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EFFECTS OF SEVERE HEMORRHAGE ON *IN VIVO* BRAIN AND SMALL INTESTINE MITOCHONDRIAL NADH AND MICROCIRCULATORY BLOOD FLOW

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Severe body stress induced by hypoxemia and hypotension may lead to total body energy state deterioration. The perfusion of the most vital organs is maintained at the expense of "less vital" organs. In the present study, we used a multi-site multi-parametric (MSMP) monitoring system for real-time evaluation of tissue blood flow (TBF) and mitochondrial NADH fluorescence of the brain and the small intestine following hemorrhage. In Group 1, uncontrolled hemorrhage, mean arterial pressure (MAP) was decreased to 40 mmHg within 2 minutes and shed blood was re-infused after 30 minutes. In Group 2, controlled hemorrhage, during the 30 minutes of hemorrhage, MAP was kept at 40 mmHg. During hemorrhage, in both groups, the intestinal TBF and NADH deteriorated, while the brain remained relatively well protected. In Group 1, all parameters partly recovered within the hemorrhage phase, while in Group 2, complete recovery occurred only after resuscitation. At the end of the experiment, both models showed a decrease in intestinal viability (TBF decreased, NADH increased), while the brain metabolic state in Group 2 declined slightly. Our unique multi-parametric monitoring device demonstrated that, under hemorrhage, the small intestine responded entirely differently from the brain. This may suggest the potential usefulness of the monitoring of less vital organs, as proxy organs, in critical conditions such as massive hemorrhage. The present study also highlights the importance of mitochondrial function monitoring in similar conditions in the clinical environment.

Keywords: Mitochondrial dysfunction; multiparametric monitoring; Laser Doppler Flowmetery; fluorometric NADH monitoring.

1. Introduction

Severe hemorrhage involves a depression of cardiac output, decrease of perfusion pressure and oxygen delivery (DO_2), decreased systemic blood pressure, and a minor change in oxygen consumption (VO_2).[1–3] Severe and sustained decreases in DO_2 eventually overwhelm the micro-vascular response, until the mitochondria cannot sustain aerobic metabolism and VO_2 declines. Under these conditions, cellular function depends on the aerobic and anaerobic mechanisms providing ATP; with insufficient ATP levels, irreversible tissue damage ensues. Under severe hemorrhage, cardiac output and perfusion pressure increase as a compensatory mechanism.[4]

However, under significant loss of intravascular volume, hemodynamic instability develops, tissue perfusion decreases, leading to cellular hypoxia, organ damage, and finally death.[1–3] The mortality rate of patients following the first 24 hours after hemorrhagic shock is 50%, and survivors are at a high risk of developing infection and organ failure.[5] Therefore, the early detection of the patients' condition is clearly important.[6,7] In cases of severe stress, such as hemorrhagic shock, blood is re-distributed to the most vital organs, such as the brain, heart, and adrenal gland, whereas the "less vital" and peripheral organs and tissues, such as the gastrointestinal (G-I) tract, muscles, and skin undergo vasoconstriction and a decrease in blood flow and oxygen supply,[8,9] making them a useful, early indicator of the body condition.

In the present study, we developed a unique multi-parametric monitoring device for real-time measurements of the cerebral cortex simultaneously with the small intestine in various hemorrhage models in rats. We also tested the effects of controlled vs. uncontrolled hypotension, on the responses of these two organs.

2. Methods

2.1. *The multi-site multi-parametric (MSMP) monitoring system*

In the present study, the MSMP system enabled simultaneous monitoring of the brain and small intestine. The MSMP (Fig. 1) monitors both NADH redox state

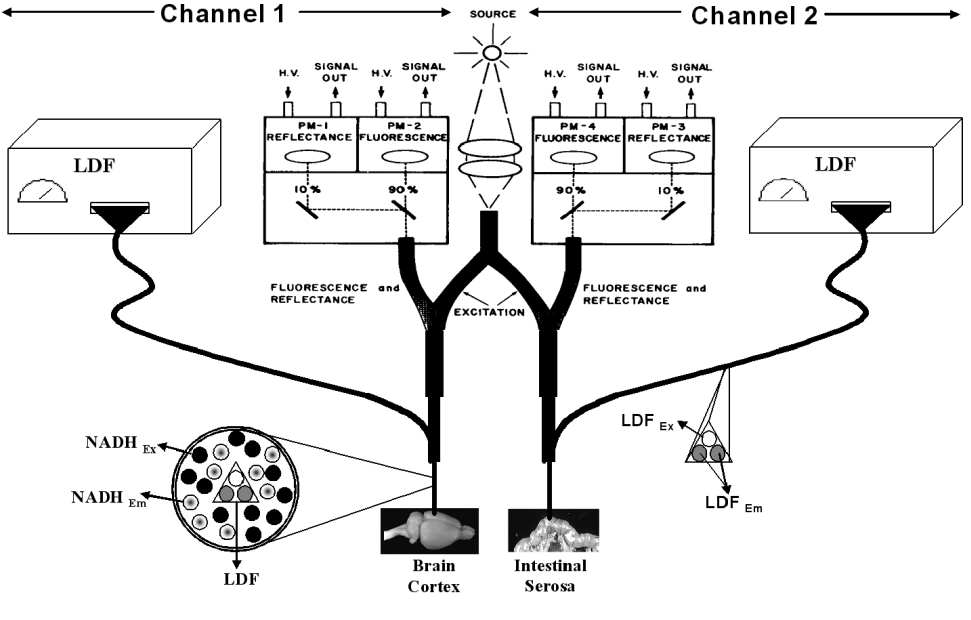

Fig. 1. Schematic representation of the MSMP probe containing optical fibers for NADH monitoring (Ex – Excitation, Em – Emission, H.V. – High Voltage) and for tissue blood flow (Laser Doppler Flowmetry – LDF).

using fluorometry[10,11] and tissue blood flow (TBF) using Laser Doppler Flowmetery (LDF) from the same location, simultaneously in each organ. More details are presented at our previously published article.[12]

2.2. *Animal preparation*

All experiments were performed in accordance with the guidelines of the Animal Care Committee of Bar-Ilan University. Wister male rats (220 g–300 g) were anesthetized by an intraperitoneal (IP) injection of 0.3 mL/100 g equithesin. A heating pad was placed under the rat to maintain the body temperature at 37°C and a rectal thermistor probe was inserted to measure body temperature. The cerebral cortex and the small intestine were exposed and the monitoring probes were placed on the two organs as detailed in our recent article.[12] The femoral arteries were cannulated for blood pressure monitoring and bleeding, and the femoral vein was cannulated for the resuscitation phase. In Group 1 (the uncontrolled hypotension group), hemorrhage was induced until blood pressure decreased to 40 mmHg and no further interference was made for a period of 30 minutes. Whereas in Group 2 (the controlled hypotension group), blood was withdrawn until mean arterial pressure (MAP) reached the level of 40 mmHg and was maintained at this level for 30 minutes, after reperfusion had begun. In both groups, shed blood was re-infused following the 30 minutes period and monitoring proceeded for additional 2 hours. At the end of the experiments, the rats were scarified by the inhalation of pure N_2.

3. Results

In order to evaluate the reliability of our continuous monitoring model, a group of 4 rats were monitored for 180 minutes with no further perturbation. The results indicated that throughout the entire experiment, there were no significant changes in any of the parameters in both organs (results not shown).

When uncontrolled hypotension was induced in Group 1 (Fig. 2), MAP immediately decreased (44 ± 5 mmHg, $p < 0.01$) and remained significantly low for 10 minutes, followed by a full recovery within the hemorrhage phase. The intestinal responses were greater and faster than those of the brain. Following bleeding, intestinal TBF immediately decreased by $5 \pm 21\%$ ($p < 0.01$) and later almost fully recovered. In contrast, in the brain, TBF increased by $13 \pm 28\%$ ($p < 0.05$) only several minutes after hypotension induction and remained high for the entire period. Intestinal reflectance increased ($3 \pm 8\%$, $p < 0.05$) and then decreased back to its basal level, while cerebral reflectance remained stable. Intestinal NADH immediately increased ($5 \pm 13\%$, $p < 0.05$), followed by full recovery, whereas cerebral NADH did not significantly change. During resuscitation and afterwards, MAP remained relatively stable. Intestinal TBF transiently recovered reaching high levels of 115% followed by a gradual decrease by $10 \pm 35.5\%$ to very low levels. In comparison, TBF in the brain slowly increased reaching a level of $0.12 \pm 147\%$. About 20 minutes after resuscitation, the intestinal reflectance decreased by $2 \pm 11\%$

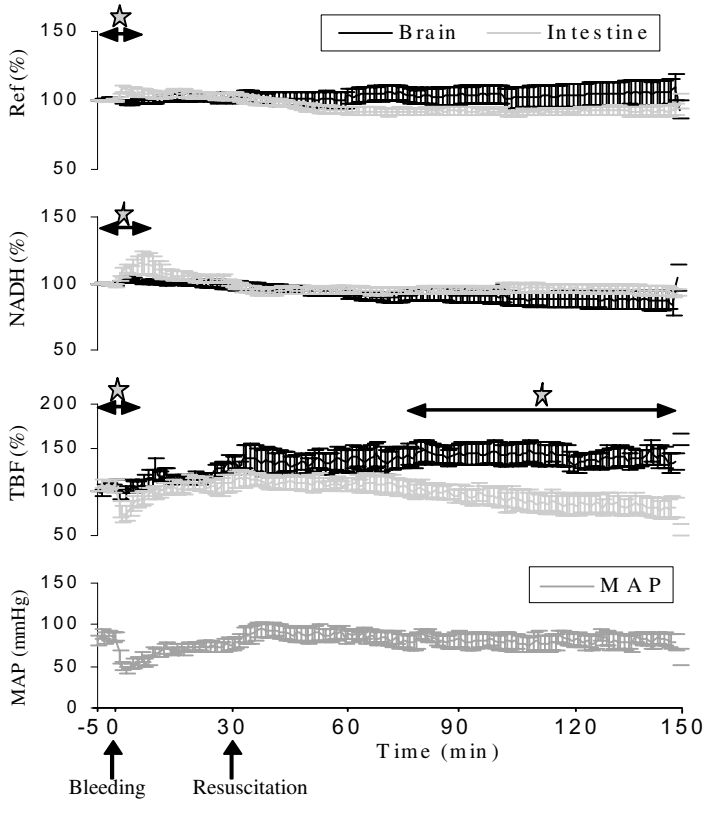

Fig. 2. The responses of the brain, small intestine, and MAP to uncontrolled hypotension for 30 minutes (Group 1, $n = 9$, mean $\pm$ S.E.). The arrows represent the period in which significant differences were found between the two organs in each minute. (*) $p < 0.05$. REF – reflectance; NADH – mitochondrial NADH redox state; TBF – tissue blood flow; and MAP – mean arterial pressure.

($p < 0.05$) and remained significantly low for an hour, while the cerebral reflection remained stable. However, NADH in both organs remained stable, with no significant changes.

The comparison between organs showed significant differences in all of the parameters during the bleeding session ($p < 0.05$). Tissue blood flow (TBF) showed major differences between the organs, during the bleeding session and an hour after resuscitation ($p < 0.05$).

Upon controlled hypotension induction in Group 2 (Fig. 3), MAP significantly decreased (62 ± 1 mmHg, $p < 0.001$). The intestine and the brain responded quite differently. Intestinal TBF significantly decreased ($7 \pm 30\%$, $p < 0.01$), while TBF in the brain remained relatively stable. Intestinal reflectance significantly increased ($7 \pm 28\%$, $p < 0.01$), while the cerebral reflectance remained stable. Intestinal NADH had two phases, an increase of $10 \pm 32\%$ ($p < 0.01$), followed by a slight decrease of 10 minutes after bleeding began. Cerebral NADH increased significantly

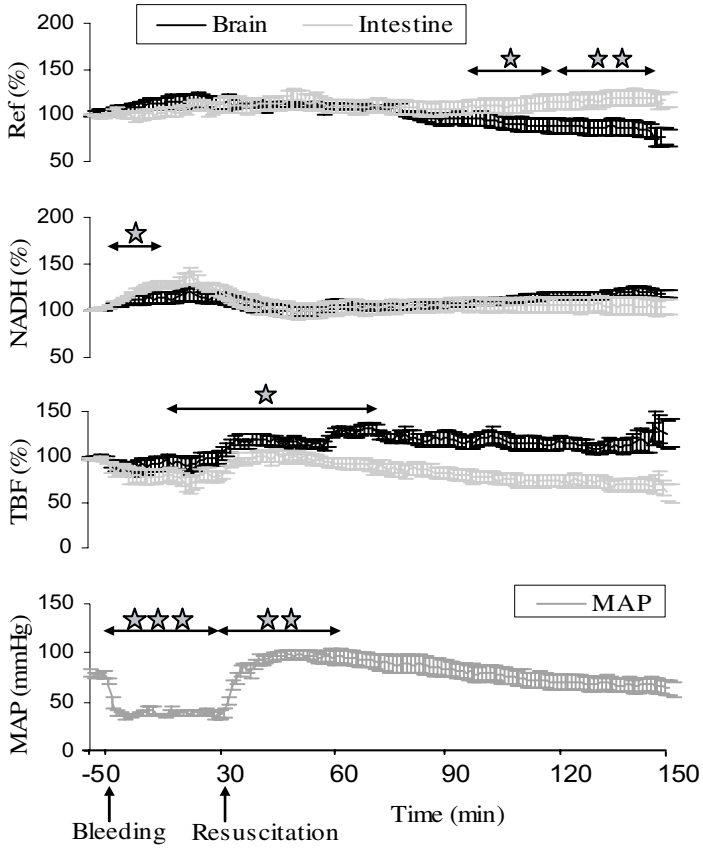

Fig. 3. The responses of the brain, small intestine, and MAP to controlled hypotension for 30 minutes (Group 2, $n = 9$, mean $\pm$ S.E.). The arrows represent the period in which significant differences were found between the two organs in each minute.

(*) $p < 0.05$, (**) $p < 0.01$, (***) $p < 0.001$. Abbreviations are as presented in Fig. 2.

($2 \pm 9\%$, $p < 0.01$) and was the only parameter in the brain that showed a significant response.

Following resuscitation, MAP levels fully recovered. However, about an hour after resuscitation, MAP decreased by 14 ± 5 mmHg ($p < 0.01$), until reaching significantly low levels. About 50 minutes after resuscitation began, intestinal TBF levels were again significantly low following a decrease of $12 \pm 39\%$ ($p < 0.01$), while TBF in the brain increased. Following resuscitation, intestinal reflectance decreased and gradually returned to its basal level (with no significant change). Cerebral reflectance also decreased gradually with no significant change. Intestinal NADH level showed a trend of decrease below the basal level, but then it increased towards the basal level and remained steady for the rest of the experiment. Cerebral NADH decreased gradually but without a significant alteration, except for resuscitation itself and several minutes afterwards where changes were significant

($p < 0.01$). When comparing the responses of the intestine and the brain to the bleeding episode, significant differences can be seen in the reflectance ($p < 0.01$) and in NADH ($p < 0.05$). During resuscitation, there were significant changes in the reflectance ($p < 0.01$) and in the TBF ($p < 0.05$).

4. Discussion

According to the global study of diseases, 30%–40% of trauma mortality is attributable to hemorrhage.[13] Moreover, patients who survive are at a high risk of developing infection and organ failure.[5] Early diagnosis of hemodynamic catastrophe and early resuscitation are crucial in hemorrhagic shock;[6,7] therefore, many experimental studies are conducted using various models of hemorrhage. In this study, we tested 2 models: uncontrolled and controlled hypotension. These 2 models actually differ in the time of hypotension, which was very short in the uncontrolled group (~ 2 minutes) due to the activation of auto-regulation mechanisms,[4] and longer (30 minutes) in the controlled hypotension group. The distinction between the organs is thus clearly emphasized. In the intestine, even a very short hypotension produced tissue-state deterioration. In contrast, in the brain, the injury level appeared to be correlated to the duration of hypotension, namely, the longer the hypotension phase, the greater the ischemic reperfusion injury. In both models, during hemorrhage, the intestinal tissue deteriorated, as reflected in its hypo-perfusion state and the elevation of NADH level. These observations suggest the use of the small intestine as a proxy organ for monitoring in similar clinical conditions. Regarding the brain, although most parameters were not significantly changed, there was still an increase in NADH level, suggesting the importance of mitochondrial function monitoring for an early detection of tissue deterioration, rather than monitoring TBF alone.

Acknowledgment

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