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Essay

Ketamine improves survival in severe burn injury in rats via the expression of heat shock protein 70, far or close to the clinical perspective?☆

*Zulma Dueñas**

Associate Professor, Physiological Science Department, School of Medicine, National University of Colombia, Bogotá DC, Colombia

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ABSTRACT

The purpose of this reflection or commentary is not to demerit the efforts of the researchers, but rather to highlight some aspects that should be taken into account in the future for implementing experimental designs that may potentially contribute to strengthen the links between basic biomedical and clinical research, referring to the research article entitled Ketamine improves survival in severe burn injury in rats via the Expression of Heat Shock Protein 70. This is a purely constructive reflection aimed at encouraging those who are one way or other involved in research, to develop a more comprehensive analysis and a proposal for an experimental design that enables the extrapolation of the results from animal models to a clinical application, with a limited number of subjects but preserving the validity of the trial, in addition to making the best possible use of the experimental subjects and of the relationship between basic and clinical research. In fact, the ethical guidelines issued by the *Council for International Organizations of Medical Sciences*, in its chapter on animal experiments emphasize that the validity of a trial allows for using the minimum number of animals in an experiment. The article that makes reference to this matter was published by Zhang Meng-yuan et al., and concludes that Ketamine therapy improves the survival of patients with severe burn injuries via the expression of heat shock proteins in the heart and the brain.

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* Correspondence to: Carrera 45 No. 44-21, Etapa II, Torre 5, Apto. 803, Bogotá, DC, Colombia.
E-mail addresses: zjduenasg@unal.edu.co, zujadugo@gmail.com

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Palabras clave:
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RESUMEN

El presente comentario o escrito de reflexión tiene por objetivo señalar, en el artículo de investigación titulado «*Ketamine improves survival in severe burn injury in rats via the expression of heat shock protein 70*», algunos aspectos que, sin pretender demeritar el trabajo de los investigadores, pudieran ser tenidos en cuenta a futuro, en aras de implementar posteriormente diseños experimentales que puedan contribuir de la mejor forma a fortalecer el vínculo entre la investigación biomédica básica y la clínica. Esta reflexión es de carácter netamente constructivo, procurando motivar en cada uno de quienes de una u otra manera mantienen un vínculo con la investigación, el desarrollo de un mejor análisis y propuesta de diseño experimental que permita proyectar resultados obtenidos en modelos animales, hacia una aplicabilidad clínica, manteniendo un número reducido de sujetos de trabajo, sin con ello quitarle validez a un estudio y buscando aprovechar de la mejor manera el ente de experimentación y el vínculo de la investigación básica con la clínica. En efecto, las pautas éticas emitidas por el *Council for International Organizations of Medical Sciences*, en su aparte referido a la experimentación con animales, instan en que la validez de un estudio per mite mantener al mínimo el número de animales a utilizarse en un experimento. El artículo sobre el cual se hace esta reflexión fue publicado por Zhang Meng-yuan et al. y se concluye que la terapia con ketamina mejora la sobrevivencia en heridas con quemaduras severas vía la expresión de proteínas de choque térmico en miocardio y cerebro.

Ketamine is a non-barbiturate dissociative anesthetic agent that induces a rapid and profound sedation, anesthesia and analgesia with a broad range of uses and effects on human beings. The use of Ketamine as an anesthetic agent began in the mid-seventies[1]; however, currently Ketamine has a wide variety of applications. One of the several usual applications is for the treatment of depression[2,3] because of its high affinity of the N-methyl D-aspartate (NMDA) receptor. Depressive patients show improved symptoms as soon as the next day following the administration of Ketamine treatment.[4,5] Another application of Ketamine is in the treatment of asthma. It has been suggested that Ketamine may relieve bronchospasm in asthmatic children who do not respond to traditional treatment[6]; however, some authors conclude that in order to prove Ketamine's effectiveness in the treatment of acute asthma in children, there is a need to undertake randomized trials with strong statistical power and a sound methodology, in addition to exploring varying doses of Ketamine and its role in children who need ventilation for severe asthma. Some of the possible applications suggested by other trials include the use of Ketamine as an agent for reducing mortality and pain, both in animal and human models in third degree burns.[7–10] Several authors validate this proposal based on the anti-inflammatory effects of Ketamine and its influence to induce protein expression in response to heat shock (HSP). In fact, the article referred to in this commentary concludes that Ketamine therapy improves survival in severe burn injuries, via the expression of heat shock proteins in the heart and the brain[11]; such conclusion however deserves careful consideration that take several factors into consideration, as discussed later on.

HSPs are a family of chaperon molecules that notwithstanding their ubiquitous expression are highly preserved in the evolutionary scale.[10] HSPs expression is regulated through several stressor events, mainly those derived from heat shock. Hence, HSPs contribute to protect the cells from heat stress and other types of stressors such as proteotoxic stress.[10] Based on HSPs functions, they are classified into six families: (1) HSP20 r sHSP, proteins (2) HSP40 or class J, (3) HSP60 or GroEL/ES, (4) HSP70, (5) HSP90, and (6) HSP100.[12] Poor function of these proteins may contribute to various lifetime disorders; i.e. Parkinson's disease, cardiovascular conditions and Alzheimer's disease, among others. These proteins have also been associated to longevity. The induction of HSP in aging may potentially extend longevity through re-folding of damaged proteins that accumulate during old age and cause cell toxicity. Models such as *Caenorhabditis elegans* have shown that HSPs overexpression extends the life span because it reduces proteotoxicity.[13] Hence, a drop in HSP during aging is associated with interrupted cell homeostasis that causes illnesses such as cancer, cellular senescence and neurodegeneration. In general, HSP levels decrease with age in most organs, including the neurons. Aging also causes attenuation or alteration of several signaling pathways, in addition to the expression of transcription factors such as heat shock factors (HSF).[13]

The most frequently studied role of heat shock proteins is that of molecular chaperons to prevent undesirable interactions among unfolded polypeptides during their synthesis or transport, preventing irreversible aggregations among the various proteins, avoiding misfolding or denaturation.[14,15] There is however evidence that link these proteins to various cellular events, including their contribution to the immune response[16]; HSPs from intracellular or extracellular sources,

may act as immune modulators and alleviate the symptoms of inflammatory disease. HSPs overexpression has also been identified in various types of cancer as a factor in tumor progression, making them the target for treatment.[17] Finally, cytoprotective effects of heat shock proteins have been reported in burn injuries[18,19]; so, keeping in mind the reports on the effects of Ketamine-induced heat shock proteins, particularly HSP70, it is precisely this relationship that served as the foundation for the article analyzed.[11]

In the study published by Zhang Meng-yuan, Wang Gongming, Li Fang-lin, Dong Ling, Xu Yan-bing, Chiang Joseph-S, entitled: "*Ketamine Improves Survival in Severe Burn Injury in Rats* via *the Expression of Heat Shock Protein 70*",[11] the authors designed an experiment to prove that Ketamine improves the survival of third degree burned subjects, via an increased expression of heat shock protein – hsp70. The article provides valuable knowledge, yet additional experiments are required for completeness, in order to elicit a solid and valid foundation to move ahead with the clinical application suggested by the researchers.

Notwithstanding the fact that the authors used a 40 mg/kg dose of Ketamine injected 15 min after the injury, the rationale for using such dose and the timing remains unclear, keeping in mind that other articles have reported the use of different doses and different time frameworks.[20–23] For instance, the researchers Carr and Farban, administer 9 mg/kg and are able to evidence an increase in HSP 70 in the olfactory mucosa,[20] while Xiao et al., demonstrate the effect of Ketamine in septic shock by injecting 80 mg/kg 20 min before and 40 mg/kg one hour after the event.[21] Another trial reported by Liao et al., indicates that in adult rats, Ketamine doses ranging from 20 mg/kg to 120 mg/kg increase the expression of HSP 70 in the hippocampal neurons, yet the effect is less evident in younger rats.[22] Based on these outcomes, the authors could have discussed the reason for selecting that dose but the injection time is still an open question. Whilst animal models are aimed at developing and studying new forms of treatment, injecting the Ketamine 15 min after the injury is barely applicable. It is quite evident that a burned patient does not get medical care in such a short time as discussed by the authors based on Gurfinkel's results; no effect would be observed either if the Ketamine were injected 1 h later.[23] Consequently, according to the literature, the authors should have considered a more realistic time framework for the administration of treatment and then the number of subjects could have been smaller. In fact, the analysis 3 h later would not have been necessary, as shown in the Western Blot tests in Figs. 2 and 3; furthermore, in line with CIOMS guidelines, the number of subjects could have been reduced and still maintain the validity of the results.[24] A more diligent approach using 20, 30 and 40-min intervals would have contributed information about the effect of Ketamine induction on HSP70 under these varied conditions that are more consistent with the actual length of time elapsed until a burned patient arrives at a healthcare center. Based on the authors' conclusion under the experimental conditions, the injection of Ketamine improves the survival of patients with third-degree burns; such effect could be mediated by HSP70, according to the myocardial and brain tissue evaluation. However, further trials are needed to put forward a treatment approach and its potential clinical application. In fact, the evaluation of the injured airway response and the observed behaviors, the effect on salivation, among several other analyses will significantly contribute to the reports. Furthermore, the comparison of the responses obtained from all the experiments in younger subjects will contribute with relevant knowledge to all areas since the percentage of hospital admissions with burns is much higher in children than in adults. The response to an intervention of a developing child, a youngster and an adult are totally different. Finally, it should be stressed that both biomedical basic research and the use of experimental models, are fundamental for developing knowledge. However, the future of biomedical sciences lies in translational research and hence keeping in mind the clinical applicability of basic research will further strengthen the link between basic research and the clinic.

A clear example of a prudent discussion of the results and their applicability is the article published by researchers Jat and Chawla.[6] Underlining the limitations of their trial, the conclusions emphasize that in order to prove the effectiveness of Ketamine in acute pediatric asthma, further randomized trials with strong statistical power are needed to explore different doses. This is a clear example of the importance of self-criticism in published papers, acknowledging the limitations and weaknesses of the trial, before advocating any clinical applicability. Along these lines, another example of acknowledgment of limitations in an article is the paper published by Norambuena et al.[8] The purpose of the article was to compare the efficacy of oral midazolam vs. Ketamine, acetaminophen and codeine to provide sedation and analgesia for children undergoing burn wound care. In this report the authors conclude that the combination of oral midazolam and Ketamine provides better analgesia than the combination of midazolam, acetaminophen and codeine for painful procedures in burned children. However, they clearly explain that one of the limitations of the trial was the limited number of patients and suggest a larger number of patients prior to suggesting a protocol for clinical practice. One further example of caution for arriving at conclusions in a trial is the conclusion of McGuinness et al.[25] in their systematic review on Ketamine as an analgesic agent in adults with burn injuries; here the authors acknowledge that although IV Ketamine is commonly used as a pain killer in burn injuries, based on the data from such systematic review they believe that there is not enough proof to make final recommendations for its clinical use.

Acknowledging the limitations and the questions about the applicability of the results of a trial provides a realistic value of the work accomplished, in addition to valid information for future endeavors. By no means the generation of knowledge should be based on the applicability of the results in the clinic; thus in most cases, the results of an experiment are not followed by immediate applicability. This would certainly be a genuine success, but that is not always the case.

As already mentioned, translational research seeks to strengthen the links between basic biomedical research and clinical research, mostly to be able to design experiments that yield results with clinical applicability. The best way to accomplish this goal is to hold a joint discussion, basic and clinical researchers, about the best strategy to strengthen the experimental design; identifying the finest methodology and generating significant and statistically powerful results to

pave the way for the next step which is formalizing a clinical protocol.

Indeed, studies such as the one reported by Zhang at al., contribute to generate knowledge and yield promising results; however, an improved design and the recognition of strengths and weaknesses will render results with a stronger potential applicability.

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Conflict of interest

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REFERENCES

1. Domino EF, Chodoff P, Corssen G. Pharmacologic effects of CI-581, a new dissociative anesthesic in man. Clin Pharmacol Ther. 1965;6:279–90.
2. Howland RH. Ketamin for the treatment of depression. J Psychosoc Nurs Ment Health Serv. 2013;51:11–4.
3. Murrough JW. Ketamine as a novel antidepressant: from synapse to behavior. Clin Pharmacol Ther. 2012;91:303–9.
4. Zarate Jr CA, Mathews D, Ibrahim L, Chaves JF, Marquardt C, Ukoh I, et al. A randomized trial of an N-methyl-d-aspartate antagonist in treatment-resistant major depression. Arch Gen Psychiatry. 2006;63:856–64.
5. Murrough JW, Perez AM, Pillemer S, et al. Rapid and longer-term antidepressant effects of repeated ketamine infusions in treatment-resistant major depression. Biol Psychiatry. 2012, http://dx.doi.org/10.1016/j.biopsych.2012.06.022.
6. Jat KR, Chawla D. Ketamine for management of acute exacerbations of asthma in children. Cochrane Database Syst Rev. 2012;11:1–20.
7. Neder Meyer T, Lázaro Da Silva A. Ketamine reduces mortality of severely burnt rats, when compared to midazolam plus fentanyl. Burns. 2004;30:425–30.
8. Norambuena C, Yañez J, Flores V, Puentes P, Carrasco P, Villena R. Oral ketamine and midazolam for pediatric burn patients: a prospective, randomized, double-blind study. J Pediatr Surg. 2013;48:629–34.
9. Kundra P, Velayudhan S, Krishnamachari S, Gupta SL. Oral ketamine and dexmedetomidine in adults' burns wound dressing – a randomized double blind cross over study. Burns. 2013. PII:S0305-4179(13)00053-3.
10. Reich DL, Silvay G. Ketamine: an update on the first twenty-five years of clinical experience. Can J Anaesth. 1989;36:186–97.
11. Zhang M-y, et al. La ketamina mejora la sobrevida en lesión por quemadura severa en ratas, a través de la expresión de la proteína de choque 70. Rev Colomb Anestesiol. 2013;41:82–7.
12. Murshid A, Eguchi T, Calderwood SK. Stress proteins in aging and life span. Int J Hyperthermia. 2013:442–7.
13. Feng PM, Chen W, Lin H, Chou KC. iHSP-PseRAAAC: identifying the heat shock protein families using pseudo reduced amino acid alphabet composition. Anal Biochem. 2013. PII:S0003-2697.
14. Vidal Magalhães W, Gouveia Nogueira MF, Kaneko TM. Heat shock proteins (HSP): dermatological implications and perspectives. Eur J Dermatol. 2012;22:8–13.
15. Richter K, Haslbeck M, Buchner J. The heat shock response: life on the verge of death. Mol Cell. 2010;40:253–66.
16. Van Noort JM, Bsibsi M, Nacken P, Gerritsen WH, Amor S. The link between small heat shock proteins and the immune system. Int J Biochem Cell Biol. 2012;44:1670–9.
17. Murphy ME. The HSP70 family and cancer. Carcinogenesis. 2013;34:1181–8.
18. White DJ, Carlson D, Ordway GA, Horton JW. Protective role of heat stress in burn trauma. Crit Care Med. 2004;32:1338–45.
19. Rodeberg DA, Meyer JG, Babcock GF. Heat shock response: presence and effects in burn patient neutrophils. J Leukoc Biol. 1999;66:773–80.
20. Carr VM, Farbman AL. Effect of ketamine on stress protein immunoreactivities in rat olfactory mucosa. J Psychosoc Nurs Ment Health Serv Neuroreport. 1993;5:197–200.
21. Xiao H, Xu HW, Liu H, Zhang L. Effect of ketamine on endotoxin-induced septic shock in rats and its mechanism. Zhongguo Wei Zhong Bing Ji Jiu Yi Xue. 2007;19:303–5.
22. Liao R, Wang QY, Zhang L, Xiao H. Expression of HSP70 induced by ketamine in the hippocampus of rat at different ages. Sichuan Da Xue Xue Bao Yi Xue Ban. 2004;35:492–5.
23. 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.
24. Howard-Jones N. A CIOMS ethical code for animal experimentation. WHO Chron. 1985;39:51–6.
25. McGuinness SK, Wasiak J, Cleland H, Symons J, Hogan L, Hucker T, Mahar PD. A systematic review of ketamine as an analgesic agent in adult burn injuries. Pain Med. 2011;12:1551–8.