Hypertonic saline and hemorrhagic shock: hepatocellular function and integrity after six hours of treatment[1]

Solução salina hipertônica e choque hemorrágico: função hepatocelular e integridade após seis horas de tratamento

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ABSTRACT

Purpose: The comparison after 6h of hemorrhagic shock (HS) treatment with NaCl 7.5% (Hypertonic Saline Solution – SSH) or Ringer Lactate (RL) on liver function and integrity. **Methods**: Male Wistar rats were submitted to HS (Mean Arterial Pressure – MAP= 45 mmHg) during 60 min and then treated with NaCl 7.5% (SSH, 10% of blood loss, n=8) or Ringer Lactate (RL, 400% of blood loss, n=8). After 6h rats were anesthetized, hepatic function was assessed by bile flow measurement and liver integrity evaluated by determination of alanine aminotransferase (ALT) and bilirubin activities. **Results**: There was no difference in MAP between the groups during the whole experiments. Biliary flow showed a significant recovery after SSH treatment ($p<0.05$), and significant decrease of ALT ($p<0.001$) and bilirubin levels ($p<0.001$) in comparison to RL. **Conclusion**: Resuscitation of HS with NaCl 7.5% promoted better recovery of liver function and lesser hepatocellular damage after 6h of treatment compared to RL. The improvement is very likely related to increased microvascular perfusion provided by small volume resuscitation.

Key words: Ischemia. Reperfusion. Shock, Hemorrhagic. Saline Solution, Hypertonic. Liver. Rats.

RESUMO

Objetivo: Comparar os efeitos após 6 horas do tratamento do choque hemorrágico (CH) com solução de NaCl 7,5% (Solução Salina Hipertônica – SSH) e Ringer Lactato (RL) sobre a função e integridade hepática em ratos. **Métodos**: Ratos Wistar (n=16) machos foram submetidos a choque hemorrágico controlado (Pressão Arterial Média – PAM = 45 mmHg) durante 60 minutos e após ressuscitados com SSH (10% da perda volêmica, n=8) ou RL (4 vezes o volume sangüíneo retirado, n=8). Após 6 horas a função hepática foi determinada pela quantificação do fluxo biliar. A integridade hepática foi avaliada pelas bilirrubinas e pela alanino aminotransferase (ALT). **Resultados**: Não foi constatada diferença de PAM entre os grupos durante os experimentos. O fluxo biliar apresentou recuperação significativa no grupo SSH em comparação ao grupo RL ($p<0,05$). O grupo SSH apresentou diminuição significativa nos níveis de ALT ($p<0,001$) e das bilirrubinas ($p<0,001$). **Conclusão**: Após 6 horas de tratamento do choque hemorrágico a SSH mostrou-se superior ao RL, recuperando a função e a integridade hepatocelular, provavelmente por melhora da perfusão nutricional hepática, e diretamente relacionada ao seu mecanismo de ação.

Descritores: Isquemia. Reperfusão. Choque Hemorrágico. Solução Salina Hipertônica. Fígado Ratos.

Introduction

Hemorrhagic shock (HS) is one of the major causes of preventable death following trauma. The liver is the second organ most frequently involved in the microvascular and cellular energetic dysfunction after severe or prolonged HS [1;2]. The goal of HS resuscitation is the restoration of blood volume and peripheral perfusion. Evidences show that to restore the peripheral perfusion with RL are necessary three or four times the volume of shed blood [1;3]. However, large volumes of RL replacement can be deleterious, resulting in worsening of the tissular oxygenation and perfusion [4;5]. On the other hand, resuscitation with small volume of Hypertonic Saline Solution (HSS - NaCl 7,5%) is also an effective method to restore the cardiac output and organic perfusion [3;6;7]. The therapy is based on the mobilization of endogenous fluids through an osmotic gradient from the intracellular and interstitial compartments to the intravascular[8]. There are strong evidences of hepatic blood supply improvement with HSS treatment [9-11]. At the present moment, the benefits of HSS resuscitation were determined only after short periods of resuscitation, and there are few

evidences about the maintenance of its effects at late time after treatment of HS [12,13]. Thus, we decided to evaluate the effects of a single dose of HSS in comparison to LR treatment after 6 hours of HS resuscitation on the liver integrity and function in rats.

Methods

Animals and procedures

The study was conducted after approval by the ethics committee of the Postgraduate Course of Surgery, and in agreement with the "Guide for the Care and Use of Laboratory Animals" (National Institutes of Health, Bethesda, EUA). Male Wistar rats bred at the Basic Health Sciences Institute of Federal University of Rio Grande do Sul (UFRGS) weighing between 225g and 265g were fasted overnight but with free access to water. They were anesthetized with ketamine sulfate 75 mg/Kg (Ketamin®, Cristalia, São Paulo, Brazil) and xylazin 10 mg/Kg (Virbaxyl®, Virbax of Brazil, São Paulo, Brazil) ip. During the whole experiment anesthetic supplementation was given when necessary. Polypropylene catheters (PE-50, Portex®, Kent, England) were used to cannulate the right carotid artery, the right jugular vein, the right femoral artery and the tail artery. The carotid artery was used for bleeding to achieve the desired degree of shock and to obtain the blood samples (T0). To maintain patent lines catheters and to compensate for ion and water losses, catheters were rinsed with 1 ml/ 100g BW/h normal saline. The volume replacement was given by the right femoral vein, and the tail artery was used for continuous recording of MAP. After shock and resuscitation, the carotid artery and jugular vein catheters were occluded and left in the cervical and torso subcutaneous. The tail artery and right femoral vein catheters were withdrawn and the skin closed and anesthetized with 1% lydocain (Cloridrato de Lidocaína1%, Geyer Medicamentos®, Porto Alegre, Brazil). The rats were allowed to recover in appropriate cages with indirect heating and free water access. After 6 hours, animals were once more anesthetized, catheters rinsed and MAP recorded. A transverse laparotomy was performed, the common bile duct was cannulated, and the bile was collected and measured over the entire length of the experiments and standardized per gram of liver wet weight. At the end of the experiments blood samples (T6) were taken and the animals were sacrificed with lethal doses of sodium thiopental. All blood samples were centrifuged, the plasma frozen and stored at -80ºC and livers were weighted.

Experimental protocol

The rats were bled at a rate of 0.5 ml/min within 10 minutes to induce shock (MAP, 45 mmHg) and this level was kept for 60 min. Further bleeding or reinfusion of shed blood was performed, when necessary to maintain MAP stable during the shock phase. At the end of the shock phase the animals were randomly assigned in two groups, according to the solution to be infused:

1. RL (n=8): received Ringer Lactated (four times shed blood volume) within 20 min.
2. HSS (n=8): received NaCl 7,5% (10% shed blood volume) within 2 min.

After the initial resuscitation phase (20 min) the animals received 40 ml/Kg/h normal saline during more 20 min, whereas the wounds were closed. An additional group of three animals served as controls. They were subjected to the surgical procedure but without shock and resuscitation. Blood samples were taken similarly and bile flow collected and recorded.

Sampling and assays
Alanine aminotransferase (ALT) and total bilirubins

The plasmatic ALT and total bilirubins assays were made with standard kits (LABTEST® - Diagnostic, Belo Horizonte, Minas Gerais, Brazil).

Statistical analysis

Data are expressed as means ± standard error of mean (± SEM). The results were tested using the Sigma Stat 3.0 and graphics made with Sigma Plot 8.0. Between the treatment groups data were compared using the Mann-Whitney U-test. Multiple comparisons among the control and treatment groups were tested at each time point by Kruskal-Wallis analysis of variance on ranks followed by a Dunn's test. Comparison within the groups to test for time effects was performed by means of ANOVA for repeated measures on ranks, followed by Tukey test, or Wilcoxon signed rank test, when appropriated. The level of significance was assigned to $p<0,05$.

Results

Mean arterial pressure

There was no difference in the MAP between the groups during the experiments. Initial MAP values were around 110 mmHg, and after 6h of HS and resuscitation the MAP averaged values near 70 mmHg.

Bile flow

Mean bile flow was 0,34 ± 0,02 ml/g liver/min in the RL group, 0,48 ± 0,03 ml/g liver /min in the HSS group and 0,64 ± 0,04 ml/g liver /min in the control group. Bile flow was significantly higher in the HSS group in comparison to the RL group ($p<0,05$), but without difference to the control group (Figure 1).

ALT and total bilirubins

In the RL group serum ALT ranged from 37,1 ± 2,2 U/l (T0) to 169,6 ± 3,9 U/l (T6) ($p<0,001$). In the HSS group, ALT varied from 39,1 ± 2,2 U/l (T0) to 99,4 ± 2,2 U/l (T6). ALT activity was significantly lower after HSS treatment ($p<0,001$) (Figure 2). Serum total bilirubins in the RL group ranged from 0,23 ± 0,01 mg/dl (T0) to 1,68 ± 0,05 mg/dl (T6) ($p<0,001$). In the HSS group the values were 0,22 ± 0,01 mg/dl (T0) and

0,62 ± 0,03 mg/dl (T6) (p<0,001). After 6h of resuscitation, the total bilirubins were significantly lower in the HSS group (p<0,001) (Figure 3).

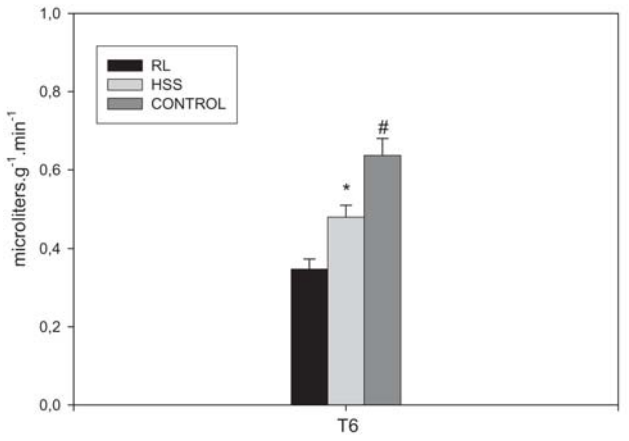

FIGURE 1 - Bile flow in RL (n=8), HSS (n=8) and control (n=3) groups. *p<0.05 vs LR. # p<0.001 vs LR. Kruskal-Wallis analysis of variance followed by Dunn's test. Data are means ± SEM.

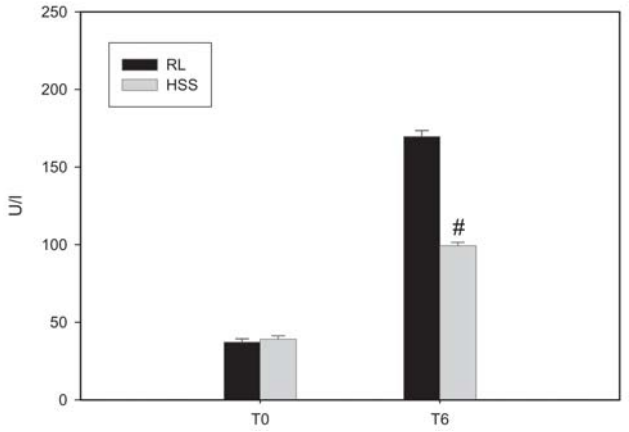

FIGURE 2 - Serum ALT levels at baseline (T0) and after 6h treatment (T6) in groups RL (n=8) and HSS (n=8). # p<0,001 vs RL. Mann-Whitney U test. Data are means ± SEM.

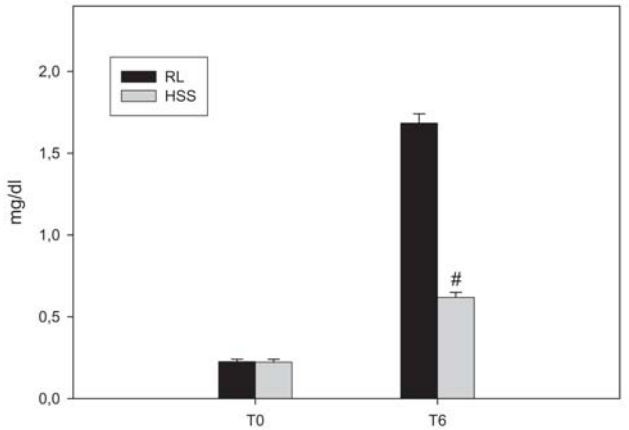

FIGURE 3 - Serum total bilirubin levels at baseline (T0) and after 6h of resuscitation (T6) in LR (n= 8) and HSS (n=8) groups. # p <0,001 vs LR. Mann-Whitney U test. Data are means ± SEM.

Discussion

Hemorrhagic shock and the consequences of its treatment (ischemia and reperfusion) are considered etiologic factors to acute hepatic liver failure. This can be noticed by the significant bile flow decrease and significant increase of the transaminases activity and plasmatic bilirubins [6,14,15]. It has been demonstrated that long periods of hepatic hypoperfusion promote structural deterioration, metabolic dysfunction and microcirculatory disorders, which are early detectable after 2 hours of ischemia. Until this moment, these phenomena are reversible. Therefore, the time to start the fluid reposition and the duration of reperfusion are determinant to the injury generation [15]. Changes in the bile flow after shock and resuscitation are directly related to the hepatocytes blood supply and are consequence of the dysfunction of Na^+/K^+ ATPase pump [12]. Thus, severe shock can decrease or even stop the bile flow, with only a partial recovery after resuscitation to levels around 70% of the baseline [6,15]. When the groups were compared after 6 hours of resuscitation, we verified a significant reduction of bile flow in the RL group compared to the controls, and it was also significantly smaller than the HSS group. The RL bile flow was only 53% of the control group, whereas the HSS bile flow corresponded to 75% of the controls, showing recovery to baseline levels. These findings are solid evidences about the superiority of the HSS therapy in the recovery of the hepatic microcirculatory blood supply and decreasing of the anaerobic cycle of the hepatocytes, while the RL treatment was not able to revert the metabolic depression of the shock state after 6 hours of the reperfusion in the same extension. The ALT is released from the hepatocytes according to the severity of cell damage and is, therefore, an useful marker of liver dysfunction [16]. In line with Jarrar et al [16], our results showed a significant increase of the plasmatic ALT activity in both treatment groups after shock and resuscitation. However, after 6 hours of resuscitation this increase was significantly attenuated in the HSS group. Some authors have demonstrated that RL resuscitation (3 times the shed blood) restore the hepatocellular function immediately after resuscitation [17-19]. However, ours results have demonstrated that after 6 hours of the resuscitation the hepatocellular function remains compromised, showing clearly a significant reduction in the bile flow. In this experimental model of HS and resuscitation we showed an improvement of the hepatic function in the HSS group, demonstrated by bile flow recovery to baseline levels, and also a maintenance of the hepatic cells integrity, determined by decreased values of plasmatic bilirubins and ALT. The bile flow recovery, the small increase of plasmatic bilirubins and ALT levels in the HSS group strongly suggest a clear improvement of the hepatic nutrition, a better cellular perfusion and less hepatocellular injury. Our results demonstrate the HSS superiority when compared to RL related to hepatic function and integrity after 6 hours of resuscitation of HS in rats.

Conclusion

Resuscitation of HS with NaCl 7.5% promoted better recovery of liver function and lesser hepatocellular damage

after 6h of treatment compared to RL. The improvement is very likely related to increased microvascular perfusion provided by small volume resuscitation.

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