

Apelin protect against multiple organ injury following hemorrhagic shock and decrease the inflammatory response

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ABSTRACT

Introduction: Hemorrhagic shock (HS) result in multiple organ injury and inflammatory response that lead to death. The exact mechanism is not clear. Apelin is an endogenous ligand of orphan G-protein coupled receptor APJ. Apelin has anti-inflammatory effects on the release of inflammatory mediators. **Objectives:** To examine the protective effects of apelin against multiple organ injury and the possible involvement of inflammatory pathways. **Methodology:** Male Sprague-Dawley rats (300–350 g) were subjected to hemorrhage over 60 min to reach a mean arterial blood pressure of 40 mmHg. Then, rats were treated or not with 1 mL of 10 nm/L apelin-13 intraarterially resuscitation was performed *in vivo* by the reinfusion of the shed blood for 30 min to restore normotension. Blood samples were collected for measurement of tumor necrosis factor (TNF) using ELISA (R and D systems). Biopsies were obtained from organs for light microscopic examination. **Results:** HS rats showed significant increase the levels of TNF. Apelin significantly lowered the production of TNF- α . Histological examination of hemorrhagic shocked untreated rats revealed structural damage. Less histological damage was observed in the organs of treated rats. Apelin-treatment decreased the number of inflammatory cells and mitochondrial swollen in cells. **Conclusion:** Treatment with apelin before resuscitation protects against multiple organ injury in HS by attenuation the inflammatory response and might be a therapeutic target for HS.

Key words: Apelin, hemorrhage, rat, shock, structure, tumor necrosis factor

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INTRODUCTION

Hemorrhagic shock (HS) and resuscitation is known to result in multiple organ injury and activate inflammatory responses that can lead to death.^[1] Despite the advances in critical care, still hemorrhage is a leading cause of death following trauma.^[2] HS, trauma, and multiple organ dysfunction remain a challenge in the treatment of trauma patients. The exact mechanism (s) is not clear. However, there are several mechanisms involved in the pathogenesis of multiple organ injury and failure

following hemorrhagic shock. One known mechanism is the activation of inflammatory pathways.^[3,4] Hemorrhagic shock and resuscitation activate an inflammatory process that can increase the risk of death after trauma,^[5] HS activate the immune system that result in the activation of monocytes and leukocytes.^[5] The immune cells hereby will produce proinflammatory markers as well as reactive oxygen species. The inflammatory response will trigger a series of reactions that lead to tissue injury.^[6,7] Activation of the inflammatory pathways is associated with multiple organ injury, dysfunction, and failure.^[8,9] Tumor necrosis factor (TNF) is one of the most characteristic inflammatory factors contributing to organ injury following HS.^[10-12]

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Apelin is a novel endogenous ligand for the G-protein-coupled receptor APJ. Apelin receptors are present in the central nervous system and the peripheral tissues, in the heart, lung, kidney, and endothelial cells^[13] and endothelial cells.^[14] Apelin has been shown to be involved in the regulation of cardiovascular function, angiogenesis, fluid homeostasis, and energy metabolic. It is becoming apparent that apelin may play a pathophysiological role in many of the regulatory systems.^[15] Progress has been made in recent years in clarifying the significance of apelin, much remains to be discovered. Apelin was shown to have anti-inflammatory effects on inflammatory mediator release.^[16,17]

This study investigated the protective effects of apelin administration before resuscitation of hemorrhagic in preventing multiple organ injury. Furthermore, we analyze the possible mechanism of protection by analyzing the potential of apelin to lower systemic TNF production following resuscitation.

METHODOLOGY

Animal preparation

The study was approved by the Ethical Committee at King Abdul-Aziz City for Science and Technology. Male Sprague-Dawley rats were injected intra-peritoneally (i.p.) with heparin sodium 2000 IU 15 min prior to anesthesia. The rats were then anesthetized using urethane 125 mg/kg i.p. The left carotid artery was cannulated using polyethylene tubing size 60, and was connected to an in-line pressure transducer for continuous blood pressure monitoring. Animals were allowed to stabilize for a period of 30 min. The animals were assigned randomly to 33 experimental groups ($n = 6$ per group): (1) Normotensive rats (N), (2) HS rats and (3) hemorrhagic shock rats treated with apelin-13 (HS-AP).

Hemorrhage and resuscitation

As previously described,^[18-21] rats were hemorrhaged using a reservoir (a 10 mL syringe) that is connected to the arterial (carotid artery) three-way stopcocks. Opening the stopcock and aspirating gently and gradually with the syringe will induce hemorrhage. Blood was aspirated at a rate of 1 mL/min. Blood was continuously withdrawn or re-infused to the animal to maintain a mean arterial pressure of approximately 40 mmHg. The same surgical procedures were performed as for the sham hemorrhage group except rats will not be hemorrhaged.

After 60 min hemorrhage period, hearts were resuscitated *in vivo*, by reinfusion of the shed blood to restore normotension and blood pressure were monitored for 30 min. Rats were either treated or not with an intra-arterial injection of apelin-13.

Experimental groups

Three experimental groups ($n = 6$) were assigned for the study:

- Normotensive rats (N) - rats will undergo the same surgical preparation and were monitored for continuous blood pressure measurements for the experimental period 120 min
- HS rats - after 30 min stabilization period, rats were hemorrhaged to 40 mmHg for 60 min. Rats were then resuscitated and monitored for 30 min
- Effect of apelin-13 during HS (HS-AP) - after 30 min stabilization period, rats were hemorrhaged to 40 mmHg for 60 min. One milliliter of 10 nm/L apelin-13 was injected intra-arterially. Rats were then resuscitated and monitored for 30 min.

Light microscopy

To perform a histological examination of multiple organs, rat heart, intestine, kidney, and liver were harvested and stored in 10% formalin solution ($n = 6$ in each group). We obtained 2 transverse sections per organ for histological examination and sections were stained with hematoxylin and eosin (H and E) stain. The areas affected by HS and resuscitation consisting of inflammatory cells, necrosis, and hemorrhage, were determined in the H and E staining. All data were analyzed in a blind fashion.

Tumor necrosis factor measurements

Blood (0.5 mL) was collected from the left carotid artery cannula before hemorrhage, before resuscitation and 30 min after resuscitation and centrifuged at 2500 g for 10 min and plasma was stored at -80°C until analysis for TNF- α measurement. Serum samples were analyzed by ELISA.

Statistical analysis

Data were initially analyzed with Bartlett's test for homogeneity. Data found not to be homogeneous were transformed and reanalyzed. Data were analyzed with multivariate analysis of variance. Means were analyzed using Duncan's test and were considered significant when yielding a $P \leq 0.05$. Data were expressed as means \pm standard deviation.

RESULTS

The animals were subjected to HS to lower the mean arterial blood pressure to the desired level of hypotension (35–40 mmHg). The total volume of blood withdrawn was 15 ± 1.1 mL/kg body weight. There was no significant difference in the amount of blood withdrawn among the groups of animals subjected to HS.

Apelin decreased the inflammatory response to hemorrhagic shock

As observed in Figure 1, HS caused a significant increase in the serum levels of TNF- α when compared to normotensive

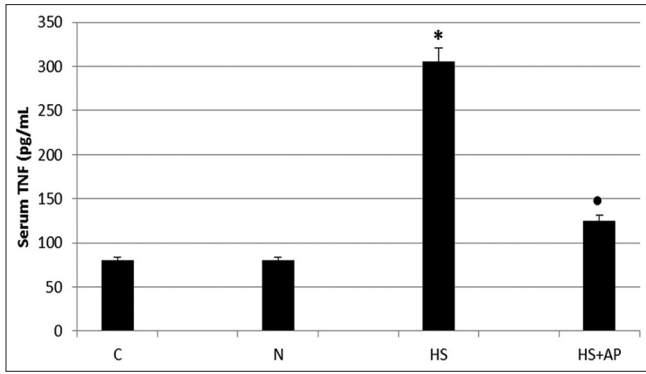


Figure 1: Hemorrhage increased tumor necrosis factor- α levels. * $P < 0.05$ versus sham group ($n = 6$ per group), and * $P < 0.05$ versus hemorrhage group ($n = 6$ per group)

group ($P < 0.05$). Treatment with apelin before resuscitation following HS significantly lowered the levels of TNF- α [Figure 1].

Apelin decrease organ injury after hemorrhagic shock

HS and resuscitation caused multiple organ injury [Figure 2]. Heart sections in the H and E-stain showed areas of hemorrhage, coagulation necrosis with diffuse edema and neutrophil infiltration [Figure 2a-c]. Blood vessels in the heart sections showed swollen endothelium with neutrophil infiltration [Figure 2d and e].

Sections from multiple organs of treated animals before resuscitation of HS showed normal tissue with no evidence of

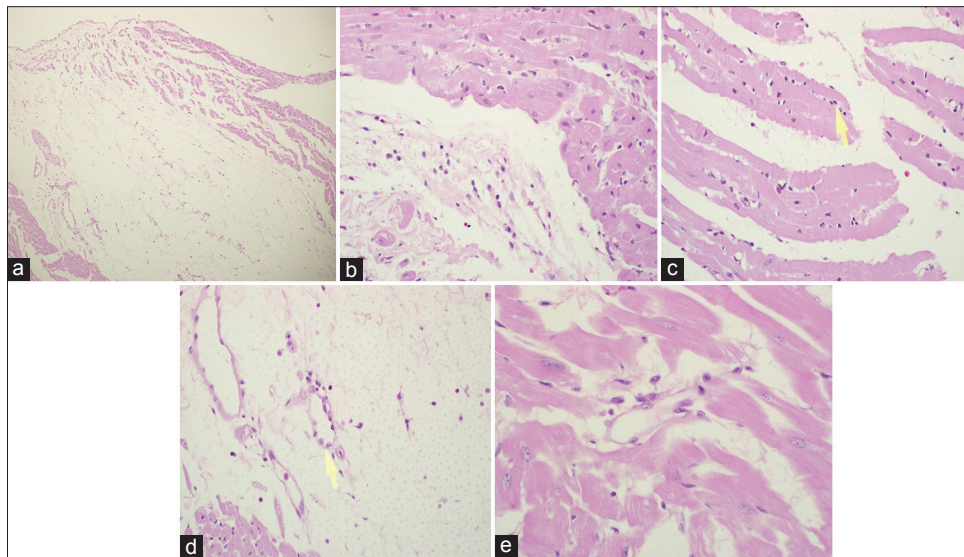


Figure 2: Light micrographs of multiple organs from hemorrhagic shocked rats. (a-c) Heart section showing inflammatory cell infiltration around blood vessels and edema (H and E, $\times 100$, $\times 400$ and $\times 400$). (d and e) Endothelium of blood vessel in the heart, showing swollen endothelium with neutrophil infiltration (H and E, $\times 400$ and $\times 600$)

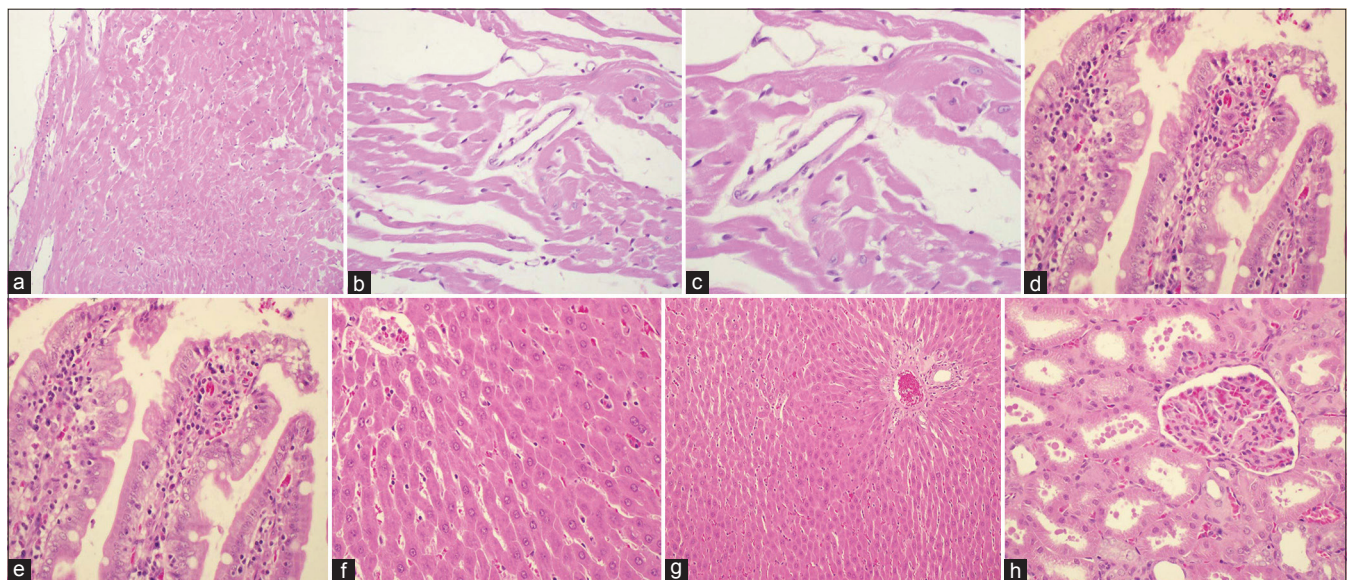


Figure 3: Light micrographs of multiple organs from hemorrhagic shocked rats treated with apelin showing normal tissue. (a) Heart section (H and E, $\times 200$). (b and c) Endothelium (H and E, $\times 400$ and $\times 600$). (d and e) Intestinal section (H and E, $\times 100$ and $\times 400$). (f and g) Liver section (H and E, $\times 400$ and $\times 200$). (h) Kidney section (H and E, $\times 600$)

inflammation and tissue injury. Figure 3 shows tissues obtained from multiple organs from animals treated with apelin before resuscitation following HS. The inflammatory infiltration and the increase in the number of neutrophils were prevented by treatment with apelin.

DISCUSSION

Despite the advances in critical care, resuscitation strategies fail to prevent deleterious inflammatory responses. Resuscitation can cause systemic inflammatory responses that can lead to multiple organ injury, dysfunction, and death.^[8] This study showed that HS and resuscitation are associated with multiple organ injury and activation of inflammatory responses. The study showed that apelin may provide a therapeutic potential to resuscitation following HS. Animals treated with apelin showed no evidence of organ injury after resuscitation. One explanation is by lowering the inflammatory response to HS and resuscitation.

The results from the present study are in agreement with previous reports that showed that resuscitation following HS results in multiple organ injury and dysfunction.^[4]

Apelin has been shown to have anti-inflammatory effects.^[22] We speculated that apelin could protect against postresuscitation organ injury by attenuating inflammatory responses. The present study showed that apelin decreased the inflammatory response to shock and protected against postresuscitation organ injury.

However, the protective mechanism of apelin against multiple organ injury is not fully understood. Our previous study showed that apelin protect against myocardial dysfunction following *in vivo* and *ex vivo* resuscitation following HS.^[23] Previous studies have shown that apelin has a cardioprotective effect.^[24,25] Apelin is an endogenous substance that may involve activation of phosphatidylalipase C, protein kinase C, and sacrolemmal sodium hydrogen exchange, and sodium-calcium exchange.^[26]

The mechanisms of the apelin protective effects may be from enhancing the activity of sodium hydrogen exchange with consequent intracellular alkalization.^[27]

However, the activation of the inflammatory pathways is the key process in organ dysfunction following HS and the production of inflammatory markers such as TNF.^[8,25]

The results of the present study and other previous studies on apelin had led to speculation that apelin could be a future target for therapeutic strategies following HS and resuscitation.

CONCLUSION

Apelin protect against multiple organ injury following resuscitation of HS by attenuation of the inflammatory response.

Apelin may attenuate the inflammatory.

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Conflicts of interest

There are no conflicts of interest.

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