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# Ketamine improves survival in severe burn injury in rats via the expression of heat shock protein 70☆

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ABSTRACT

Ketamine, a general anesthetic, has been shown to elicit the heat-shock response (HSR) in some of the animal models. We examined whether ketamine improves survival in severe burn injury in rats via the expression of heat shock protein 70. 124 male Wistar rats were randomly divided into three groups: a control group (group C, $n=20$), burned group (group B, $n=52$), and burned + ketamine group (group K, $n=52$). The rats in groups B and K had full-thickness burns of 30% of their total body surface. The rats in group K were treated with ketamine (40 mg/kg, i.m.) 15 min after injury, and those in group B were injected with saline at the same volume. After the rats were euthanized, HSP70 expression in myocardium and brain samples was examined by Western blot analysis. Survival status was evaluated for the rats not euthanized. After 10 days, survival rate of rats in group K was higher than that of group B (70% versus 30%). Western blot analyses revealed that HSP70 protein expression in myocardium in response to ketamine administration is stronger than that in response to saline administration at 3 h (158% *versus* 65%) and 6 h (165% versus 68%). Compared with that in group B, ketamine strongly increased HSP70 protein expression level in cerebral tissue at 3 h and 6 h (79% *versus* 51%, at 3 h; 123% *versus* 98%, at 6 h). We concluded that ketamine therapy improves survival in severe burn injury via the expression of heat shock protein 70 in myocardial and cerebral tissues.



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## La ketamina mejora la sobrevida en lesión por quemadura severa en ratas, a través de la expresión de la proteína de choque 70

*Palabras clave:*
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Choque
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RESUMEN

Se ha demostrado que la ketamina, una anestésico general, produce una respuesta de choque térmico (HSR) en algunos modelos en animales. Examinamos si la ketamina mejora la sobrevida en lesión por quemadura severa en ratas, a través de la expresión de la proteína de choque 70. Un total de 124 ratas Wistar machos se dividieron aleatoriamente en 3 grupos: un grupo de control (grupo C, n = 20), un grupo quemado (grupo B, n = 52) y un grupo quemado + ketamina (grupo K, n = 52). Las ratas de los grupos B y K presentaban quemaduras de espesor completo en el 30% del total de su superficie corporal. Las ratas del grupo K se trataron con ketamina (40 mg/kg, i.m.) a los 15 min después de la lesión y las del grupo B se inyectaron con igual volumen de solución salina. Luego de practicar la eutanasia a las ratas, se examinó la expresión de HSP70 en muestras del miocardio y del cerebro con análisis Western blot. En las ratas que no se sacrificaron se evaluó el estado de sobrevida. Luego de 10 días, la tasa de sobrevida en las ratas del grupo K era superior a las del grupo B (70% versus 30%). Los análisis Western blot mostraron que la expresión de proteína HSP70 en el miocardio en respuesta a la administración de ketamina es más fuerte que en respuesta a la administración de solución salina a las 3 h (158% versus 65%) y a las 6 h (165% versus 68%). En comparación con el grupo B, la ketamina aumentó marcadamente el nivel de expresión de la proteína HSP70 en tejido cerebral a las 3 h y a las 6 h (79% versus 51% a las 3 h; 123% versus 98% a las 6 h). Concluimos que el tratamiento con ketamina mejora la sobrevida en lesión por quemadura severa, mediante la expresión de la proteína de choque 70 en los tejidos del miocardio y del cerebro.



## Introduction

The heat shock response (HSR) is a cellular defense system that is highly conserved throughout evolution.[1,2] It can be found in a wide spectrum of organisms ranging from prokaryotes to human beings. The underlying molecular mechanism is based on the ability of the transcription factor heat shock factor (HSF)-1 to display inducible DNA binding activity to the consensus heat shock element (HSE) and involves multiple steps, including nuclear translocation, oligomerization, and inducible serine phosphorylation of HSF-1, the latter being an important determinant of the transactivating potency of HSF-1.[3] The HSR is characterized by the expression of heat shock proteins (hsps), which are synthesized by cells in response to heat, hence the name, as well as to various other stressful stimuli.[4,5] Expression of hsps, which is classified by their function and size, has been shown to protect cells from a broad range of cellular stressors such as hypoxia, oxygen radicals, endotoxin, infections, and fever.[6] The cytoprotective capacity of heat shock proteins may be attributed in part to their ability to stabilize intracellular protein structures, which allows resumption of normal cellular and physiological activities by cells and organisms facing life-threatening insults.[7]

Ketamine, a general anesthetic, has been shown to elicit the heat-shock response (HSR) in some of the animal models: it has increased the cellular concentration of heat-shock protein 70 (HSP70) in the olfactory mucosa[8] and in the brain cortex of rats.[9] According to other authors,[10] swine anesthetized with ketamine and pentobarbital have shown increased expression of HSP70 in the liver. The protective potential of HSR in burns has been previously demonstrated; rats preconditioned by heat had reduced mortality after severe burns, when compared to unburned animals.[11] Therefore, the hypothesis of this study was to determine whether ketamine would also be able to improve survival in severely burnt rats by inducing the expression of heat shock protein 70 in myocardial and cerebral tissues.

## Methods

### *Animal preparation*

The experimental protocol used for this study was approved by the Animal Experimental Center of Shandong Provincial Hospital and was performed in accordance with the Regulations for the Administration of Affairs Concerning Experimental Animals (the State Council, China, 1988). Rats were housed in individual cages in a temperature-controlled room with alternating 12 h light–dark cycles and were acclimated to the laboratory for 2 weeks before the study. Food was removed 12 h prior to the study, but all the animals were allowed free access to water.

### *Burn injury protocol*

Rats were anesthetized with inhaled halothane 4%. The dorsum of the rats were shaved and immersed in water at 92 °C

for 20 s, which resulted in a full-thickness burn involving 30% of the total body surface area. The hemodynamic effect of edema formation and serum exudation at the burn site was minimized by administration of 5 mL of normal saline IP immediately after burn injury. In preliminary studies, histopathologic examination of the burned area indicated that this injury model produces a reproducible, full-thickness skin burn, generally with no direct injury to underlying internal organs.

### *Sampling procedure*

Male Wistar rats ($n = 124$) weighing 250–350 g were randomly allocated to 3 groups: control group (group C) ($n = 20$); burned group (group B) ($n = 52$), and burned + ketamine group (group K) ($n = 52$). Rats in group K were intramuscularly injected with katemine (40 mg/kg) after 15 min of burn injury, while those in group B were injected with saline of the same volume. Eight rats, each from groups K and B, were killed at 3 h, 6 h, 12 h and 24 h after administration. Myocardium and brain samples were collected from all groups for HSP70 detection by Western blot analysis. Survival status of the remaining mice in each group was evaluated at 3 h, 6 h, 12 h, 24 h, 48 h, 96 h, 192 h and 240 h.

### *HSP70 detection (Western blot analysis)*

Myocardium and brain tissue were thawed, finely minced, weighed, rinsed with cold phosphate-buffered saline, and homogenized on ice. The homogenates were centrifuged at 20,000 rpm at 4 °C for 30 min. Protein samples were boiled for 10 min at 100 °C; they were then separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and transferred to a nitrocellulose membrane. After blocking with 5% dry nonfat milk in Tris-buffered saline with 0.1% Tween 20 (TBS/T) for 1 h at room temperature, membranes were washed 3 times for 10 min in TBS/T and then incubated overnight at 4 °C with anti-HSP70 monoclonal antibody conjugated to alkaline phosphatase (SPA-810AP, StressGen) at 1:1000 concentration. Membranes were washed 3 times in TBS/T for 10 min and incubated with appropriate peroxidase-conjugated secondary antibodies. After another 3 washes with TBS/T for 10 min, membranes were reacted with the enhanced chemiluminescence system and then exposed onto films. Protein levels were quantified by scanning densitometry using Quantity One V4.62 (Bio-Rad).

### *Statistical analysis*

All values are expressed as mean ± standard error of mean (SEM). Statistical significance in differences between groups was assessed by one-way analysis of variance and that with groups was by unpaired Student's *t*-test using SPSS 11.0 software. The Fisher's exact test was applied to survival data. $P < 0.05$ was considered statistically significant.

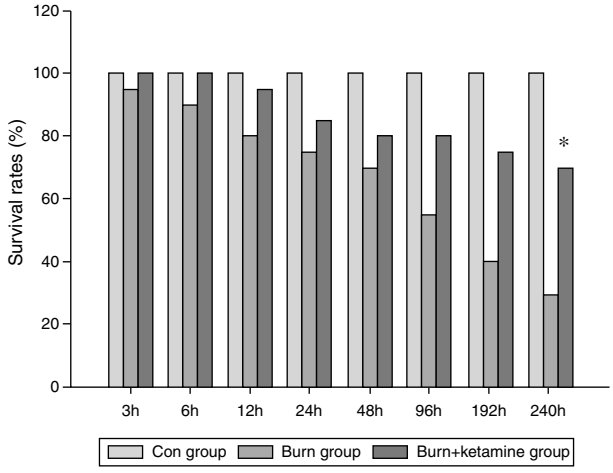


**Fig. 1 – Comparison of survival rates in control group (Con), Burn group and Burn + ketamine group.**
***P < 0.05 *versus* Burn group.**

## Results

### *Survival status*

No animals in group C died within the first 240 h. The survival rate of severely burned rats in group B was 95% (19 of 20 rats) at 3 h, 90% (18/20) at 6 h, 80% (16/20) at 12 h, 75% (15/20) at 24 h, 70% (14/20) at 48 h, 55% (11/20) at 96 h, 40% (8/20) at 192 h and 30% (6/20) at 240 h. Survival in group K was 100% (20/20) at 3 h, 100% (20/20 animals) at 6 h, 95% (19/20) at 12 h, 85% (17/20) at 24 h, 80% (16/20) at 48 h, 80% (16/20) at 96 h, 75% (15/20) at 192 h and 70% (14/20) at 240 h. The differences of survival rates between group K and group B were statistically significant at 240 h ($P < 0.05$) (seen in Fig. 1).

### *HSP detection*

Western blot analyses revealed that HSP70 protein expression in myocardium in response to ketamine administration is stronger than that in response to saline administration at 3 h (158% versus 65%, $P = 0.008$) and 6 h (165% versus 68%, $P = 0.005$). In addition, the HSP70 protein expression in myocardium reached the peak at 24 h (seen in Fig. 2).

Compared with that in Burn group, ketamine strongly increased HSP70 protein expression level in cerebral tissue at 3 h and 6 h (79% versus 51%, $P = 0.023$, at 3 h; 123% versus 98%, $P = 0.015$, at 6 h). Further, Western blot analyses revealed that the HSP70 protein expression in myocardium reached the peak at 12 h (seen in Fig. 3).

## Discussion

In this work, no animals died due to immobilization and injection procedures. The burn model, being commonly used,[12]

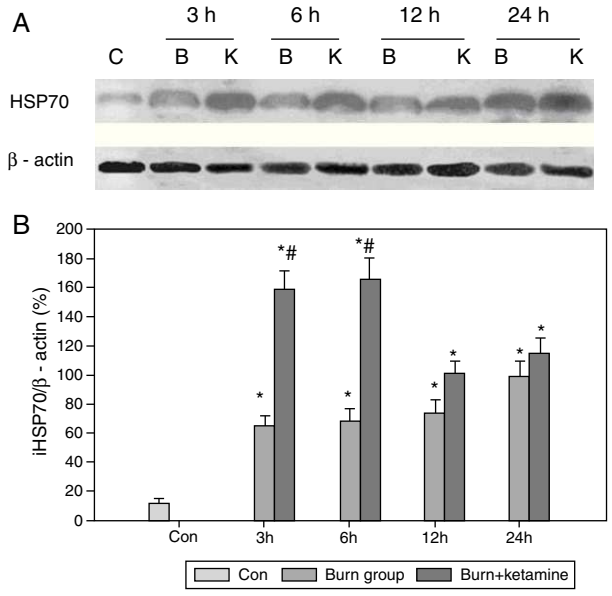


**Fig. 2 – Representative Western blot experiment on HSP70 expression in myocardium of rats of control group (Con), Burn group and Burn + ketamine group (A) and densitometric evaluation of HSP70 expression of eight experiment rats (B) are shown. Data (mean [SEM]) show the fold of iHSP70 to β-actin.**
***P < 0.05 *versus* group Con; #P < 0.05 *versus* group B.**

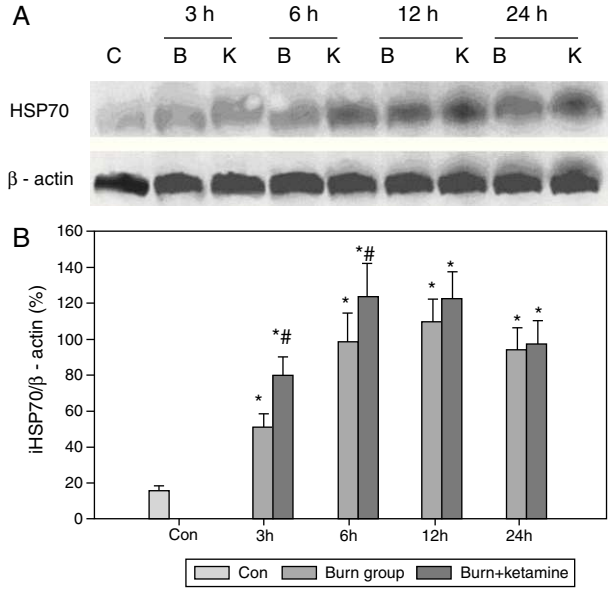


**Fig. 3 – Representative Western blot experiment on HSP70 expression in brain of rats of control group (Con), Burn group and Burn + ketamine group (A) and densitometric evaluation of HSP70 expression of eight experiment rats (B) are shown. Data (mean [SEM]) show the fold of iHSP70 to β-actin.**
***P < 0.05 *versus* group Con; #P < 0.05 *versus* group B.**

allowed for a more precise graduation of burn extent according to body surface as obtained by Meeh's formula. This process produced standard third-degree animal burns. Wistar rats have been found to be more susceptible to thermal injury than Sprague-Dawley.[13]

Ketamine, a general anesthetic, has been shown to elicit the heat-shock response (HSR) in some animal models: it has increased the cellular concentration of heat-shock protein 70 (HSP70) in the olfactory mucosa[8] and in the brain cortex of rats.[9] However, little is known about whether ketamine administration improve survival in severe burn injury. The data presented here provide evidence that ketamine therapy improves survival in severe burn injury. This is also supported by the reports of Neder et al. wherein animals anesthetized with ketamine had a mortality rate that was significantly lower than that of animals anesthetized with the association of midazolam and fentanyl.[14]

Our finding that ketamine given 15 min after burn injury helped in improved survival was unexpected, considering a report that ketamine given 1 h after burn injury did not improve survival.[15] The apparent inconsistency between our results and those of the previous study may relate to differences in the severity of burn injury and dose of ketamine. In the previous study, the burn caused 15–20% mortality without ketamine treatment; whereas, in our study, the burn caused 70% mortality. In the previous study, the dose of ketamine was 10 mg/kg, considerably smaller than the dose used in our study, 40 mg/kg. The combined results from our study and the previous one suggest that a reduction of burn-induced mortality can be demonstrated when a large dose of ketamine is given in a model of severe burn injury but not when a smaller dose of ketamine is given after a less severe burn injury.

The data presented here support the hypothesis that ketamine induces a heat shock response and that it mediates cytoprotection against severe burn injury at clinically relevant tissue concentrations by both of evidences: (1) Western blot analyses revealed that ketamine administration induced the HSP70 protein expression in myocardium and cerebral tissue; (2) After 10 days, survival rate of rats in group K was higher than that of group B.

During burn stress response, the heart is particularly susceptible to hypoxia, because it has only a limited reserve of high-energy phosphates. Both the severity and duration of hypoxia determine cardiac response to a decreased oxygen supply. Hypoxia and oxidative stress induce biochemical and functional changes, despite which the heart attempts to maintain its function to counteract oxygen tension changes.[16] A number of studies in recent years have suggested that the expression of HSP70 may play an important role in protecting myocardium against ischemia, hypoxia, endotoxin, reperfusion injury, and oxidative damage. Currie et al.[17] showed that a rise in levels of a particular HSP70, induced by heat stress, is associated with protection against ischemia–reperfusion injury. Wischmeyer et al.[18] found that administering glutamine could significantly reduce the deleterious changes in myocardial metabolism and preserve cardiac output after reoxygenation, which was due to the enhanced expression of

HSP70 induced by glutamine. Burn trauma can produce a twofold increase in myocardial HSP70 and heat stress 1 h before burn trauma causes an increase in left ventricular developed pressure and can improve ventricular performance.[19]

Ischemia and hypoxia are the primary causes of brain injury after a severe burn. A number of studies have shown that HSP70 plays a neuroprotective role in animal models of ischemic and traumatic brain injury.[20,21]

In addition to the well-studied role of HSP70 as a molecular chaperone assisting in correct protein-folding, several new mechanisms by which HSP70 can prevent cell death have been described.[22,23] HSP70 is now known to regulate apoptotic cell death both directly by interfering with the function of several proteins that induce apoptotic cell death and indirectly by increasing levels of the anti-death protein bcl-2. Lee et al.[23] found that HSP 70 knockout mice had a significantly greater myocardial infarction volume than wild-type mice. Other research on mechanisms of this protection in vitro has shown that ketamine exerts neuroprotective effects by inhibiting the phosphorylation of transcription factor c-Jun, which plays significant role in cell apoptosis inhibition and cell survival.[24] Based on our observation in this study, we suggest that ketamine plays an important role in myocardial and neural protection by increasing HSP 70 expression in rats after severe burns and thus prolonging the rats survival. Yu et al.[25] reported that ketamine can suppress the production and activity of proinflammatory cytokines TNF-alpha, interleukin-6, nuclear factor-kappaB, and Toll-like receptor 2 or 4 in the lungs. These effects correlated with longer survival in injury-related sepsis.

## Conclusions

Ketamine therapy improves survival in severe burn injury. This beneficial effect is probably achieved through eliciting the heat-shock response (HSR), as evidenced by the expression of heat shock protein 70 in myocardial and cerebral tissues. More extensive and specific experiments are required to discover the exact molecular mechanisms by which ketamine exerts its effects. Such knowledge might lead to therapeutic approaches that could ultimately be used in clinical practice.

## Funding

None

## Conflict of interest

There are no conflicts of interest to declare.

## REFERENCES

1. Welch WJ. Mammalian stress response: cell physiology, structure/function of stress proteins, and implications for medicine and disease. Physiol Rev. 1992;72: 1063–81.
2. Malhotra V, Wong HR. Interactions between the heat shock response and the nuclear factor-κB signaling pathway. Crit Care Med. 2002;30:S89–95.
3. Westerheide SD, Morimoto RI. Heat shock response modulators as therapeutic tools for diseases of protein conformation. J Biol Chem. 2005;280:33097–100.
4. De Maio A. Heat shock proteins: facts, thoughts, and dreams. Shock. 1999;11:1–12.
5. Kregel KC. Heat shock proteins: modifying factors in physiological stress responses and acquired thermotolerance. J Appl Physiol. 2002;92:2177–86.
6. Hightower LE. Heat shock, stress proteins, chaperones, and proteotoxicity. Cell. 1991;66:191–7.
7. Hendrick JP, Hartl FU. Molecular chaperone functions of heat-shock proteins. Annu Rev Biochem. 1993;62:349–84.
8. Carr VM, Farbman AL. Effect of ketamine on stress protein immunoreactivities in rat olfactory mucosa. Neuroreport. 1993;5:197–200.
9. Renis M, DiGiacomo C, Sorrenti V, Russo A, la Delfa C, Reale S, et al. Heat shock proteins following rat cerebral ischemic/reperfusion episode: effect of ketamine. Biochem Mol Biol Int. 1994;33:345–54.
10. Pannen BH, Maeda K, Ayuse T, Brienza N, Revelly JP, Robotham JL, et al. Hepatic heat shock and acute-phase gene expression are induced simultaneously after celiotomy in the anesthetized pig. Anesthesiology. 1995;83:850–9.
11. Meyer TN, Silva AL, Vieira EC, Alves ACV. Heat shock response reduces mortality after severe experimental burns. Burns. 2000;26:233–8.
12. Walker HL, Mason Jr AD. A standard animal burn. J Trauma. 1968;8:1049–51.
13. Rapaport FT, Bachvaroff RJ, Grullon J, Kunz H, Gill TJ. Genetics of natural resistance to thermal injury. Ann Surg. 1982;195:294–304.
14. Neder Meyer T, Lázaro da A, Silva. Ketamine reduces mortality of severely burnt rats, when compared to midazolam plus fentanyl. Burns. 2004;30:425–30.
15. Gurfinkel R, Czeiger D, Douvdevani A, Shapira Y, Artru AA, Sufaro Y, et al. Ketamine improves survival in burn injury followed by sepsis in rats. Anesth Analg. 2006;103: 396–402.
16. Yagmurdur H, Aksoy M, Arslan M, Baltaci B. The effects of propofol and ketamine on gut mucosal epithelial apoptosis in rats after burn injury. Eur J Anaesthesiol. 2007;24:46–52.
17. Currie RW, Karmazyn M, Kloc M, Mailer K. Heat-shock response is associated with enhanced postischemic ventricular recovery. Circ Res. 1988;63:543–9.
18. Wischmeyer PE. Glutamine and heat shock protein expression. Nutrition. 2002;18:225–8.
19. White DJ, Carlson D, Ordway GA, Horton JW. Protective role of heat stress in burn trauma. Crit Care Med. 2004;32:1338–45.
20. Gaspary H, Graham SH, Sagar SM, Sharp FR. HSP70 heat shock protein induction following global ischemia in the rat. Brain Res Mol Brain Res. 1995;34:327–32.
21. Tsuchiya D, Hong S, Matsumori Y, Kayama T, Swanson RA, Dillman WH, et al. Overexpression of rat heat shock protein 70 reduces neuronal injury after transient focal ischemia,transient global ischemia, or kainic acid-induced seizures. Neurosurgery. 2003;53:1179–87.
22. Krueger-Naug AM, Plumier JC, Hopkins DA, Currie RW. Hsp27 in the nervous system: expression in pathophysiology and in the aging brain. Prog Mol Subcell Biol. 2002;28:235–51.

23. Lee SH, Kwon HM, Kim YJ, Lee KM, Kim M, Yoon BW. Effects of HSP70 gene knockout on the mitochondrial apoptotic pathway after focal cerebral ischemia. Stroke. 2004;35:2195–9.
24. Wang L, Jing W, Hang YN. Glutamate-induced c-Jun expression in neuronal PC12 cells: the effects of ketamine and propofol. J Neurosurg Anesthesiol. 2008;20: 124–30.
25. Yu M, Shao D, Yang R, Feng X, Zhu S, Xu J. Effects of ketamine on pulmonary inflammatory responses and survival in rats exposed to polymicrobial sepsis. J Pharm Pharm Sci. 2007;10:434–42.