

2. Summary

2.1. Preconditioning with heme-based solutions: influence on heme oxygenase-1 expression and organ failure after hemorrhagic shock

In clinical practice, blood transfusion is limited due to a lack of potential donors and the risk of transmission of infectious diseases. Thus, during the last decades several artificial oxygen carriers have been developed as alternatives to allogeneic blood transfusions. Diaspirin-cross-linked hemoglobin (DCLHb) seemed to be promising due to its oxygen carrying characteristics well as the independence of the blood group in case of need for blood products. However, in several studies primarily using DCLHb, several side effects of acellular haemoglobin have been identified, among which the pronounced effect of nitric oxide (NO) scavenging seems to be of particular importance. In addition, in clinical trials, treatment with DCLHb after hemorrhagic shock or ischemic stroke increased mortality. In contrast, heme arginate (HAR), a heme based solution without oxygen-carrying capacity and without NO-scavenging effect does not increase blood pressure. However, both classes of substances contain heme, which is the substrate and one of the most potent inducers of heme oxygenase 1 (HO-1). This microsomal enzyme catalyzes the initial and rate limiting reaction of heme catabolism, that is, the oxidative cleavage of heme molecules to yield equimolar quantities of biliverdin-IXa (antioxidative properties), carbon monoxide (vasodilatation), and iron. The induction of HO-1 may exert tissue protection against ischemic events such as hemorrhagic shock through vasodilatation or antioxidative properties of the enzyme system

Therefore, in the present study we investigated the effect of DCLHb and HAR on HO-1 protein expression in liver, kidney, heart, lungs, and aorta. Furthermore, we tested whether preconditioning with DCLHb or HAR reduces organ failure and mortality in a model of hemorrhagic shock and resuscitation.

The study was performed on anaesthetised, spontaneously breathing Sprague-Dawley rats.

In the first set of experiments, 24 hours after preconditioning with DCLHb (1, 2, or 3 g/kg body weight) or HAR (5, 25, or 75 mg/kg body weight) the induction of HO-1 and HSP-70 protein expression in the organs of interest was analyzed by Western blot analysis.

In the second set of experiments, 24 hours after preconditioning with DCLHb (1 or 3 g/kg body weight) or HAR (5 mg/kg body weight) the animals were subjected to hemorrhagic shock with a mean arterial blood pressure (MAP) of 30-40 mmHg for 1 or 2 hours followed by fluid resuscitation over 5 or 4 hours, respectively. Animals pre-treated with Ringer's solution (Vehicle, 30 ml/kg body weight) served as controls.

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In the third set of experiments, in order to evaluate the protective role of HO induction, HO activity was blocked in one Vehicle- and one HAR-group for each shock period, by intravenous injection of tin-mesoporphyrin-IX (SnMP-IX) a specific blocker of the heme oxygenase pathway. To quantify organ injury arterial blood samples were taken at baseline, end of the shock, and at the end of experiment. In addition, at the end of the experiment liver tissue was harvested by freeze-clamping and later analyzed for adenosine triphosphate (ATP) content.

DCLHB and HAR dose-dependently induced HO-1 protein in the tested organs compared to Vehicle at 24h after pre-treatment. Simultaneously, pre-treatment with DCLHb and HAR led to a dose-dependent increase in total bilirubin concentrations in serum assessed at 24h compared to Vehicle injection, which may reflect increased HO-activity due to induction of HO-1 protein.

Preconditioning with HAR resulted in decreased shock-induced organ injury after 1 or 2 hours of hemorrhagic shock compared to DCLHb and Vehicle. This was reflected in higher shed blood volumes for inducing hemorrhagic shock, delayed onset of decompensation while shock period, higher oxygenation index at the end of the shock period, and earlier recompensation after shock period. In contrast, DCLHb-preconditioning worsened shock-induced organ injury compared to Vehicle. In addition, DCLHb-preconditioning resulted in a further decrease of ATP-levels in liver tissue as compared to Vehicle and HAR. In total, DCLHb-preconditioning increased mortality in contrast to HAR-pre-treatment, which reduced shock-mediated mortality compared to Vehicle. Blockade of HO-activity by SnMP-IX abolished the protection mediated by HAR.

Although DCLHb as well as HAR dose-dependently induced HO-1 protein in the absence of an unspecific stress response only HAR-preconditioning protects against shock induced organ failure and mortality. Although the underlying mechanisms are not completely understood, the NO-scavenging and vasoactive effects of DCLHb may increase shock-mediated microcirculatory failure and organ dysfunction.

Thus, HAR-preconditioning prevents organ failure and decreases mortality after hemorrhagic shock and resuscitation, and may be a means of pharmacological protective preconditioning through induction of HO-1.