

Influence of S(+)-ketamine analgesia in renal intraoperative ischemia. Histological study in rats¹

Influência da analgesia com S(+)-cetamina em isquemia renal intra-operatória. Estudo histológico em ratos

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ABSTRACT

Purpose: To study in rats the effect of S(+)-ketamine on the renal histology after intraoperative hemorrhage. **Methods:** Twenty male Wistar rats, anesthetized with sodium pentobarbital, were randomly divided in 2 groups: G1 - control (n=10) and G2 - S(+)-ketamine (n=10), both submitted to arterial hemorrhage of 30% of volemia in 3 moments (10% each 10 min) 60 min after anesthesia. G2 received S(+)-ketamine, 15 mg. kg⁻¹, i.m., 5 min after anesthesia and 55 min before the 1st hemorrhage moment (M1). Medium arterial pressure (MAP), rectal temperature (T) and heart rate were monitored. The animals were sacrificed in M4, 30 min after the 3rd hemorrhage moment (M3) and the kidneys and blood collected from hemorrhage were utilized for histological study and hematocrit (Ht) determination. **Results:** There were significant reduction of MAP, T, and Ht. The histological study verified G1 = G2 for tubular dilation, congestion, and necrosis. The total score addition were significantly different and G2 > G1. **Conclusion:** Hemorrhage and hypotension determined changes in kidney histology. The rise in catecholamine blood concentration probably was the cause of S(+)-ketamine-induced higher score of histological changes.

Key words: Anesthesia and Analgesia. Pentobarbital. Kidney. Ischemia. Hemorrhage. Hypotension. Hypovolemia.

RESUMO

Objetivo: Investigar, em ratos, o efeito da S(+)-cetamina na histologia renal após hemorragia intra-operatória. **Métodos:** Vinte ratos Wistar machos, anestesiados com pentobarbital sódico, foram divididos, aleatoriamente, em 2 grupos: G1 – controle (n=10) e G2 - S(+)-cetamina (n=10), submetidos a hemorragia de 30% da volemia em 3 momentos (10% a cada 10 min) 60 min após anestesia. G2 recebeu S(+)-cetamina, 15 mg. kg⁻¹, i.m., 5 min após anestesia e 55 min antes do 1.^o momento de hemorragia (M1). Foram monitorizadas a pressão arterial média (PAM), temperatura retal (T) e frequência cardíaca. Os animais foram sacrificados (M4) 30 min após o 3.^o momento de hemorragia (M3). Os rins e o sangue das hemorragias foram utilizados para estudo histológico e do hematócrito (Ht). **Resultados:** Houve redução significativa da PAM, T e Ht. Na histologia, G1=G2 na dilatação tubular, congestão e necrose. A soma total dos escores foi significativamente diferente e G2>G1. **Conclusão:** Hemorragia e hipotensão determinaram alterações na histologia renal. O aumento da concentração sanguínea de catecolaminas provavelmente determinou escores mais altos de alterações histológicas com o uso de S(+)-cetamina.

Descritores: Anestesia e Analgesia. Pentobarbital. Isquemia Renal. Hemorragia. Hipotensão. Hipovolemia.

Introduction

S(+)-ketamine, a ketamine S(+)-enantiomer, is a noncompetitive N--methyl-D-aspartate (NMDA) receptor ion channel blocker. It is a short acting anesthetic used as an inducing agent or during surgical and short diagnostic procedures, providing rapid dissociative anesthesia followed by rapid recovery.

Anesthetics should be chosen to protect renal function for those patients with coexisting renal disease, under some type of surgical procedures or with risk factors for

development of perioperative renal failure, but the ideal anesthetic has not been developed to protect renal function. So, these patients are predisposed to perioperative morbidity and mortality.

Ketamine inhibits cytokines-induced mesangial cells (MC) proliferation. These cells regulate glomerular blood flow. Many cytokines and vasoactive hormones, such as angiotensin II, have been identified in the modulation of MC proliferation¹⁻³. Angiotensin II affects acute renal hemodynamics under pathological conditions and induces cell proliferation in a cultured murine MC line³ and stimulates

the proliferation of cultured human fetal MC⁴. But ketamine also has inhibitory effects on the angiotensin II-induced MC proliferation, and so, with renal function under some clinical conditions.

In the kidney, the early compensatory response to moderate to severe hemorrhage from surgery is vasoconstriction (with elevated activity of the renin-angiotensin system or renal sympathetic nerves) in order to support the blood pressure and maintain blood flow to more vital organs⁵. The kidney releases, then, prostaglandins, vasodilators that play an important role in the preservation of its function. They act to maintain glomerular filtration rate and renal blood flow by modulating the effects of vasoconstrictors such as angiotensin II or norepinephrine on the renal vasculature⁶. But as S(+)-ketamine also has sympathomimetic action, the aim of this study was to determine whether this agent alters renal histology during hypotension due to acute hemorrhage, with hypovolemia.

Methods

The study was approved by the Animal Care and Ethics Committee of our College of Medicine. Twenty adult male Wistar rats (>250 g) had anesthesia induced with sodium pentobarbital - 50 mg. kg⁻¹ by intraperitoneal injection. Two groups of 10 rats were studied after anesthesia induction: the control group (G1) which underwent arterial hemorrhage and the S(+)-ketamine group (G2) which underwent arterial hemorrhage and received S(+)-ketamine, 15 mg. kg⁻¹, by intramuscular injection, immediately after the anesthesia installation. The rats were maintained with spontaneous ventilation and supplemental oxygen (1L. min⁻¹) via a mask. Rectal temperature (T) was monitored with alcohol thermometer. Surgery comprised a longitudinal incision in the neck and blunt dissection to expose and cannulate with 24 GA venocath: 1) internal jugular vein in order to maintain administration of Ringer lactate solution, infused at a rate of 0.5 mL. kg⁻¹. h⁻¹; 2) carotid artery to allow blood pressure and heart rate (HR) to be monitored via a transducer and recorder (Datex Engstrom, Finland). Sixty minutes after the anesthesia administration in G1 and G2 (about 55 minutes after S(+)-ketamine administration), 30% of the volemia were collected through the carotid artery, in the animals of both groups, in three moments (M1, M2, and M3) with 10 minutes interval. The volemia of the animals was calculated as 6% of bodyweight⁷. In each moment, medium arterial pressure (MAP), T, and HR were recorded. The arterial blood collected was utilized as samples for analysis of hematocrit (Ht) (microhematocrit method, Centremicro, Fanem, Brazil). After each moment of hemorrhage, if the MAP was lower than 80 mmHg, blood loss could be replaced by Ringer lactate solution, 1.6 mL. kg⁻¹. When ceased the periods of blood loss, the rats remained anesthetized during 30 min, when they were sacrificed with intravenous overdose of pentobarbital (moment M4). The kidneys of animals have gone for histological analysis and so the fresh tissue was fixed in Duboscq-Brasil solution, during 24 hours. After that, they remained in 70% alcohol. A full cross-sectional face of each kidney was processed and stained with hematoxylin and eosin stains. The kidneys were identified by a study number,

and the code was not known to the research pathologist who made histological assessments and scores. The kidneys were assessed on three criteria: 1) evidence of dilated tubules, 2) evidence of renal congestion, and 3) evidence of tubular necrosis. For the diagnosis of tubular necrosis, identification of necrotic nuclear and cytoplasmic debris within proximal tubular lumens was required. Dilated tubules, renal congestion, and necrotic tubules were scored on the scale 0 to 3 (0 = no evidence of change from normal, 3 = the most severe in the spectrum of changes noted).

Statistical analysis

MAP and T were studied in M1, M2, M3, and M4. Ht was studied in M1 and M4. For each attributes the Profile Analysis was performed⁹. The histological data were also analyzed (Mann-Whitney proof). Differences were considered significant at the 0.05 level ($p < 0.05$).

Results

Data presented in Table 1 represent values of MAP and T in M1, M2, M3, and M4. Hematocrit data are presented in M1 and M4. There was significant reduction of Ht, MAP and T during the experiment period, but G1 didn't differ statistically from G2. HR results are not presented because after hemorrhage some animals had values above the maximum value recorded by Datex Engstrom (heart rate above 250 beats per minute).

Kidney sections of both groups showed some cortical histological abnormalities (Table 2) as dilated tubules (Figure 1) in all kidneys except one in the control group. This difference was not statistically significant. Parenchymal congestion of variable degree was observed more in the S(+)-ketamine group (5 animals) than in the control group (2 animals) and that difference wasn't statistically significant. Necrosis was seen in the kidneys of 9 animals of each group (G1 and G2) (Figure 2). When the addition of the scores of all histological changes was obtained, the S(+)-ketamine group (G2) showed statistically significant higher scores than the control group (G1).

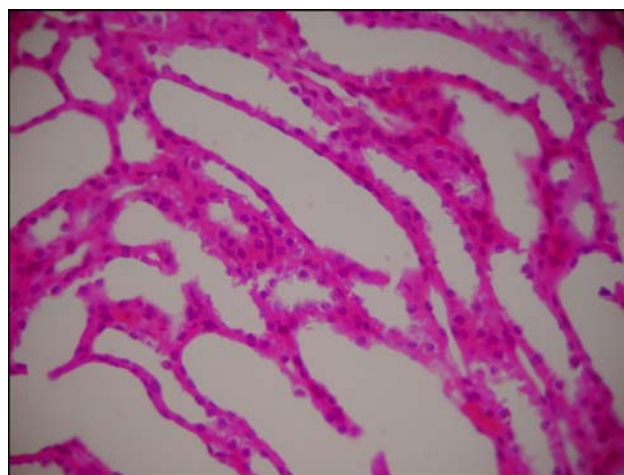


FIGURE 1 - Hemorrhage of 30% of volemia produced tubular dilation in the kidney rat. G1 = G2

TABLE 1 - Medium arterial pressure (MAP), rectal temperature (T) and hematocrit (Ht). Values in Means \pm S.E.M

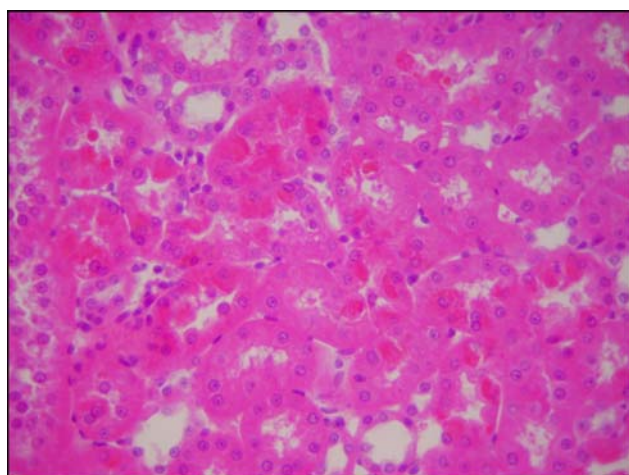
Moments Parameters	M1	M2	M3	M4	Statistics
MAP (mmHg)	90.95 \pm 22.01	74.95 \pm 15.61	59.10 \pm 17.77	64.70 \pm 25.78	In all moments: G1 = G2
	107.10 \pm 14.62	81.25 \pm 20.03	59.10 \pm 17.77	68.40 \pm 27.14	In G1 ⁸ M1 > M2 > (M3 = M4) In G2 ⁸ M1 > (M3 = M4)
T (°C)	34.1 \pm 0.84	33.7 \pm 0.91	33.2 \pm 0.95	32.4 \pm 1.21	In all moments G1 = G2
	33.5 \pm 0.71	33.2 \pm 0.83	32.8 \pm 0.88	32.0 \pm 0.87	In G1 and G2 ⁸ (M1 = M2) > M4
Ht (%)	41.9 \pm 3.4			37.6 \pm 4.7	In all moments G1 = G2
	40.5 \pm 5.7			33.3 \pm 6.5	In G1 and G2 ⁸ M1 > M4

* p < 0,05

TABLE 2 - Histological changes. Median (Md) and mean (x) values

Histological Changes Groups	Tubular Dilation (D)	Congestion (C)	Necrosis (N)	Score addition (D + C + N)
G1	Md = 2.0 x = 1.6	Md = zero x = 0.3	Md = 2.0 x = 1.5	Md = 4.0 x = 3.4
G2	Md = 3.0 x = 2.3	Md = 0.5 x = 0.7	Md = 2.0 x = 1.9	Md = 5.5 x = 4.9
Statistics	G1 = G2	G1 = G2	G1 = G2	G1 < G2*

* p < 0,05

**FIGURE 2** - Hemorrhage of 30% of volemia produced tubular necrosis in the kidney rat. G1 = G2

Discussion

The cardiovascular changes produced by ketamine are well documented also for the pure enantiomers. Blood pressure and heart rate are increased during ketamine anesthesia in humans and experimental animals. These sympathomimetic actions are produced primarily by direct stimulation of central nervous system. There is also an inhibition of peripheral catecholamines reuptake⁷. The rats of S(+)-ketamine group had higher values of blood pressure than control group, which received only sodium pentobarbital (Table 1).

Sodium pentobarbital, when given orally, do not produce significant overt cardiovascular effects except for a slight decrease in blood pressure and heart rate such as occurs in normal sleep. Apparently, a decrease in cardiac output usually is sufficient to offset an increase in total calculated peripheral resistance, which sometimes is accompanied by an increase in heart rate. In presence of hypovolemic state the reflexes are already operating maximally, and barbiturates can cause an exaggerated fall in blood pressure⁷. Our animals received the barbiturate by intraperitoneal route, which has rapid diffusion. So, a higher blood concentration and a more accentuated response were waited (Table 1).

Among the determinants of renal perfusion in the clinical situation are cardiac output, arterial blood pressure, and intravascular volume state. Renal blood flow is dependent on renal perfusion pressure, so systemic hypotension should be promptly and aggressively corrected to avoid the development of ATN. In the normal mammalian kidney, loss of autoregulation generally occurs at a MAP of 75-80 mmHg⁸. A blood pressure less than 60 mmHg is likely to be inadequate. The rats of this study, both in G1 and G2, remained with MAP values below 60 mmHg after the last hemorrhage moment and below 70 mmHg 30 minutes after that, without correction of volemia. Severe renal hypoperfusion, if not corrected, will ultimately lead to ischemic ATN⁸.

The oxygen tension in the renal cortex is about 50 mmHg higher than that of the inner medulla, as a result of heterogeneity of flow and oxygen requirement⁹. Medullary oxygenation is strictly balanced by a series of control mechanisms, which match regional oxygen supply and consumption. Failure of these controls renders the outer medullary region susceptible to acute or repeated episodes of hypoxic injury, which may lead to acute tubular necrosis (ATN) especially of the thick ascending limbs (the mTAL regions). In these regions ATN can be induced by as little as a 40-50% decreases in renal blood flow⁹.

In the kidney, any increase in circulating catecholamines (especially epinephrine) will cause vasoconstriction via the α_1 -receptors and activation of the rennin-angiotensin system. As the result, despite a normal renal blood flow, intramedullary ischemia may occur, especially in the region of the mTAL, where the sodium-potassium ATPase enzymes are very sensitive to the effects of ischemia. These increases in sympathomimetic hormones lead to renal cortical vasoconstriction, which is a compensatory attempt by the body to redistribute blood flow to the renal medulla, but in fact, it causes ischemia⁹. Ketamine exhibits no stereoselectivity on norepinephrine and serotonin transporters. Its enantiomer S(+)-ketamine is about three to four times more potent as an anesthetic than R(-)-ketamine, but an equipotent dose of S(+)-ketamine alone may show weaker norepinephrine and serotonin uptake inhibition than R(-)-ketamine at anesthetic effective doses¹⁰. Our results show the same type of histological changes for both groups, but the S(+)-ketamine group had significantly higher addition of scores. The sympathomimetic action of S(+)-ketamine probably is the responsible for this performance. Decreased renal perfusion, which causes include hypovolemia, like we sought to determine in our animals, determines acute renal failure (ARF). Intrarenal ARF that is not the result of primary vascular, glomerular or interstitial disorders has been referred to as acute tubular necrosis (ATN). The most common cause of ATN is pre-renal ARF that is not treated in a timely manner^{11,12}. Prolonged ischemia, after hypovolemia, for instance, causes cell death both through necrosis and through sublethal cell damage which triggers apoptosis of tubular cells, resulting in ATN^{12,13}. Apoptosis, a programmed cell death, is characterized by progressive cell shrinkage with nuclear condensation and fragmentation, and rapid clearance by phagocytosis¹⁴. Due to the rapid phagocytosis, it is not

associated with release of reactive mediators from the affected cells. Necrosis is the result of severe cellular ATP depletion, leading to biochemical collapse of the cell and release of cytosolic inflammatory mediators¹⁵. In many models necrosis and apoptosis may coexist and their proportion is often determined by the severity of injury. Otherwise, a study of human tubular cells in cold storage (4°C) provided evidence that very low temperature per se does not result in apoptosis, but is primarily necrotic¹⁶. Moderate hypothermia (28°C) during cardiopulmonary bypass, by stimulating IL10 synthesis and suppressing TNF α production during this period of cardiac operations, might provide organ protection¹⁷. The temperature reached by our animals was of light to moderate hypothermia (31-32°C). So, further studies are necessary to answer questions about the role of the body temperature to protect or not the kidney from ischemic injury.

The development of ATN is most often multifactorial in origin, involving both ischemic and cytotoxic injury to proximal tubular cells. ATN may therefore be caused by one or a combination of insults, including hypoperfusion, the patient risk factors and the type of surgical procedure also playing significant role¹⁴.

Summarizing, the changes observed in kidney histology probably were determined by hemorrhage and hypotension. We conclude that if S(+)-ketamine--induced higher score of histological changes in the rat kidney occurred, the rise in catecholamine blood concentration probably is the cause. Otherwise, further studies are necessary to completely answer this question.

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