Studies Regarding The Effect Of Statins On The Inflammatory Process Using An Experimental Sepsis Model

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Background

Sepsis is a disease that is mostly found in the *Intensive Care Units* (ICU) where it accounts for the majority of deaths. Its main characteristic is the systemic inflammatory response syndrome (SIRS) triggered by an infection. Until recently it was widely accepted that although the syndrome is triggered by a specific pathogen, its course is dominated by the exaggerated response of the immune system and release of a number of factors including pro-inflammatory cytokines, chemokines and complement activators whose action turns out to be destructive for the host – this is known as the *uncontrolled inflammatory response theory.*

Numerous studies have shown, however, that the immune reaction of the human body is much more complex and multiple dysfunctions that occur are due to an imbalance and lack of coordination between pro-inflammatory and anti-inflammatory factors which drives the septic patient into a *hyper-immune* initial state followed by an *immunologic paralysis*.

Moreover, the host response may be different depending on the virulence of the pathogen, the inoculum size, concomitant illness of the patient, age or gene polymorphism responsible for the synthesis of different cytokines. Although multiple therapeutic targets were identified (high-mobility group B proteins, HMGB; C5a complement; C5aR complement receptor; macrophage migration inhibitory factor) and new methods of treatment developed (corticosteroids, activated protein C, tumor necrosis factor antagonist, interleukin-1 receptor antagonists, anti-endotoxin antibodies; ibuprofen) the number of deaths attributed to sepsis continues to grow. This could be explained by the wide variability of host response and the dynamic and unpredictable course of the disease.

In this context, the scientific community has raised the question whether *statins* can be the silver bullet that everyone awaits. The HMG-CoA-reductase inhibitor drugs (or statins) were introduced into clinical practice in the 1980s to lower cholesterol levels in patients with heart disease. HMG-CoA reductase is an enzyme receiving multiple control mechanisms and is also a limiting step in cholesterol biosynthesis. Statins inhibit this enzyme in a competitive and dose-dependent manner. Although this explains their lipid-lowering effect for which they were originally introduced in practice also provides clues about other biological effects and their potential toxicity. Inhibition of steroid precursors may have biological implications which are not limited to cell membrane lipid layers. The same metabolic pathway is involved in the production of several inflammatory, vasoactive or regulating compounds with impact throughout the entire body such as in gene expression, cell cytoskeleton, the trans-membrane transport, cell proliferation, migration, transformation and programmed cell death. The various biological effects, appart from the lipid-lowering one, are called "*pleiotropic*" from the Greek "pleion" meaning *many* and "tropos" which means *crossroads* and include an array of actions like cell proliferation, immuno-modulatory effects, anti-oxidant, anti-thrombotic, protection of endothelial function and the activation of vitamin D.

Experimental and clinical data have shown the beneficial effects of statins during sepsis, acute respiratory distress syndrome (ARDS) or sub-arachnoid haemorrhage (HSA). Because statin activity is not limited to some particular inflammatory mediators, these drugs may act by modulating the magnitude of the entire immune response. Several studies have reported a reduction in 30 days and 1 year mortality in critical patients who received statins before admission. Even patients who developed multiple organ dysfunction (MODS) but were on statin therapy had better

outcome than those with MODS of the same age and sex group but without statins.

Aims and objectives.

We intend to obtain an experimental model of sepsis using rats and further study the effects of Simvastatin and Rosuvastatin on the inflammatory process in a septic syndrome induced by cecal ligation and puncture. At the same time we directly compared the effects of the two statins. The data obtained will be used to identify protection mechanisms against multiple organ dysfunction syndrome (MODS) which characterizes the septic patient in order to improve the overall prognosis and survival.

The paper is based on two studies. The first aims at validating the experimental model and establish baseline values for the parameters further evaluated. The second study quantifies the effects of statins on the inflammatory process and compares them for Simvastatin and Rosuvastatin.

Material and Methods.

The general methodology involved the use of 30 Wistar rats thus respecting the rules and requirements in force for limiting the number of animals used in experiments. All stages of this research were approved by the Ethics Committee of the University of Medicine and Pharmacy Tirgu Mures and comply with EU directives on the protection of experimental animals. Rats were housed under controlled conditions, fed with standard rat chow (administered *ad libitum*) and kept on a sleep-wake cycle of 12 hours.

All animals were subjected to a period of 18 hours of fasting before the first dose of statin. Then they were allowed to feed during one hour and a second period of 18 hours of fasting was employed before the second dose of the statin. Same period of fasting was used for the group that didn't receive statins.

Anesthesia was obtained using Ketamine 100 μg/g and Xylazine 10 μg/g administered intraperitoneally. After induction of anesthesia the animals were placed under a heat lamp and a rectal probe was inserted for temperature monitoring. Fur was removed from the abdomen and a 1 cm incision was performed 2-3 cm left from the midline under aseptic and antiseptic conditions. After opening the abdominal cavity the cecum was identified and carefully retracted.

Then we used the cecal ligation and puncture (CLP) technique which consists in placing a ligature at 1 cm from the cecal end, distal from the ileo-cecal valve (ie without causing bowel obstruction) and a double perforation of the intestinal wall using a 19G needle. Then the cecum was gently pressed to extrude a small amount of faeces in the peritoneal cavity which will confirm the existence of a patent puncture and also initiate the septic process. The cecum was then re-introduced into the peritoneal cavity and the wound sutured.

All animals received fluid resuscitation with 0.9% sodium chloride (normal saline, NS) 0.05 ml/g injected subcutaneously with a 26G needle. This fluid dose was repeated every 24 hours throughout the duration of the experiment. Pain control was obtained using Tramadol 20 μg/g every 24 hours administered subcutaneously with the first dose immediately after surgery. If animals showed clinical or specific behavior signs of insufficient analgesia, pain medication was supplemented.

Statins were administered orally in two doses 18 and 3 hours before induction of anesthesia. The dose was 80 μg/g Simvastatin or 20 μg/g Rosuvastatin. Until complete reversal of anesthesia animals were continuously monitored ensuring a normal body temperature. Surveillance continued for 14 days after which the animals were euthanized with a dose higher than 100 mg/kg intra-cardiac Sodium Pentobarbital.

We analyzed body weight modifications, total leukocyte count (WBC), the percentage of lymphocytes (LYM%), serum Procalcitonin (PCT), Interleukin 1 β (IL-1β), Interleukin 6 (IL-6) and Tumor Necrosis Factor alpha (TNFα) concentrations.

Conclusions

We concluded that the experimental model is suitable for such studies and the data obtained can be used for a better understanding of the inflammatory process during sepsis. Also the inflammatory process plays a central role in abdominal sepsis and is characterized by increased levels of IL-6, IL-1β and TNF. Regardless of statin used in the model of sepsis by CLP, this decreases the total number of leukocytes, Procalcitonin levels, IL-6 and TNFα. In particular, Simvastatin had a more pronounced anti-inflammatory effect by decreasing levels of PCT, TNF and IL-1β compared to Rosuvastatin. However, Simvastatin failed to decrease the level of IL-6 to such levels as those determined by Rosuvastatin. Rosuvastatin although it seems to have a lower anti-inflammatory effect, significantly decreased the levels of IL-6 starting from day 4 and kept them at a constant value throughout the experiment. The data suggest that both Simvastatin and Rosuvastatin effects vary in a time depending manner during the course of the disease.
Taking everything into account, Simvastatin appears to be superior to Rosuvastatin by promoting the inhibition of well known pro-inflammatory biomarkers.

Future studies should confirm these observations and further assess the extent to which these data can be extrapolated and used to treat the septic patient.

Keywords: *sepsis; statins; rat; inflammation*