Isoflurane: An Ideal Anesthetic for Rodent Orthotopic Liver Transplantation Surgery?

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ABSTRACT

Purpose. Because the choice of anesthetic affects the rodent orthotopic liver transplantation (OLT) model, we compared the effects of isoflurane, ketamine, chloral hydrate, and pentobarbital on the OLT model.

Basic Procedures. OLT was performed using the two-cuff technique. Two hundred male rats were randomly divided into five groups: control, isoflurane, ketamine, chloral hydrate, and pentobarbital groups. Rectal temperatures, respiratory rates, arterial blood values (pH, $PaCO_2$, PaO_2, and $SatO_2$), liver function tests and histopathology, recovery times, and anhepatic stage mortality rates were assessed.

Main Findings. Compared with controls, respiratory rates decreased by 20% in the isoflurane group, and decreased by 40%-50% in the ketamine, chloral hydrate, and pentobarbital groups. The PaO_2, $SatO_2$, and pH levels in the ketamine, chloral hydrate, and pentobarbital groups were significantly lower than those in the isoflurane and control groups ($P < .05$). Only the pentobarbital group displayed significant liver histopathologic changes along with significantly higher levels of serum alanine aminotransferase and total bilirubin, but a significantly lower level of serum albumin, compared with the control group ($P < .05$). The isoflurane group had a 0% anhepatic stage mortality rate compared with rates of 30%-40% in the other anesthetic groups.

Principal Conclusions. Isoflurane should be the preferred anesthetic for rodent OLT surgery due to its minimal respiratory and hepatic physiological effects as well as its low anhepatic phase mortality rate. Secondary to isoflurane, ketamine and chloral hydrate may be administered as donor anesthetics. Pentobarbital use should be avoided entirely in rodent OLT surgery due to its significant hepatotoxic effects.

LEE ET AL initially constructed the orthotopic liver transplantation (OLT) model in 1973 [1]. Since then, the OLT model has been incrementally modified and improved. As anesthetics have been shown to affect hepatic physiology in rats [2,3], the choice of anesthetic has impacted the development of the OLT model and its results. Anesthetics may also play a role in the anhepatic stage, which has a high mortality rate in the OLT model.

In this study, we compared the effects of four common anesthetics–isoflurane, ketamine, chloral hydrate, and pentobarbital–on the OLT model. We recorded physiological indices (respiratory rate, rectal temperature, and blood analysis), liver indices (enzymatic and histopathologic changes), anhepatic stage mortality rates, safe dose of each anesthetic, induction and maintenance time after surgery, postoperative recovery time, and preanesthetic medication dose. A better understanding of the effects of different

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Table 1. Power Analysis for the Statistical Analysis Comparing Indices From the Five Comparison Groups

Index	Comparison	X1	X2	S1	S2	Sc	Zβ	β	1-β
Rectal temperature	ISO vs KET	35.420	32.440	1.610	0.840	1.649	8.419	0.000	1.000
Rectal temperature	ISO vs CHL	35.420	33.560	1.610	2.420	4.224	2.087	0.018	0.982
Rectal temperature	ISO vs PEN	35.420	32.210	1.610	1.860	3.026	6.293	0.000	1.000
Respiratory rate	CON vs ISO	105.260	84.560	5.230	4.210	22.539	17.539	0.000	1.000
PaO_2	CON vs ISO	15.210	14.350	1.280	2.140	3.109	0.221	0.413	0.587*
$PaCO_2$	CON vs ISO	6.470	6.980	1.280	0.870	1.198	0.124	0.452	0.548*
$PaCO_2$	CON vs KET	6.470	7.870	1.280	1.560	2.036	2.428	0.008	0.992
$PaCO_2$	CON vs CHL	6.470	7.120	1.280	1.640	2.164	0.016	0.492	0.508*
$PaCO_2$	CON vs PEN	6.470	8.020	1.280	1.450	1.870	3.108	0.001	0.999
$PaCO_2$	ISO vs KET	6.980	7.870	0.870	1.560	1.595	1.191	0.117	0.883
$PaCO_2$	ISO vs CHL	6.980	7.120	0.870	1.640	1.723	−1.483	0.069	0.069*
$PaCO_2$	ISO vs PEN	6.980	8.020	0.870	1.450	1.430	1.930	0.027	0.973
pH	CON vs ISO	7.410	7.390	0.120	0.480	0.122	−1.704	0.045	0.045*
$SatO_2$	CON vs ISO	98.120	96.790	2.140	1.560	3.507	1.216	0.111	0.889
ALT	CON vs ISO	23.170	24.190	2.350	0.360	2.826	0.753	0.227	0.773
ALT	CON vs KET	23.170	22.470	2.350	1.680	4.172	−0.427	0.334	0.334*
ALT	CON vs CHL	23.170	25.400	2.350	2.560	6.038	2.099	0.018	0.982
ALT	ISO vs KET	24.190	22.470	0.360	1.680	1.476	4.371	0.000	1.000
ALT	ISO vs CHL	24.190	25.410	0.360	2.560	3.342	1.025	0.154	0.846
TBIL	CON vs ISO	3.560	5.880	0.420	1.570	1.321	7.068	0.000	1.000
TBIL	CON vs KET	3.560	4.150	0.420	0.230	0.115	5.833	0.000	1.000
TBIL	CON vs CHL	3.560	4.490	0.420	2.280	2.687	0.577	0.281	0.719*
TBIL	ISO vs KET	5.880	4.150	1.570	0.230	1.259	4.935	0.000	1.000
TBIL	ISO vs CHL	5.880	4.490	1.570	2.280	3.832	1.216	0.111	0.889

*1-β values of <0.75. β is the probability of making a type II error. The measure of statistical power 1-β represents the likelihood that an experiment will detect an effect when there is an effect to be detected. Therefore, as 1-β approaches unity, the probability of a type II error decreases.

anesthetics should enable researchers to select the most appropriate anesthetic for OLT model construction.

METHODS

Subjects and Materials

The protocols of this study were examined and approved by the Chongqing Medical University Ethics Committee on Animal Experimentation. All efforts were made to minimize animal suffering and to reduce the number of animals used. Two hundred male Sprague Dawley rats, weighing 200 to 300 g each, were supplied by Chongqing Medical University (Chongqing, China). The donor rats were slightly heavier than recipient rats. All rats were fasted 12 hours prior to surgery but had ad libitum access to water.

Four anesthetics (isoflurane, ketamine, chloral hydrate, and pentobarbital) were purchased from Sigma Chemical Co. (St. Louis, Mo, United States). The microsurgical equipment package, automatic blood gas analyzer, and digital electronic thermometer were purchased from Shanghai Medical Instruments (Shanghai, China).

OLT Model Construction and Experimental Groupings

Kamada's two-cuff technique was used to construct the OLT model (ie, portal vein and hepatic inferior vena cava anastomosis using the casing method and hepatic inferior vena cava anastomosis using the suture method). OLT model development consisted of the following four steps as reported previously [4]: (1) vascular cuff and biliary stent preparation; (2) donor liver preparation and trimming; (3) recipient preparation; and (4) graft implantation. The two hundred male rats were randomly separated into the following five groups (n = 40 per group): control (CON) group, isoflurane (ISO) group, ketamine (KET) group, chloral hydrate (CHL) group, and pentobarbital (PEN) group. Donor and recipient rats in each group were provided the same anesthetic.

Recording of Physiological Indices

Respiratory rates and rectal temperatures 5 hours postsurgery were determined in all five groups. Arterial blood sampled from the abdominal aorta was tested (pH, $PaCO_2$, PaO_2, and $SatO_2$) in all five groups. Venous blood sampled from the inferior vena cava was

Table 2. Anesthetic Characteristics Across the Experimental Groups

Index	Group			
	ISO	KET	CHL	PEN
Premedication	0.03 mg IM atropine	None	None	None
Anesthetic method	Inhalation	IP injection	IP injection	IP injection
Anesthetic dose	3% in induction phase 2% in maintenance phase	120 mg/kg	400 mg/kg	45 mg/kg
Induction time (min)	2–4	2–3	2–3	1–2

Abbreviations: IM, intramuscular; IP, intraperitoneal.