= 0.149$, P value = 0.701). Although anesthesia with halothane reduced the threshold dose of epinephrine for induction of PVC in sham-operated rats but this effect was not statistically significant (P > 0.05).

(Figure 4) shows the threshold dose of epinephrine for induction of VT in different experimental groups. Halothane anesthesia and bile duct ligation significantly reduced the threshold dose of epinephrine for induction of VT (two way ANOVA: effect of bile duct ligation in halothane treated groups: $F_{(BDL\ vs\ sham)}= 19.623$, P value < 0.001; effect of halothane in BDL groups: $F_{(halothane\ vs\ pentobarbital)}= 13.136$, P value = 0.001; interaction of bile duct ligation and halothane: $F_{(BDL\ vs\ sham)}= 12.416$, P value = 0.001). Although anesthesia with halothane significantly reduced the threshold dose of epinephrine for induction of VT in cirrhotic rats, it had no significant effect on sham-operated animals (P > 0.05).

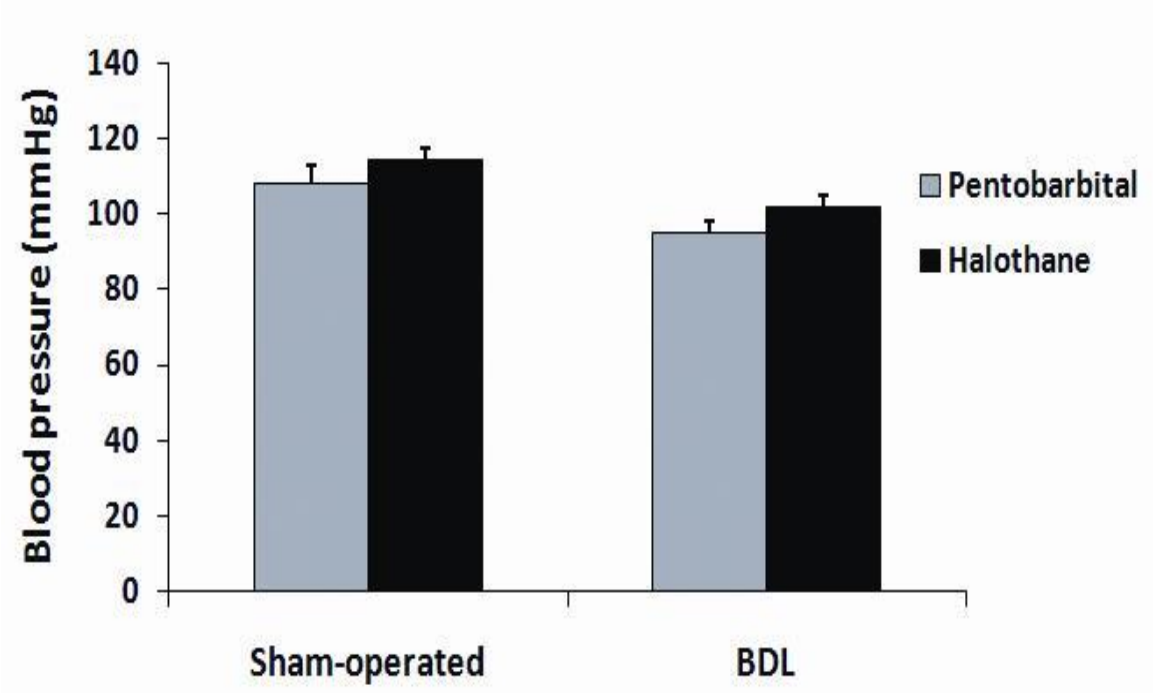
(Figure 5) depicts the threshold dose of epinephrine for induction of VF in different experimental groups. Halothane anesthesia and bile duct ligation significantly reduced the threshold dose of epinephrine for induction of VF (two way ANOVA: effect of bile duct ligation in halothane treated group: $F_{(BDL\ vs\ sham)}= 42.532$, P value < 0.001; effect of halothane in BDL groups: $F_{(halothane\ vs\ pentobarbital)}= 10.690$, P value <0.001; interaction of bile duct ligation and halothane: $F_{(BDL\ vs\ sham)}= 28.066$, P value < 0.001). Despite the fact that anesthesia with halothane significantly reduced the threshold dose of epinephrine for induction of VF in cirrhotic rats, it had no significant effect on sham-operated animals (P> 0.05).

Figure 1- Comparison of QTc interval durations between pentobarbital- and halothane-anesthetized rats in sham-operated and bile duct ligated (BDL) groups.



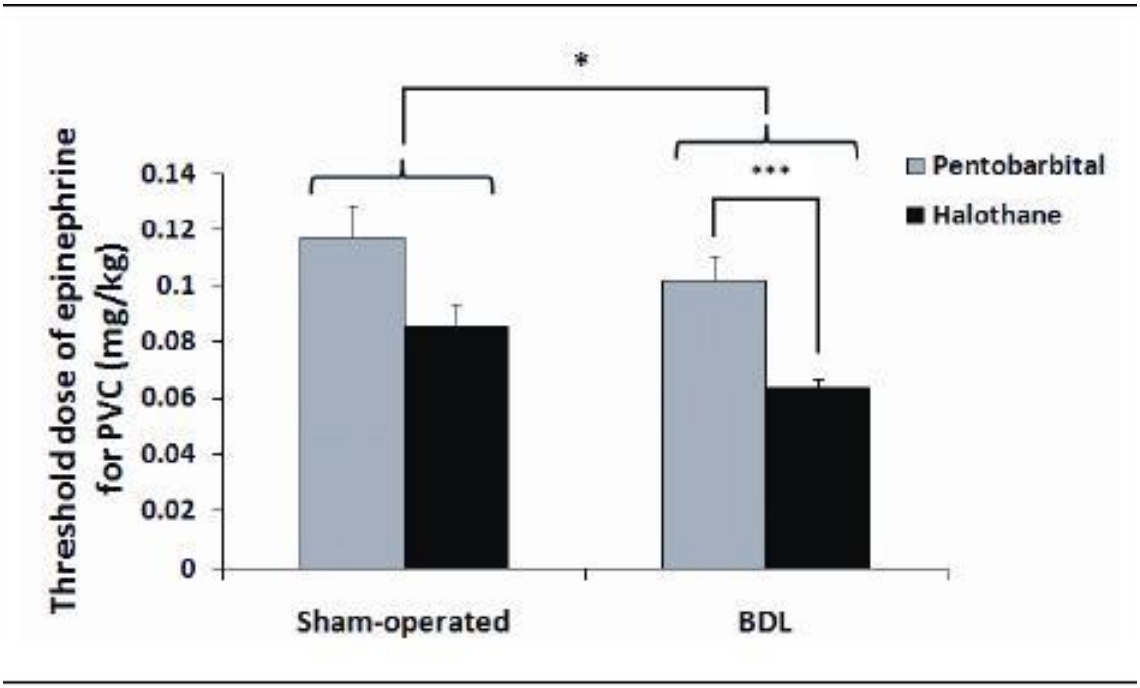
Values are expressed as mean ± S.E.M. Each group consisted of 12 rats. Differences were analyzed by two-way ANOVA. * P< 0.05 and *** P < 0.001.

Figure 2- Comparison of tail blood pressures (BPs) between pentobarbital- and halothane-anesthetized rats in sham-operated and bile duct ligated (BDL) groups.



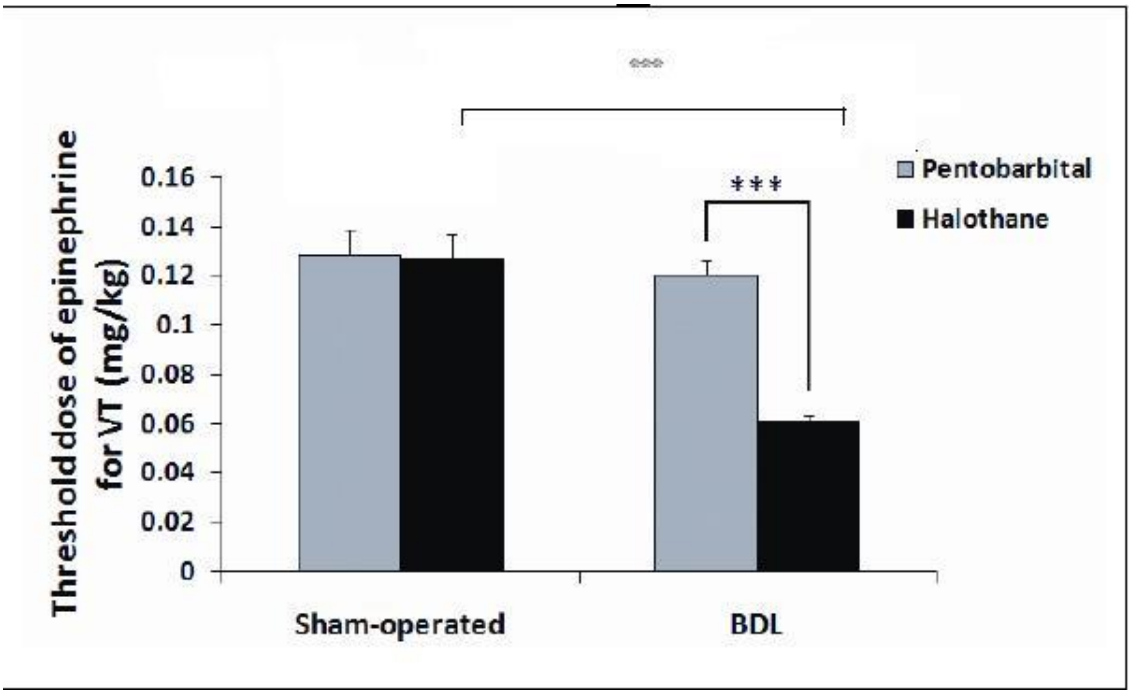
Data are expressed as mean ± S.E.M. Each group consisted of 12 rats. Differences were analyzed by two-way ANOVA.

Figure 3- Comparison of threshold doses of epinephrine for induction of premature ventricular contraction (PVC) between pentobarbital- and halothane-anesthetized rats in sham-operated and bile duct ligated (BDL) groups.



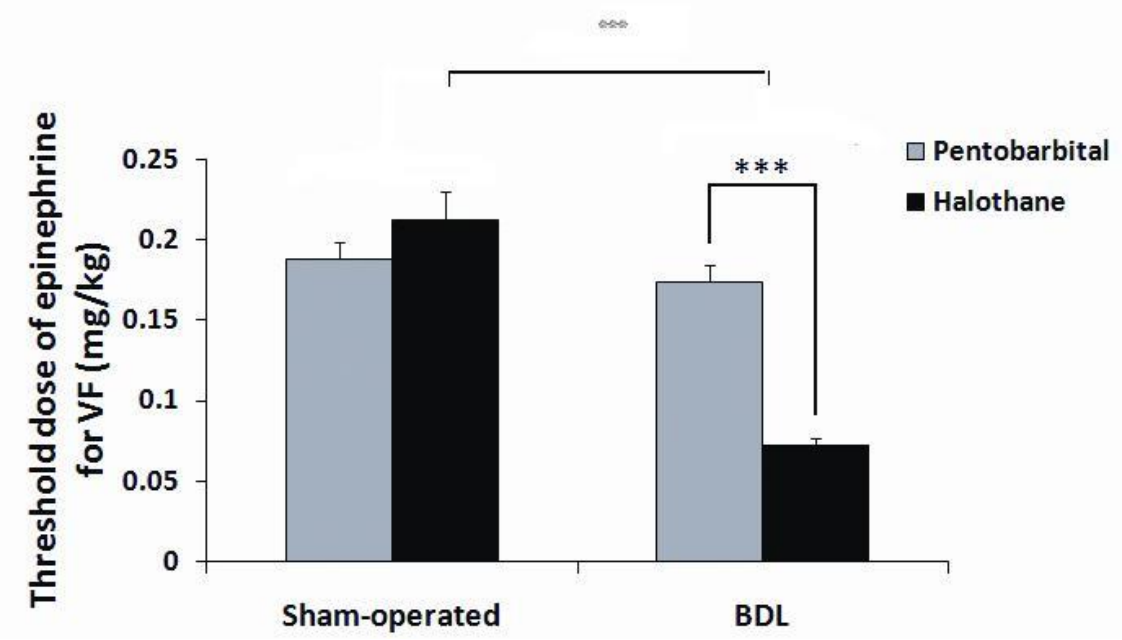
Values are expressed as mean ± S.E.M. Each group consisted of 12 rats. Differences were analyzed by two-way ANOVA. * P< 0.05. *** P<0.001

Figure 4- Comparison of threshold doses of epinephrine for induction of ventricular tachycardia (VT) between pentobarbital- and halothane-anesthetized rats in sham-operated and bile duct ligated (BDL) groups.



Values are expressed as mean ± S.E.M. Each group consisted of 12 rats. Differences were analyzed by two-way ANOVA. *** P< 0.001.

Figure 5- Comparison of threshold doses of epinephrine for induction of ventricular fibrillation (VF) between pentobarbital- and halothane-anesthetized rats in sham-operated and bile duct ligated (BDL) groups.



Values are expressed as mean ± S.E.M. Each group consisted of 12 rats. Differences were analyzed by two-way ANOVA. *** P< 0.001.

Discussion

In this study, we found that anesthesia with halothane increases the susceptibility of BDL rats to epinephrine-induced VT and VF. Additionally, it was observed that BDL rats had longer QTc intervals and lower heart rates in comparison to sham-operated controls. The present study raises concerns regarding the use of halothane as a general anesthetic in cirrhotic patients.

Topical epinephrine solutions are currently used for hemostasis during surgeries [12]. However, this drug has proven to be arrhythmogenic, even when used topically in low doses, especially in individuals anesthetized with halothane. For this reason, animal models of epinephrine-induced arrhythmia have been used to study the pathophysiology of this lethal side effect [10].

Bile duct ligation is a widely accepted model for inducing cirrhosis in rats [17]. Cirrhosis can cause a battery of cardiovascular abnormalities such as hyperdynamic systemic circulation, chronotropic incompetence, left ventricular failure, QTc prolongation and cardiac arrhythmia [18-19,2]. A considerable proportion of cirrhotic patients suffer from atrial fibrillation and flutter. Moreover, diuretics, which are the mainstay treatment for ascites, can cause hypo- and hyperkalemia, which might lead to atrioventricular block in cirrhotic patients [6]. Prolonged QT interval duration is seen in as much as 47% of patients with cirrhosis and its prevalence increases with the severity of cirrhosis [3]. This condition predisposes cirrhotic patients to polymorphic ventricular arrhythmia [4], and therefore, drugs that affect cardiac repolarization should be used with extreme caution in patients with liver disease. It is noteworthy that cirrhotic patients who have QTc prolongation are at an increased risk of sudden cardiac death [5].

In this study, we found that halothane-anesthetized rats had shorter QTc intervals in comparison to pentobarbital-treated controls. Since the QT interval is the period during which all ventricular fibers are depolarized [20], halothane-induced QTc interval shortening might be due to the shortened action potential duration that is generally seen in cardiac ventricular fibers which have been exposed to halothane. Additionally, we observed that anesthesia with halothane increased the susceptibility of sham-operated and BDL rats to epinephrine-induced PVC. This finding is in accordance with previous studies suggesting that halothane diminishes the duration of refractory period in cardiac purkinje and ventricular fibers, and thus, increases the possibility of electrical re-entry [21]. It has been demonstrated that halothane-anesthetized rats, cats and dogs are much more sensitive to epinephrine-induced PVC in comparison to sevoflurane- or isoflurane-anesthetized animals [10].

Moreover, a large number of clinical studies have revealed that anesthesia with halothane is associated with a high rate of arrhythmia, especially VT [11]. Twenty-eight days after cholestasis, cirrhosis occurs in a high proportion of BDL rats [17]. Thus, it is very likely that the majority of BDL rats used in our study had impaired liver function during assessment of arrhythmia. In contrast to other inhalational anesthetics, such as enflurane and isoflurane, halothane is extensively metabolized in the liver. Trifluoroacetic acid is one of the main hepatic metabolites of halothane which is known to activate cardiac adenosine tri-phosphate sensitive potassium channels and possesses cardio-protective effects [22]. Based on this fact, impaired hepatic metabolism of halothane and low levels of trifluoroacetic acid might be the reason for increased sensitivity of BDL rats to epinephrine-induced arrhythmia. However, further studies are required to assess this hypothesis.

In essence, the present study shows that BDL rats have longer QTc intervals in comparison to sham-operated animals. Additionally, it was shown that halothane-anesthetized rats had shorter QTc intervals in comparison to pentobarbital-anesthetized animals. Moreover, it was demonstrated that anesthesia with halothane decreases the thresholds for epinephrine-induced PVC, VT and VF in cirrhotic animals. Considering the fact that halothane is still used as a general anesthetic in developing countries [8-9], we suggest that this general anesthetic should be used with extreme caution or, if possible, avoided in patients with cirrhosis.

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