Title

Peptidomic characterization of hemorrhagic shock plasma samples: Effects of tranexamic acid

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digestive enzymes play a central role in the pathogenesis. Digestive enzymes are synthesized in the pancreas and transported with food along the small intestine, but can escape across the mucosal barrier out of the lumen into the wall of the intestine. This leakage of digestive enzymes in oxygen and energy-depleted states, such as hemorrhagic shock, is due to breakdown of the mucosal epithelial barrier in the intestine. Digestive enzymes are transported not only into the wall of the intestine but also further into the systemic circulation via the portal venous system, the intestinal lymphatics and via the peritoneal space.

Methods: Preclinical experiments with hemorrhagic and endotoxic shock in the rat and in pigs.

Results: The presence of digestive enzymes in tissues outside the intestine can have major consequences. Intestinal villi break down, leaving the digestive enzymes in the lumen of the intestine directly exposed to all intestinal tissues. In the lung and in the heart overt lesions in the form of microhemorrhages in the interstitial space are seen. The lung also becomes edematous compromising pulmonary function.

Digestive enzymes such as trypsin and chymotrypsin can activate other pro-enzymes from an inactive to an active state. One such family is the matrix metalloproteinases (MMPs), whose activation leads to further degradation of extracellular proteins and membrane structures. Digestive proteases and MMPs cleave important membrane receptors, thereby undermining the cellular functions carried out by these receptors. For example, cleavage of the extracellular domain of the insulin receptor leads to loss of insulin binding with intracellular signaling and therefore to insulin resistance, a phenomenon observed in post shock periods. Similarly, cleavage of the alpha-adrenergic receptor in vascular smooth muscle leads to a lack of cell signaling by adrenergic vasopressors, such a phenylephrine, and decreased smooth muscle contraction that results in an associated drop in central artery blood pressure, ie, vasopressor resistance. Besides these actions there is concomitant formation of shorter peptides by proteolytic degradation of plasma proteins including proteins of the coagulation cascade, complement proteins, serine protease inhibitors, C-reactive proteins, heat shock proteins and others.

Conclusions: Blockade of digestive enzymes in the lumen of the intestine serves to reduce the tissue destruction and loss of cell functions seen otherwise in shock and other low-flow states. This type of enteral blockade requires high levels of enzyme inhibitors, since digestive enzymes are present in high concentrations. Furthermore it requires placement of inhibitors over the full length of the small intestine, since even short segments of the intestine that allow entry of digestive enzymes may lead to autodigestion with systemic organ dysfunctions. The clinical efficacy of such an enteral approach remains to be determined.

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Peptidomic characterization of hemorrhagic shock plasma samples: Effects of tranexamic acid

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Objectives: Hemorrhagic shock is still one of the main causes of mortality in the intensive care units. Previous studies enlighten the fundamental role of the intestine in shock: according to the so called "Autodigestion hypothesis" the intestinal mucosal barrier, damaged by hypoperfusion, allows digestive enzymes, proteases and lipases to escape the lumen and reach the systemic circulation leading to a strong inflammatory reaction and multi-organ dysfunction. Moreover, the resulting increased proteolytic activity causes the cleavage of several membrane receptors on the endothelial cells with consequent loss of cellular functions. A label-free quantitative proteomic investigation, conducted in our laboratory, demonstrated for the first time that plasma displays an increase in peptides possibly generated by serine proteases after hemorrhagic shock, linking proteases to the larger presence of circulating peptides [1]. To further confirm these findings, we adopted the same "peptidomic" approach to verify the possible protective effects of protease inhibitors, such as TXA (tranexamic acid), against the uncontrolled proteolytic activity.

Methods: We performed a LC-ESI MS/MS analysis to compare the peptidome of plasma samples from hemorrhagic shock rats treated with or without TXA. For each animal, 2 plasma samples were collected: at the beginning (BL) and at the end of the experiment (END). The computational analysis of peptidomics data was performed with MaxQuant and Perseus software. Peptides were also searched in SATPdb, a database of structurally annotated therapeutic peptides.

Results: The analysis shows a significantly decreased number of peptides in HS plasma treated with TXA compared to HS plasma. This effect is more evident in the "END" samples. In addition, SATPdb analysis suggests the possible presence of antihypertensive peptides.

Conclusions: Our data show a large decrease of circulating peptides in HS samples in the presence of TXA. These results allow quantitatively supporting the "Autodigestion hypothesis", suggesting an increased proteolytic activity in plasma samples after hemorrhagic shock. Further analyses are in progress to better elucidate the possible proteases involved and to test protease inhibitor-based strategies, which could interfere with shock lethal course.

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Reference

[1] F. Aletti, E. Maffioli, A. Negri, M.H. Santamaria, F.A. De Lano, E.B. Kistler, Peptidomic Analysis of Rat Plasma: Proteolysis in Hemorrhagic Shock. Shock. 2016;45:(5):540-554

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Clustering shock in the acute phase using graphical models

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Objectives: Cardiogenic shock is defined as the state of inadequate circulation of blood due to ventricular failure caused by acute cardiac