The effects of anesthetic regimen in 90% hepatectomy in rats[1]

Os efeitos do regime anestésico na hepatectomia de 90% em ratos

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ABSTRACT

PURPOSE: To evaluate the influence of the anesthetic regimen on anesthetic recovery, survival, and blood glucose levels following a 90% partial hepatectomy in rats.

METHODS: Thirty adult male Wistar rats were divided into two groups according to their anesthetic regimens: intraperitoneal ketamine and xylazine or inhaled isoflurane. In order to prevent hypoglycemia, glucose was administered intraperitoneally and glucose (20%) was added to the drinking water.

RESULTS: Anesthetic recovery time was longer in the ketamine and xylazine group. The survival rate after 72 hours was lower (log rank=0.0001) in the ketamine and xylazine group (0.0%) than in the isoflurane group (26.7%). The blood glucose after six hours was lower (p=0.017) in the ketamine and xylazine group (63±31.7 mg/dL) than in the isoflurane group (98±21.2 mg/dL). The prolonged anesthesia recovery time associated with ketamine and xylazine decreased the survival rate and blood glucose levels after 90% hepatectomy.

CONCLUSION: Isoflurane anesthesia reduced the recovery time and incidence of hypoglycemia and increased the survival rate in the early hours, providing a therapeutic window that is suitable for experimental studies.

Key words: Liver Failure, Acute. Hepatectomy. Isoflurane. Ketamine. Xylazine. Ratos.

RESUMO

OBJETIVO: Avaliar a influência do regime anestésico sobre a recuperação anestésica, a sobrevida em 72 horas e a glicemia após hepatectomia parcial de 90% em ratos.

MÉTODOS: Trinta ratos Wistar machos adultos foram distribuídos em dois grupos conforme o regime anestésico: combinação de ketamina e xilazina intraperitoneal ou isoflurano inalatório. Para prevenção de hipoglicemia foi administrada glicose intraperitoneal e adicionado glicose (20%) na água de beber.

RESULTADOS: A recuperação anestésica no grupo ketamina e xilazina foi mais prolongada. Durante primeira hora após hepatectomia, nenhum rato anestesiado com ketamina e xilazina despertou. Todos do grupo isoflurano estavam ativos minutos após final da cirurgia.

A sobrevida em 72 horas foi menor (Log rank=0,0001) no grupo ketamina e xilazina (0,0%) que no grupo isoflurano (26,7%). Glicemia em 6 horas do grupo ketamina e xilazina (63±31,7 mg/dL) foi menor (p=0,017) que no grupo isoflurano (98 ±21,2 mg/dL). Prolongado tempo de recuperação anestésica com ketamina e xilazina diminuiu sobrevida e glicemia após hepatectomia 90%.

CONCLUSÃO: Anestesia com isoflurano reduziu tempo de recuperação e hipoglicemia, além de aumentar a sobrevida nas primeiras horas, possibilitando uma janela terapêutica adequada para estudos experimentais.

Descritores: Falência Hepática Aguda. Hepatectomia. Isoflurano. Quetamina. Xilazina. Ratos.

Introduction

Acute liver failure (ALF) is a rare clinical condition that develops unpredictably and is potentially fatal due to massive liver necrosis[1,2]. Despite the advances in clinical management that have been developed in recent decades, the mortality of ALF patients who do not receive transplantation remains high, reaching 70%[1,3]. One of the main factors that limit the success of ALF patient clinical treatment is an incomplete knowledge of its pathophysiology[4].

Animal models are important tools for improving our understanding of the pathogenesis of ALF. The progression of the disease, management of complications and mechanisms involved in liver regeneration are also necessary for the development and evaluation of new therapeutic approaches[5]. The anatomical characteristics of the livers of rats and mice enable varying degrees of liver mass resection, depending on the combination of lobes that are removed[6]. The primary model that is used in studies of liver regeneration after resection includes 70% hepatectomy procedures, whereas 90% hepatectomy procedures constitute an experimental model of ALF[6-8].

The reduction in metabolic capacity following extensive hepatectomy can alter the metabolism of the anesthetics used, which has systemic repercussions and effects on the parameters that are being studied [9]. The aim of the study is to evaluate the effects of two anesthetic regimens on anesthesia recovery, survival rate and blood glucose levels in rats following 90% hepatectomy.

Methods

Thirty adult male Wistar rats were studied, housed in boxes and subjected to 12-hour light and dark cycles at a controlled temperature of 22ºC. The animals were fed a standard diet and received water *ad libitum*. After surgery, they received glucose support via the administration of 20% glucose in their drinking water and standard laboratory chow *ad libitum*. The animals were weighed on a digital scale before the surgical procedure and daily thereafter in order to calculate the doses of ketamine, xylazine and glucose.

Anesthetic regimen

The rats (n=30) were divided into two groups according to their anesthetic regimens: 15 with a ketamine and xylazine combination (KX group) and 15 with isoflurane (ISO group). A combination of ketamine (Cetamin®, Sespo Ltda, Paulinia/SP; 50 mg/kg) and xylazine (Anasedan®, Rhobifarma Ltda, Cotia/SP; 20 mg/kg) was intraperitoneally administered to the KX group. Isoflurane (Forane®, Abbott SA, Buenos Aires; 3%) was inhaled via a calibrated vaporizer to ISO group.

Experimental protocol

After anesthetic induction, rat were placed on an operating table and warmed with a heating pad so as to maintain the animal's body temperature. Partial hepatectomy was performed by a single surgeon, as previously described[10,11]. With the rat in a supine position, a midline abdominal incision was followed by xyphoid cartilage retraction in order to adequately expose the liver and completely liberate of all of the liver ligaments so as to permit an accurate ligation of the pedicle of each lobe. A 90% hepatectomy included the removal of the left lateral (30%), median (40%) and right superior (20%) lobes[8]. Postoperatively, animals were allowed to recover from the anesthesia in cages inside an incubator with a 25ºC ambient temperature for 24 hours. During the first hour after the surgery, each rat was closely observed and its recovery time recorded. One hour post operation, six hours post operation and daily thereafter, each animal was administered 2 mL of a glucose solution (50 mg glucose/100 g of body weight) via an intraperitoneal injection. Six hours and daily thereafter until three days after the hepatectomy, each animal's activity was observed, and a drop of blood was obtained from a tail vein for glucose quantification with a glucometer (Accu-Chek Active®, Roche CO, Germany).

The rats were kept according to the guidelines of the Guide for the Care and Use of Laboratory Animals (Institute for Laboratory Animal Research, 2011) and according to the ethical principles of the Brazilian College on Animal Experimentation (COBEA). The study was approved by Animals Ethics and Research Committee of the Clinics Hospital of Porto Alegre,

Brazil.

Statistical analysis

All blood glucose values are presented as the mean ± standard deviation (SD) for the selected time points in each group. Statistical differences were assessed using Student's t-test. The survival rate was analyzed via the Kaplan-Meier assay. The survival rates of the two groups were compared via the log-rank test. P-values of less than 0.05 were considered statistically significant.

Results

Thirty rats (average body weight, 342.8±43.4g) were divided into two groups and underwent 90% hepatectomies, with a mean surgical time of less than 21 minutes (13.7±2.9 minutes).

One hour post surgery, all of the rats that had been anesthetized with ketamine and xylazine were still under anesthesia. In contrast, in the ISO group all of the animals were awake and active. The recovery time from the isoflurane anesthesia was 16.5±8.1 minutes. Immediately after regaining consciousness, rats began to ingest the provided glucose-water and food. Six hours after the hepatectomy, of the 15 rats in the KX group, only four were alive, with one active, two hypoactive and one still under anesthesia. In the group of animals that underwent hepatectomies under isoflurane, 14 rats were alive and active after six hours.

The survival rate after 72 hours was different between the two groups, being significantly higher (log rank=0.0001) in the ISO group (Figure 1). In the KX group, mortalities after one, six, 24 and 48 hours were 20.0%, 73.3%, 93.3% and 100%, respectively (Table 1). Hepatectomy under isoflurane resulted in a significantly lower mortality after 6 (6.7%), 24 (40%), 48 (66.7%) and 72 hours (73.6%) (Table 1).

TABLE 1 - Survival after hepatectomy depending on the anesthetic regimen in 90% hepatectomized rats.

Time after PH	Ketamine and xylazine group		Isoflurane group		p value
Hours	N (15)	%	N (15)	%	
1	12	80.0	15	100.0	0.0726
6	4	26.7	14	93.3	0.0002
24	1	6.7	9	60.0	0.0001
48	0	0.0	5	33.3	0.0001
72	0	0.0	4	26.7	0.0001

PH: partial hepatectomy

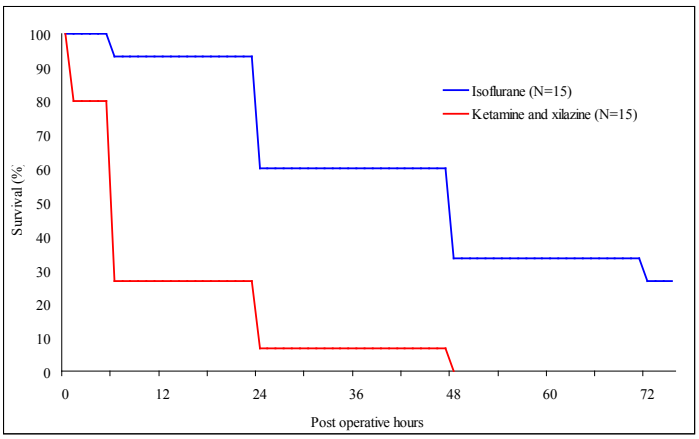

FIGURE 1 - Spontaneous survival depending on the anesthetic regime in 90% hepatectomized rats. Log rank=0.0001.

The blood glucose levels at six hours were significantly lower (p=0.017) in the KX group (63.0±31.7 mg/dL) (Table 2). In the ISO group, the average blood glucose level was 98.6±21.2 mg/dL. After 24 hours, only one rat from KX group was alive (6.7%), and its blood glucose was 48 mg/dL. In the animals that had been anesthetized with isoflurane, the mean blood glucose levels were 65.3±18.0 after 24 hours, 78.8±16.3 after 48 hours and 71.5±20.6 mg/dL after 72 hours (Table 2).

TABLE 2 - Changes in blood glucose (mg/dL) after a 90% hepatectomy in rats depending on the anesthetic regimen.

Time after PH	Ketamine and xylazine group		Isoflurane group		p value
Hours	N	Mean±SD	N	Mean±SD	
6	4	63.0 ±31.7	14	98.6 ±21.2	0.017
24	1	48.0	9	65.3 ±18.0	
48	0	-	5	78.8 ±16.3	
72	0	-	4	71.5 ±20.6	

PH: partial hepatectomy
SD: standard deviation

Discussion

The development of an experimental model for evaluating ALF therapies requires that at least two criteria be considered: high mortality prior to hepatocyte regeneration and an appropriate therapeutic window[12]. The liver regeneration that occurs after hepatectomy rapidly replaces the hepatocytic mass removed, limiting ALF to three or four days[10,13,14]. During this period, in animal models of ALF, there should be a large number of deaths, which is characteristic of ALF; however, the mortality rate in the early hours cannot be excessive, or else it shortens the

therapeutic window[12]. The anesthetic regimen and the prevention of severe hypoglycemia are among the primary determinants of early mortality in animals that have been subjected to partial hepatectomy.

Defining the anesthetic regimen for surgical procedures in animals can be a challenge. In experimental procedures that involve liver resection or transplantation, anesthesia will ideally have a minimal effect on liver function[9]. The combination of intraperitoneal ketamine and xylazine administration is the usual anesthesia for laboratory animals, producing an anesthetic effect for at least one hour[15,16] followed by a two- to four-hour period of sleep[18].

In several recent studies on liver regeneration using partial hepatectomy (70%) models, a combination of ketamine and xylazine was used as the anesthetic regimen[20-22]. The experience of our group (data not shown) has confirmed the possibility of using ketamine and xylazine as an anesthetic for performing partial (70% and 85%) hepatectomy in rats; however, increasing the liver resection to 90% renders this regimen inadequate. Ketamine and xylazine are extensively metabolized by liver mitochondrial enzymes, and their metabolites are excreted in the urine[9,21]. In our study, the decrease in the remaining liver mass probably led to a reduction in the hepatic metabolic capacity for anesthetics and carbohydrates. The deepening of the anesthetic plane, causing cardiorespiratory depression and severe hypoglycemia, were possibly the determinants of death within the first six hours in the group of rats that had been anesthetized with ketamine and xylazine. In contrast, the shorter recovery from anesthesia with isoflurane permitted the animals to awaken within minutes. In the evaluation during the first hour after surgery, all of the rats were awake and active.

Isoflurane is an inhalational halogenated methyl ethyl ether anesthetic with a high molecular stability. Thus, less than 0.2% of the inhaled dose is metabolized because it is almost completely eliminated in the exhaled air[19,20]. Its induction and recovery times are very fast, and its depth can be adjusted easily and quickly[18]. It is considered to be the ideal anesthetic for studies of metabolism and toxicity due to its minimal systemic metabolism, the small effect it has on liver enzymes and the reduced potential risk of liver and kidney damage that arise from its use[18-21]; however, isoflurane anesthesia has been used in a few studies of models of extended hepatectomy in murine rodents[8,17-19]. The disadvantages of isoflurane are related to the need for enhanced anesthetic monitoring, particularly during induction, by trained personnel and with specialized equipment, which results in increased research costs[16].

The observed increased liver resection and reduced survival rate[10,11,23] were accompanied by a reduction in blood glucose levels in the early hours[10]. The liver is the key organ in the production, storage and distribution of nutrients and energy. The loss of hepatocytic mass during ALF is accompanied by reductions in glycogen stores, the capacity for gluconeogenesis and the ability to maintain blood glucose during fasting[24]. The increase in early mortality that is associated with larger resections can be partially reduced by avoiding severe early hypoglycemia by administering 20% glucose in the drinking water[11,25,26]. In our study, the prolonged recovery time of ketamine and xylazine anesthesia rendered this procedure ineffective because only 20% of the animals had awakened from anesthesia six hours after the end of the procedure. In the group that had been anesthetized with isoflurane, the intake of water with glucose was possible immediately after the surgery, there was no hypoglycemia in the first hours after surgery and the survival rate after six hours was high (93.3%).

Supplementing glucose via drinking water may be insufficient for preventing severe hypoglycemia in hepatectomies of greater than 90%[11]. The strict monitoring of blood glucose and correcting hypoglycemia via the intraperitoneal administration of 5% glucose (in addition to adding it to the water) in rats with 90% resections can increase the survival rate to 80%[13,27]. In our study, the prophylactic administration of intraperitoneal glucose to rats that underwent 90% hepatectomy with ketamine and xylazine did not prevent hypoglycemia across the entire monitoring period and did not impede the progression to death in 73.3% of the animals.

Although ketamine and xylazine present technical and economic advantages in comparison to isoflurane[9] and are widely used in experiments with rodents[15,16], their pharmacological characteristics limit their use in models of 90% hepatectomy. Inhalation anesthesia, particularly isoflurane, demands minimal hepatic metabolism, enables better anesthetic control and should be the preferred anesthetic in murine models of ALF.

Conclusion

Isoflurane anesthesia reduced the recovery time and incidence of hypoglycemia and increased the survival rate in the early hours, providing a therapeutic window that is suitable for experimental studies.

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