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## A comparative analysis of isotonic versus hypertonic solution volume replacement in septic rats

*Análise comparativa da reposição volêmica com solução isotônica e solução hipertônica em ratos com sepse*

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Received from the Surgical Technique Laboratory/ Medical/ CCBS. Pontifícia Universidade Católica do Paraná - PUCPR - Curitiba (PR), Brazil.

Submitted on August 17, 2010  
Accepted on October 26, 2010

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### ABSTRACT

**Purpose:** Sepsis severity and mortality risk require aggressive therapy which includes hemodynamic support. The best fluid for volume replacement, however, is controversial. This study aimed to compare 0.9% isotonic saline solution versus 7.5% hypertonic saline solution as volume replacement fluid in sepsis induced by cecum ligation plus puncture rats.

**Methods:** This experimental trial included 30 rats divided into three groups: Control group (CG, n = 10), isotonic (ISG, n = 10) and hypertonic solution (HSG, n = 10). Fifteen hours after cecum ligation and puncture, all animals underwent respiratory rate, mean arterial pressure, renal and hepatic blood flow and weight evaluation, plus blood collection for TNF- $\alpha$  measurement. The ISG and HSG treatment groups received volume replacement 60 minutes before the procedure with either 0.9% or 7.5% saline solu-

tion, respectively.

**Results:** Two animals died. Significant differences were found for the animals' mean weight after 15 hours ( $p=0.018$ ), particularly relevant when ISG and HSG are compared ( $p=0.003$ ). Renal blood flow was also significantly different for the CG versus HSG ( $p=0.002$ ) and CG versus ISG ( $p=0.008$ ), but not significantly different for ISG versus HSG. No mean arterial pressure improvement was found for HSG (0.054). Other variables were not significant.

**Conclusions:** Although no mean blood pressure, hepatic flow or TNF- $\alpha$  improvements were detected, the rats with sepsis 15 hours after cecum ligation and puncture showed significantly increased renal blood flow which was 0.9% isotonic saline solution or of 7.5% hypertonic solution use independent.

**Keywords:** Saline solution, hypertonic; Isotonic solutions; Sepsis; Rats, Wistar

### INTRODUCTION

Microorganisms or they toxic byproducts in blood stream, hemodynamic changes and local plus generalized inflammatory response are distinctive processes, however together characterize and define sepsis as a systemic inflammatory response syndrome (SIRS) caused by a microorganism infection.<sup>(1,2)</sup>

Nevertheless recent diagnosis and therapy progresses, sepsis has still high mortality rates, between 30% and 80%,<sup>(3)</sup> and is the main cause of death in intensive care units (ICU).<sup>(4)</sup> In Brazil, according the BASES study,<sup>(5)</sup> sepsis and septic shock account for 24.3% and 52.2% of ICU deaths, respectively.

Septic patients show marked hemodynamic disorders, mainly evidenced by its 35% to 40% of the cases progression to septic shock.<sup>(6)</sup>

Complex changes involve myocardial contractility, micro- and macrocirculation, leading to reduced perfusion and poor tissue oxygen offer.<sup>(7)</sup>

Therefore, as a frequent, expensive and potentially fatal disease, with little mortality progress evidenced,<sup>(8)</sup> aggressive therapy is mandatory, and includes antibiotics therapy plus supportive measures, mainly hemodynamic support, therefore involving volume replacement aimed to improve tissue perfusion.

However, the literature analysis evidences controversy on the best volume replacement fluid. According to the Brazilian Sepsis Consensus (2003),<sup>(9)</sup> crystalloids are the first choice; among them, isotonic saline (0.9% NaCl) is most frequently used, however requires large volume infusions. On the other hand, hypertonic solutions improve heart contractility, provide pre-capillary dilation, however have risks of hypertonic conditions development.<sup>(9-11)</sup>

Hypertonic saline use in sepsis was poorly studied, and its main mechanisms of action include fast intracellular to extracellular compartment fluid mobilization, endothelial and tissue edema reduction, myocardial contractility increase, cardiac output improvement, arterioles vasodilation, microcirculation improvement, hemodilution and immunomodulation.<sup>(10,11)</sup>

Thus, considering the lack of clear definition of hypertonic fluids in sepsis management use, this study was aimed to compare the hemodynamic responses to 0.9% isotonic saline (ISS) versus 7.5% hypertonic saline (HSS) volume replacement in rats with sepsis induced by cecum ligation and puncture (CLP), the most similar to human sepsis abdominal injury animal model.<sup>(1,12)</sup>

## METHODS

After approval by the Animal Studies Ethics Committee (CEUA) of the Pontifícia Universidade Católica do Paraná (PUCPR), Brazil, an experimental trial was conducted involving 30 Wistar male rats (*Rattus norvegicus albinus*), provided by the PUCPR's vivarium.

Animals weighting average 310 grams were divided into three groups: control group (CG, n=10), isotonic saline group (ISG, n=10) and hypertonic saline group (HSG, n=10).

All animals underwent cecum ligation and puncture (CLP) procedure, conducted under intramuscular ketamine (90 mg/kg) plus xylazine (10 mg/kg) anes-

thesia, abdominal trichotomy, weight and respiratory rate (RR) measurement. Next, a supra-umbilical median laparotomy was conducted followed by ligation of the free portion of cecum with a 3-0 cotton thread and three 40-12 needle punctures. The abdominal wall was then sutured in two layers using nylon 4-0 thread, followed by analgesia with Banamine for injection (flunixin meglumine) 0.1 mL in 0.9 mL saline.

All animals were kept in cages with free access to water and food, at room temperature for 15 hours.

Fifteen hours after cecum ligation and puncture, the CG underwent intramuscular ketamine (90 mg/kg) and xylazine (10 mg/kg) anesthesia, weight measurement, 1 cm incision at inguinal region, femoral artery and vein dissection, femoral vein anticoagulation with 5000 IU/mL sodium heparin 0.2 mL femoral artery catheterization with 24G catheter and mean arterial pressure (MAP) measurement using a mercury sphygmomanometer connected with intravenous access tubing. Next, a new laparotomy was conducted for 90 seconds hepatic and renal (left kidney) blood flow evaluation using the LASERFLO Blood Perfusion Monitor BPM<sup>2</sup>, available at the PUCPR's surgical technique laboratory.

After measuring the blood flows, blood was drawn from the femoral artery catheter, centrifuged for 15 minutes at 2600 RPM in a CT-6000R centrifuge obtaining plasma that was frozen at -80°C for later TNF- $\alpha$  analysis, determined using the Rat TNF- $\alpha$  ELISA Development kit protocol.

Finally, the animals were euthanized with thiopental 250 mg/100g, each 1 g diluted in 40 mL normal saline, and the left kidney, liver and lung removed.

For the ISG and HSG groups the same procedure performed for the CG was conducted after 15 hours plus volume replacement 60 minutes before. For the ISG, 32 mL/kg 0.9% saline solution were subcutaneously infused, while for the HSG group the subcutaneous injection consisted of 4 mL/kg 7.5% saline solution.

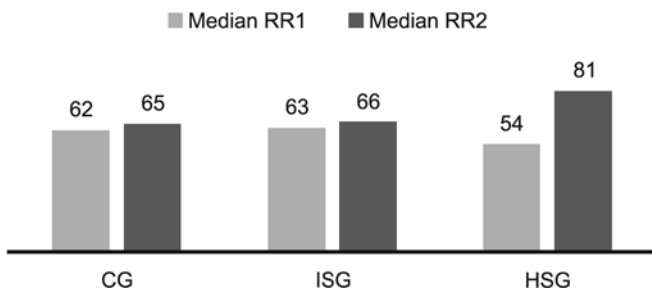
The groups' quantitative variables were compared using the non-parametrical Kruskal-Wallis test, being  $p < 0.05$  values considered statistically significant.

## RESULTS

The experimental procedure was conducted for the three study groups, monitoring RR, MAP and hepatic plus renal blood flow, followed by blood collection and liver, left kidney and lungs removal.

Death before euthanasia occurred in only two rats, both in the ISG group.

Regarding RR, a median increase was observed for the second procedure (RR2), particularly for the HSG, also versus ISG (Figure 1), although it should be highlighted that no statistical significance was found for the groups' comparison, with 0.199 for RR1 and 0.767 for RR2.



RR – respiratory rate 1; CG – control group; ISG – isotonic saline group; HSG – hypertonic saline group.

**Figure 1 – Groups comparative respiratory rate analysis. Median respiratory rates increase is shown for the second procedure, highlighting HSG. Data expressed as medians.**

A MAP improvement was observed with hypertonic solution, with average 40.9 mmHg for CG, 42.8 mmHg for ISG, and 57.9 mmHg for HSG, however not significant, p=0.054 (Table 1).

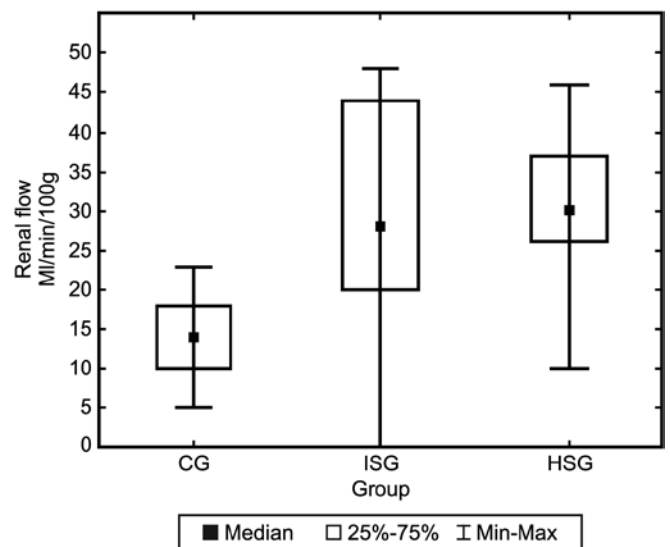
TNF- $\alpha$  inflammatory mediator measurement didn't show statistical significance, with p=0.107 for all groups.

Hepatic blood flow evaluation evidenced higher CG flow, median 51.5 mL/min/100g, while for ISG and HSG median liver blood flows of 29 and 30 mL/min/100g, respectively, were shown. However these were not statistically significant, p=0.678 for the three groups.

Finally, significant differences were shown only for renal blood flow and weight variables. For the pairwise groups' comparison, the weight difference between both surgical procedures (Weight 1 by the cecum ligation and puncture procedure time, and Weight 2 by the

hemodynamic evaluation procedure time), a median 2.7% weight decrease was found for the CG within the 15 hours, median 315.50 grams before the ligation and 307.0 grams 15 hours later. For the ISG, the weight increased by 0.47% and for HSG, was reduced by 0.85%. On the groups' comparison, these results were statistically significant for Weight 2, p=0.018, particularly for ISG and HSG comparison, p=0.003.

The renal flow had significant differences for CG versus HSG (p=0.002), and CG versus ISG (p=0.008), however was not significant for ISG versus HSG, with median CG 14 mL/min/100 g, ISG 28 mL/min/100g and HSG 30 mL/min/100g (Figure 2).



**Figure 2 – Renal blood flow for all trial groups (G1 = ISG, G2 = HSG), median CG 14 mL/min/100g, ISG 28 mL/min/100g and HSG 30 mL/min/100g.**

Table 2 displays mean, median, maximal and minimal, and standard deviations of renal blood flow and Weight 1 and 2 variables. These variables pairwise groups comparison was significant for weight 2 CG versus ISG and HSG comparison, p=0.008 and 0.002, respectively. For the renal blood flow variable, only the ISG versus HSG comparison was statistically significant, p=0.003.

**Table 1 – Groups mean arterial pressure comparison**

Variable	Group	N	Mean $\pm$ standard deviation	Median (minimum-maximum)	p value
MAP	CG	10	40.9 $\pm$ 16.74	41 (22-70)	0.054
	ISG	10	42.8 $\pm$ 16.26	49 (0-54)	
	HSG	10	57.9 $\pm$ 13.30	59 (39-78)	

MAP – mean arterial pressure; ISG – isotonic saline solution; HSG – hypertonic saline solution; CG – control group.

**Table 2 – Groups renal blood flow and weight variables comparison**

Variable	Group	N	Mean ± standard deviation	Median (minimum-maximum)	p value
Renal flow (mL/min/100g)	CG	10	13.9 ± 6.30	14 (5-23)	0.008
	ISG	10	28.0 ± 17.64	28 (0-48)	
	HSG	10	29.8 ± 9.99	30 (10-46)	
Weight1 (g)	CG	10	318.0 ± 13.56	315.5 (296-342)	0.108
	ISG	10	321.0 ± 16.68	319 (298-346)	
	HSG	10	293.6 ± 33.16	296.5 (245-348)	
Weight2 (g)	CG	10	308.9 ± 13.30	307 (291-339)	0.018
	ISG	10	318.1 ± 15.45	320.5 (288-340)	
	HSG	10	285.2 ± 31.44	294 (236-335)	

ISG – isotonic saline group; HSG – hypertonic saline group; CG – control group; CG versus ISG renal flow:  $p=0.008$ , Weight 2:  $p=0.145$ .

## DISCUSSION

Much was discovered on sepsis' pathophysiology,<sup>(13)</sup> therefore improving therapeutic perspectives. However, some topics remain unclear,<sup>(14)</sup> among them hypertonic saline solution volume replacement therapy.<sup>(11)</sup>

Aiming to evaluate the literature-described hemodynamic benefits of hypertonic saline solution therapy in sepsis, this study compared volume replacement using hypertonic 7.5% saline solution versus isotonic 0.9% saline solution in cecum ligation and puncture septic rats, using a control non-intervention septic group.

Cecum ligation and puncture was proposed as the most similar to human sepsis animal model, because it has an abscess as local septic focus, mimicking human appendicitis or perforated diverticulitis, in addition of being a simple procedure that provides a bacterial mix, quickly promotes septic shock, although a difficult-to-control sepsis magnitude.<sup>(12)</sup> Also, heparinization was required to prevent coagulation during the procedure.<sup>(15)</sup>

Death before euthanasia occurred in only two ISG group animals, only one after cecum ligation and puncture, 6.66% of the total sample, without significant impact on the study results. This was only possible after some pilot experiments, becoming clear that the post-operative period should be reduced from 18 to 15 hours, as discussed in high sample mortality studies.<sup>(7)</sup> Otherwise, the sample would be insufficient for a post-volume replacement comparison.

The choice of 32 mL/kg of 0.9% and 4 mL/kg 7.5% saline solutions replacement was to provide approximately similar NaCl amounts for both groups (0.075 g NaCl), neither excessively influencing the hydro-electrolytic balance, nor requiring large volumes infusion.

Thus, to show hypertonic saline solution effects on

septic rats volume correction, we measured the respiratory rate, mean arterial pressure, hepatic and renal blood flow, and TNF- $\alpha$ .

The weight difference between the procedures was relevant. For ISG mean 0.47% weight increase from CLP to the after 15 hours evaluation was found. HSG had a mean 0.85% weight reduction, compared to mean 2.7% reduction for CG. A  $p=0.003$  value was found for the ISG versus HSG comparison (statistically significant), in agreement with the literature-reported hypertonic solution benefits.<sup>(10,11)</sup> ISG weight may have increased due to broader extracellular space distribution, therefore requiring larger volumes, leading to interstitial overflow and tissue edema.<sup>(11)</sup> The weight reduction for CG was larger than for HSG, as CG had no volume replacement, showing that one of the most important, if not the most important benefit, of hypertonic saline solution is quick intracellular compartment fluids mobilization into the extracellular space, endothelial and tissue edema reduction, without large volumes infusion requirement.<sup>(10,11)</sup>

Considering respiratory rate results, all groups had increases, although not statistically significant, thus failing to show the RR improvements reported by Garrido et al.<sup>(14)</sup> and Friedman et al.<sup>(11)</sup> in a review article, which would characterize improved oxygen extraction. However, in an eventual future study more detailed oxymetry could be interesting.

In a literature review by Friedman et al.,<sup>(11)</sup> MAP improvement from hypertonic saline solution use was described, with CG having mean 40.9 mmHg, ISG mean 42.8 mmHg and HSG mean 57.9 mmHg, however not statistically significant,  $p=0.054$ . This shows that, even with no statistical significance, saline solution replacement lead to tentative volume replacement.

Serum TNF- $\alpha$  inflammatory mediator measurement was carefully conducted, however showed no

statistical significance ( $p=0.107$ ). No literature references were found on TNF measurement and hypertonic saline volume replacement in septic rats. TNF is an important cytokine in sepsis, being the first to be identified in circulation,<sup>(1)</sup> and induces neutrophils release from the bone marrow and muscle catabolism, stimulates angiogenesis, promotes inflammatory proteins synthesis, stimulates coagulation, and additionally, its administration to animals induces a similar to sepsis syndrome.<sup>(1)</sup>

Hepatic blood flow evaluation showed higher CG flow, median 31.5 mL/min/100g, likely due to the sepsis magnitude, as no therapeutic intervention was adopted for these rats, differently from ISG and HSG where lower flows were seen, 29 and 30, respectively. However, no statistical significance for the three groups comparison was found ( $p=0.678$ ).

The renal blood flow showed significant differences for CG versus HSG and CG versus ISG comparisons, however was not significant for ISG versus HSG, probably reflecting improved renal function. Garrido et al.<sup>(14)</sup> suggest that hypertonic saline solution is able to improve the blood flow redistribution and microcirculatory perfusion.

## CONCLUSION

Although no MAP, hepatic blood flow and TNF- $\alpha$  improvement was shown, septic rats 15 hours after CLP had significant renal blood flow increase, which was independent of isotonic 0.9% or hypertonic 7.5% saline use.

## RESUMO

**Objetivo:** Há controvérsias sobre o melhor fluido a ser utilizado na reposição volêmica no tratamento da sepse. O objetivo do estudo foi comparar a reposição volêmica com solução salina isotônica à 0,9% ou com solução salina hipertônica à 7,5% , em ratos com sepse induzida pela ligadura e punção do ceco.

**Métodos:** Estudo experimental com 30 ratos Wistar, divididos em três grupos: controle (GC, n=10), solução isotônica (GSI, n=10) e solução hipertônica (GSH, n=10). Em todos os grupos foi realizada a ligadura e punção do ceco, sendo avaliadas, após 15 horas, a frequência respiratória, pressão arterial média, fluxos sanguíneos hepático e renal, peso e coleta de sangue para dosagem de TNF- $\alpha$ . No grupo solução isotônica e grupo solução hipertônica houve reposição volêmica 60 minutos antes com solução salina a 0,9% e solução salina a 7,5%, respectivamente.

**Resultados:** Ocorreram dois óbitos na amostra total. Houve diferença significativa do peso médio dos ratos após 15 horas de sepse ( $p=0,018$ ), principalmente se comparados GSI e GSH ( $p=0,003$ ). O fluxo sanguíneo renal também apresentou diferenças significativas entre os grupos GC e GSH ( $p=0,002$ ), e GC e GSI ( $p=0,008$ ), mas sem significância entre GSI e GSH. A melhora da PAM com o uso de SSH não foi constatada ( $p=0,054$ ). As demais variáveis não apresentaram significância para o estudo.

**Conclusões:** Embora não tenham sido evidenciadas melhorias na pressão arterial média, bem como no fluxo hepático e TNF- $\alpha$ , ratos submetidos à sepse por ligadura e punção do ceco, após 15 horas, apresentaram aumento significativo do fluxo sanguíneo renal independente do uso de solução salina isotônica à 0,9 % ou hipertônica à 7,5%.

**Descritores:** Solução salina hipertônica; Soluções isotônicas; Sepse; Ratos Wistar

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