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Comparing the anti-inflammatory effects of Simvastatin and Rosuvastatin by measuring IL-1 β , IL-6 and TNF- α levels using a murinic caecal ligation and puncture induced sepsis model

Compararea efectelor anti-inflamatoare ale Simvastatinei și Rosuvastatinei măsurând nivelele serice ale IL-1 β , IL-6 și TNF- α folosind un model de sepsis la șobolan indus prin ligatură și puncție cecală

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Abstract

Background: Sepsis is a systemic host response to an infection which may evolve into severe sepsis and septic shock. It raises many health care related concerns around the world, carrying almost 30% mortality rates and a high financial burden. The disease is characterized by the triggering of some inflammatory pathways that are ultimately proven deleterious to the host organism. Although antibiotics, fluid administration, vasopressor therapy and infectious source control remain the recommended management strategies, emerging scientific data proposes statins as a new line of treatment. These drugs were first introduced in clinical practice for their cholesterol-lowering effect but the inhibition of HMG-CoA reductase and cholesterol biosynthetic pathway exhibits some less studied effects generally referred to as pleiotropic: anti-inflammatory, antithrombotic, immunomodulatory and antioxidant properties. **Objective:** To assess and compare the anti-inflammatory effect of two statins – Simvastatin and Rosuvastatin – measuring blood levels of IL-1 β , IL-6 and TNF α using a previously described murinic model of sepsis. **Methods:** We compiled four groups (C, n=7; SEP, SV, RV, n=8). Statins were administered in two doses 18 and 3 hours before surgical intervention. Sepsis was induced using the caecal ligation and puncture technique. Blood samples were obtained by venepuncture from each subject in day 1, 4, 7 and 14 (the last samples were obtained by cardiac puncture). Complete blood count, Procalcitonin, IL-1 β , IL-6 and TNF- α levels were assessed. **Results:** White blood cell counts differed across the groups showing a higher count for the septic but untreated group. Procalcitonin reacted in all septic groups but both statin treated groups had lower levels when compared to untreated group. IL-1 β levels were higher in the Rosuvastatin treated group. IL-6 levels were more heterogeneously

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dispersed but higher levels were noticed in the untreated septic group. The Simvastatin treated group had higher levels compared to the Rosuvastatin treated one. TNF α levels were higher in the septic untreated group and in the Rosuvastatin treated one. For the Simvastatin treated subjects, the level of TNF α was similar with the control group. **Conclusion:** We concluded that both drugs showed anti-inflammatory effects on the murinic CLP-induced sepsis model. Between the two, Simvastatin had greater impact by lowering blood levels of established pro-inflammatory markers.

Keywords: sepsis; experimental model; rat; HMG-CoA reductase; Simvastatin; Rosuvastatin; interleukins; cytokines.

Rezumat

Introducere: Sepsisul este o boala caracterizată printr-un răspuns sistemic inflamator cauzat o infecție care poate evolua către sepsis sever și șoc septic. Această patologie ridică mari probleme la nivel global din cauza mortalității ridicate (care în anumite situații atinge 30%) precum și din cauza încărcăturii financiare mari. Sepsisul se caracterizează prin declanșarea unor căi imune pro-inflamatorii cu rol de apărare a organismului dar care ulterior se dovedesc a fi extrem de dezavantajoase. Deși antibioterapia, resuscitarea volemică, terapia vasopresoare și controlul sursei de infecție rămân strategiile recomandate în tratamentul sepsisului, date publicate recent propun statinele ca o noua linie de tratament. Aceste medicamente au fost introduse în practica clinică datorită efectului hipocolesterolemiant însă inhibarea reductazei HMG-CoA și a biosintezei colesterolului induce o serie de efecte mai puțin studiate denumite generic pleiotrope: anti-inflamatoare, antitrombotice, imunomodulatoare și antioxidante. **Obiective:** În cadrul acestui experiment ne-am propus să evaluăm și să comparăm efectul anti-inflamator al Simvastatinei și Rosuvastatinei măsurând nevelle serice ale IL-1 β , IL-6 și TNF- α folosind un model consacrat de sepsis la șobolan. **Metode:** Am alcătuit patru grupuri (C, n=7; SEP, SV, RV, n=8). Statinele au fost administrate în două doze cu 18 și 3 ore înaintea intervenției chirurgicale. Sepsisul a fost obținut prin tehnica ligaturării și puncției cecale. De la fiecare subiect au fost recoltate eșantioane de sânge prin puncție venoasă în zilele 1, 4, 7 și 14 (ultimele eșantioane au fost obținute prin puncție cardiacă). Au fost analizați următorii parametri: hemoleucograma, Procalcitonina, IL-1 β , IL-6 și TNF- α . **Rezultate:** Numărul total al leucocitelor a fost mai mare pentru grupul septic dar netratat. Procalcitonina a fost reacționată în toate grupurile septice însă valori mai mici au fost observate în grupurile tratate cu Simvastatină și Rosuvastatină în comparație cu grupul netratat. Nivelul de IL-1 β a fost mai mare în grupul tratat cu Rosuvastatină. Nivelele de IL-6 au avut o dispersie heterogenă în cadrul experimentului însă au fost înregistrate niveluri mai înalte în cadrul grupului septic netratat. Grupul tratat cu Simvastatină a avut nivele serice mai mari comparativ cu grupul tratat cu Rosuvastatină. Nivelele de TNF- α au fost mai mari pentru grupul septic netratat și cel tratat cu Rosuvastatină. Pentru grupul tratat cu Simvastatină nivelele TNF- α a fost similare cu cele din grupul control. **Concluzii:** Ambele statine au efecte anti-inflamatorii în cadrul acestui model de sepsis la șobolan indus prin tehnica ligaturării și puncției cecale. Dintre cele două, Simvastatina a avut un impact mai mare prin scăderea nivelelor circulante a unor markeri pro-inflamatori consacrați.

Cuvinte cheie: sepsis; model experimental; șobolan; murinic; statine; reductază HMG-CoA; Simvastatină; Rosuvastatină; interleukine; citokine.

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Introduction

Sepsis is a systemic host response to an infection which may evolve into *severe sepsis* (sepsis-induced organ dysfunction or tissue hypoperfusion) and *septic shock* (severe sepsis with hypotension that is not reversed by fluid re-

suscitation)(1). Severe sepsis and septic shock, in spite of medical and technological advances, remain one of the main concerns of health care providers around the World carrying almost 30% mortality rates and a high financial burden. (2) This disease is characterized by the triggering of

some inflammatory pathways that are ultimately proven deleterious to the host organism by generating reactive oxygen species, inflammatory cytokines as well as nitric oxide production and antithrombin III depletion(3). Although antibiotics, fluid administration, restoring systemic vascular resistance with vasopressor therapy and infectious source control remain the main treatment options, emerging scientific data proposes the *3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase) inhibitors* (or statins) as a new line of treatment. This class of drugs was first introduced in clinical practice for their cholesterol-lowering effect but the inhibition of HMG-CoA reductase and cholesterol biosynthetic pathway exhibits some less studied effects generally referred to as *pleiotropic*: the reduced expression of class II MHC (major histocompatibility complex), effects on cell signaling and consequent transcriptional level changes, inhibiting vascular smooth muscle growth, reducing vascular inflammation, the direct alteration of leukocyte–endothelial cell interaction, the induction of haem oxygenase, enhancing the stability of atherosclerotic plaques, beneficial effects on endothelial function and blood flow, decreasing LDL-C oxidation and modulating platelet aggregation (4,5). These anti-inflammatory, antithrombotic, immunomodulatory and antioxidant properties were documented in a number of observational studies (6–14) but also in six large trials (15–20) with published results. In spite of the ongoing efforts of understanding these pleiotropic properties and establishing the role of statins in the management and treatment of the septic patient, there are only few authors that offer clear recommendations. Some of them concluded that statins may prove useful if administered before the onset of sepsis and during the resolution of this disease when the pro-inflammatory state of the host organism could be balanced by the anti-inflammatory properties of the statin therapy (4,21). Others stated that established statin treatment should be continued

because the cessation of such treatment is associated with worse survival rates (22). But the majority agree that more data is needed before a definitive conclusion may be drawn (23,24). The main questions that still remain to be answered are which statin is best for the septic patient, when should this be administered and what dose would be safe for this group of patients.

Our objective was to assess and compare the anti-inflammatory effect of two statins – Simvastatin and Rosuvastatin – measuring blood levels of Interleukin 1-beta (IL-1 β), Interleukin 6 (IL-6) and Tumour Necrosis Factor – alpha (TNF α) using a previously described murine model of sepsis (25–27). Given the fact that a previously established sepsis model was used, we did not include the whole array of parameters that would otherwise be needed to prove the presence of sepsis (1). Nonetheless, the parameters that were assessed for this experiment sustain the presence of sepsis for the groups inflicted with this disease, Procalcitonin level being the most important indicator (28–30).

Material and Method

Subjects and group compiling

We used 31 Wistar rats housed in a temperature controlled environment and fed under standard conditions (*ad libitum* standard rodent chow and water) with a 12 hour imposed sleep-wake cycle. They were randomized into four groups: control group (C, $n=7$), sepsis group (SEP, $n=8$), sepsis+Simvastatin (SV, $n=8$) and sepsis+Rosuvastatin (RV, $n=8$). For this experiment ethical committee approval has been obtained and European Union directives regarding the protection of animals used for scientific purposes were respected (31).

Experimental protocol

All animals were subjected to an 18-hour period of fasting prior to the first dose of statin. Food was then administered and a second period

of fasting was employed before the second dose of statin. For the control group (C) the same periods of fasting were employed. They were anaesthetized using intraperitoneal Ketamine 100 µg/g and Xylazine 10 µg/g (32). After induction of anaesthesia animals were placed under a heating lamp and a rectal probe was inserted for temperature monitoring. Fur was removed from abdomen and a sterile surgical field created. A 2 to 3 cm incision was made 1 cm left from the mid-line. After penetrating the abdominal cavity the *caecum* was identified and carefully extracted. For the control group (C) we performed a *sham* operation – the caecum was placed back inside the peritoneal cavity and the abdominal wall was closed in two layers using surgical sutures. For all other experimental groups we applied the *caecal ligation and puncture* technique (25–27). This consists of placing a tight ligature using nonresorbable suture 1 cm from the caecum base, distal from the ileo-caecal valve (without interrupting normal intestinal transit) and perforating it *twice*, on the same side, using a 19-gauge needle. Then the caecum is gently squeezed to extrude a small amount of faeces; this will initiate the septic process and also confirm that the puncture was efficient. The caecum is then replaced in the peritoneal cavity and sutures applied as described before. All animals were then resuscitated using pre-heated normal saline (NaCl 0.9%) 0.05 ml/g injected subcutaneously using a 26-gauge needle. This fluid resuscitation was repeated every 24 hours (33). Pain control was obtained using Tramadol 20 µg/g every 24 hours subcutaneously, first dose being administered immediately after the operation. If the animal showed behavioural or clinical modifications consistent with inadequate analgesia (34–36) supplemental doses were given. For the statin groups (SV and RV) two oral doses of the drugs were administered 18 and 3 hours before induction of anaesthesia combined with usual standard food. The doses were 80 µg/g

Simvastatin (37) or 20 µg/g Rosuvastatin. Until full recovery from anaesthesia was attained, the animals were monitored and normal body temperature assured. Monitoring continued for 14 days for each animal after which euthanasia was performed using intra-cardiac administration of a high dose of Sodium Pentobarbital (over 100 mg/kg) (38).

Blood sampling

While the animals were still under anaesthesia, we obtained blood samples by caudal vein puncture. Before attempting venepuncture the tail was submersed in 40 °C water for 10 minutes. A tourniquet was placed at the base of the tail and a Heparine-flushed 23-gauge needle was used. After successful intra-vascular needle placement, blood was allowed to drip freely inside a commercially available capillary blood collector (K₂EDTA coated tubes for complete blood count and standard plain tubes for cytokine and PCT testing). 1 to 2 ml of blood was obtained during each phlebotomy session. For each animal we obtained three blood samples using the described method and a forth by cardiac puncture: the *first* immediately after the intervention (day 1), the *second* in day 4, *third* in day 7 and *fourth* in day 14. Each time anaesthesia was performed as described before. Total blood count was completed within 4 hours from the sampling and the rest of the blood was centrifuged, plasma was separated and kept at -20 °C until further use.

Laboratory testing

We performed a complete blood count, Procalcitonin, IL-1β, IL-6 and TNF-α plasma concentration measurements.

Blood cell analysis was performed using a Sysmex KX21-N automated haematology analyser. Cytokines and Procalcitonin (PCT) tests were made using commercially available ready-to-use ELISA rat kits (KOMABIOTECH® for IL-

6, IL-1 β and TNF- α ; Elabscience® for PCT).

For some of the samples we did not obtain the absolute value for cytokine concentration because the absorbance values could not be plotted. This is why all statistical analysis for IL-6, IL-1 β and TNF- α was made using the *absorbance* values.

Statistical analysis

We compared the number of white blood cells (WBC), lymphocyte percentage (LYM%), Procalcitonin (PCT), Interleukin 6 (IL-6), Interleukin 1-beta (IL-1 β) and Tumour Necrosis Factor alpha (TNF- α) values, as follows: the control group (C) vs. sepsis group (SEP) at all four selected moments (day 1, 4, 7 and 14); the SEP group vs. sepsis+Simvastatin (SV) and SEP vs sepsis+Rosuvastatin (RV) at all moments; SV vs. RV at all moments. Student's t-test was employed and results presented as mean \pm standard deviation (SD). A *p* value under 0.05 was considered significant. For statistical data management we used commercially available software (*GraphPad Prism* version 5.03 for Windows®, GraphPad Software, San Diego California USA, www.graphpad.com).

Sepsis diagnosis

We used Procalcitonin levels, white blood cell count, clinical observations (social behaviour, food and water intake, day-light activi-

ty, general appearance) (34,35) and body weight measurements. Also, as previously described (25–27), we initiated the septic process, after the caecal puncture, by extruding a small amount of faeces directly inside the peritoneal cavity.

Results

White Blood Cells (WBC)

When comparing C group with SEP we obtained significant differences at all tested moments. Differences were also found between SEP and SV groups and SEP and RV ones (with two exceptions, SEP vs. SV, day 4 and SEP vs. RV, day 1). We did not find statistical differences between SV and RV groups in the first day but all other measurements differed significantly (*Figure 1*) (*Table 1*).

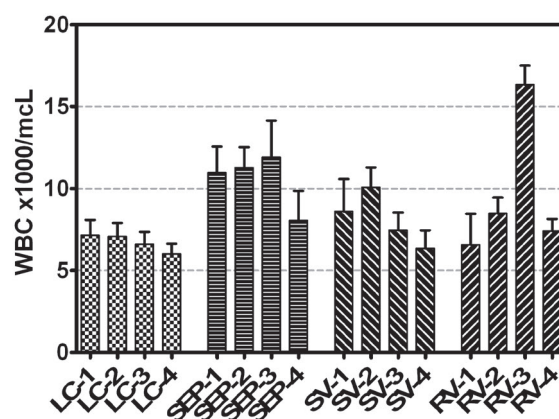


Figure 1. White blood cell count. (mean and SD)

Table 1. White blood cell counts and *p* values for statistical comparison across groups.

Day	WBC (mean \pm SD)				p value			
	C	SEP	SV	RV	C vs. SEP	SEP vs. SV	SEP vs. RV	SV vs. RV
1	7.2 \pm 0.93	11.0 \pm 1.6	8.6 \pm 2.0	6.6 \pm 1.9	< 0.0001*	0.0199*	0.0002*	0.05
4	7.1 \pm 0.82	11.0 \pm 1.2	10 \pm 1.2	8.5 \pm 0.97	< 0.0001*	0.07	0.0002*	0.0114*
7	6.6 \pm 0.75	12.0 \pm 2.2	7.5 \pm 1.1	16 \pm 1.2	< 0.0001*	0.0002*	0.0002**	< 0.0001*
14	6.0 \pm 0.64	8.1 \pm 1.8	6.4 \pm 1.1	7.4 \pm 0.74	0.0084*	0.0371*	0.35	0.0418*

* the highest mean is in the RV group; * achieved statistical significance

Table 2. Lymphocyte percentage and p values for statistical comparison across groups.

Day	LYM% median (range)				p value			
	C	SEP	SV	RV	C vs. SEP	SEP vs. SV	SEP vs. RV	SV vs. RV
1	70.3 (12.8)	51.9 (25.5)	59 (38.4)	69.8 (27)	< 0.0001*	0.07	0.0005*	0.17
4	65.5 (17.5)	42.8 (27.2)	55.6 (31.3)	44.5 (20.3)	0.0002*	0.09	0.61	0.14
7	56.1 (16.3)	31.8 (51.6)*	44.9 (43.9)*	46 (7.9)	0.0383*	0.77	0.31	0.41
14	55.3 (22.2)	53.3 (20.5)	55.8 (25.1)	55.1 (4.5)	0.46	0.81	0.96	0.78

* Coefficient of variation > 30%; * achieved statistical significance

Lymphocyte percentage (LYM%)

We obtained differences between the C and SEP groups (except day 14) and one isolated difference between SEP and RV group in the first day (Figure 2) but no other difference between the studied groups was found. (Table 2)

Procalcitonin (PCT)

When comparing C group with SEP one we recorded differences at all tested moments. The SV group compared to SEP showed a significant difference in day 1, day 4 and 7 but not day 14. The RV and SEP groups showed no difference in day 1, day 4, or 14 but significant in day 7. Regarding the comparison between SV and RV, we found statistical difference in day 1 and 4 but no difference in day 7 and 14. (Table 3) (Figure 3)

Interleukin 1-beta (IL-1 β)

When comparing C group with SEP group we found significant difference for day 4, 7 and 14 but no difference in the first day. For the SEP and SV groups we found statistical difference for all studied moments with consistent lower means in the SV group. The RV group had consistent

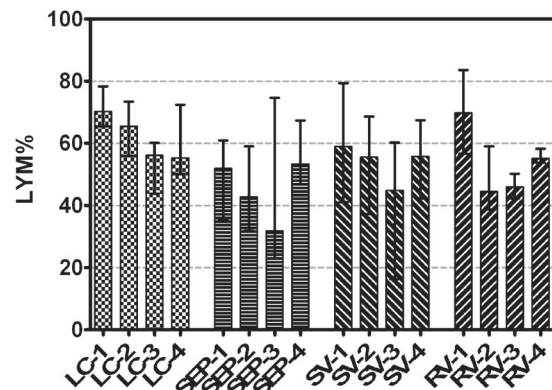


Figure 2. Lymphocyte percentage.
(median and range)

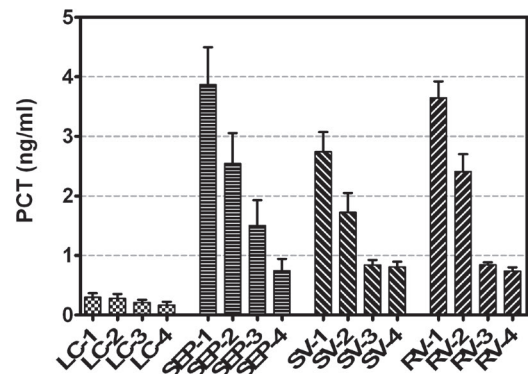


Figure 3. Procalcitonin. (median and range)

Table 3. Procalcitonin and p values for statistical comparison across groups.

Day	PCT median (range)				p value			
	C	SEP	SV	RV	C vs. SEP	SEP vs. SV	SEP vs. RV	SV vs. RV
1	0.305 (0.171)	3.77 (1.73)	2.79 (0.91)	3.68 (0.77)	< 0.0001*	0.0005*	0.37	< 0.0001*
4	0.276 (0.198)	2.5 (1.5)	1.71 (1.08)	2.4 (0.97)	< 0.0001*	0.0019*	0.53	0.0006*
7	0.193 (0.102)	1.34 (1.27)	0.85 (0.232)	0.843 (0.106)	< 0.0001*	0.0008*	0.0007*	0.96
14	0.172 (0.161)*	0.645 (0.445)	0.785 (0.259)	0.743 (0.174)	< 0.0001*	0.43	0.94	0.09

* Coefficient of variation > 30%; * achieved statistical significance

Table 4. Interleukin 1beta and p values for statistical comparison across groups.

Day	IL-1 β (mean \pm SD)				p value			
	C	SEP	SV	RV	C vs. SEP	SEP vs. SV	SEP vs. RV	SV vs. RV
1	0.14 \pm 0.018	0.168 \pm 0.036	0.127 \pm 0.018	0.391 \pm 0.061	0.08	0.0127*	< 0.0001*	< 0.0001*
4	0.108 \pm 0.014	0.139 \pm 0.022	0.105 \pm 0.019	0.283 \pm 0.082	0.0084*	0.0067*	0.0003*	< 0.0001*
7	0.105 \pm 0.018	0.153 \pm 0.037	0.11 \pm 0.02	0.159 \pm 0.033	0.0089*	0.0126*	0.73	0.0030*
14	0.103 \pm 0.014	0.129 \pm 0.009	0.1 \pm 0.016	0.158 \pm 0.033	0.0008*	0.0007*	0.0321*	0.0006*

* achieved statistical significance

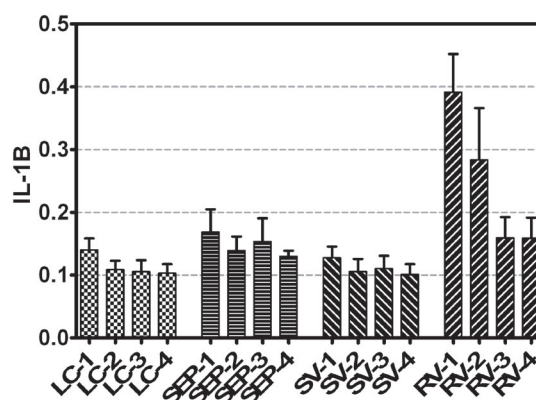
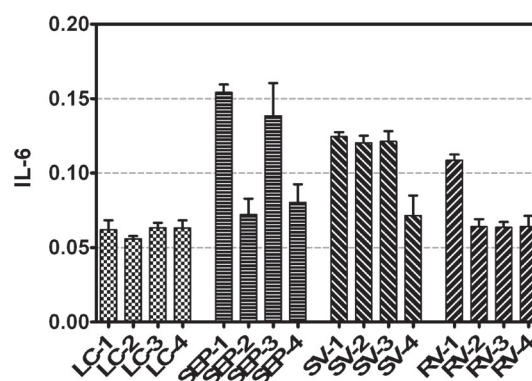
higher means with statistical significance in day 1, 4 and 14. We also found statistical difference between SV and RV for all measurements. (Table 4) (Figure 4)

Interleukin 6 (IL-6)

We recorded differences for all studied moments when comparing C and SEP groups. Regarding SV group compared to SEP one, we found statistical difference in day 1 and 4 but not in day 7 and 14. The RV group means were statistically different from SEP ones in day 1, 7 and 14 but no difference was noted in day 4. The means from SV and RV differed significantly for the first three measurements (day 1, 4 and 7) but no difference was found in day 14. (Table 5) (Figure 5)

Tumour Necrosis Factor – alpha (TNF- α)

We found obvious differences between C group and SEP for all measurements. Also the SV group showed smaller means than the SEP ones with significant differences for all four measurements (day 1, 4, 7 and 14). In the RV group, only in day 4 and 7 TNF- α levels were significantly lower, while day 1 and day 14 lev-

**Figure 4. Interleukin 1beta. (mean and SD)****Figure 5. Interleukin 6. (mean and SD)****Table 5. Interleukin 6 and p values for statistical comparison across groups.**

Day	IL-6 (mean \pm SD)				p value			
	C	SEP	SV	RV	C vs. SEP	SEP vs. SV	SEP vs. RV	SV vs. RV
1	0.062 \pm 0.006	0.154 \pm 0.005	0.125 \pm 0.002	0.109 \pm 0.003	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*
4	0.055 \pm 0.001	0.072 \pm 0.01	0.12 \pm 0.004	0.064 \pm 0.005	0.0017*	< 0.0001*	0.07	< 0.0001*
7	0.063 \pm 0.003	0.139 \pm 0.021	0.121 \pm 0.007	0.063 \pm 0.003	< 0.0001*	0.05	< 0.0001*	< 0.0001*
14	0.063 \pm 0.005	0.08 \pm 0.012	0.071 \pm 0.013	0.064 \pm 0.007	0.0041*	0.18	0.0057*	0.19

* achieved statistical significance

Table 6. Tumour Necrosis Factor -alpha and p values for statistical comparison across groups.

Day	TNF- α (mean \pm SD)				p value			
	C	SEP	SV	RV	C vs. SEP	SEP vs. SV	SEP vs. RV	SV vs. RV
1	0.131 \pm 0.003	0.43 \pm 0.042	0.177 \pm 0.048	0.389 \pm 0.061	< 0.0001*	< 0.0001*	0.14	< 0.0001*
4	0.106 \pm 0.021	0.414 \pm 0.035	0.137 \pm 0.017	0.269 \pm 0.065	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*
7	0.099 \pm 0.025	0.339 \pm 0.071	0.124 \pm 0.014	0.159 \pm 0.033	< 0.0001*	< 0.0001*	< 0.0001*	0.0171*
14	0.094 \pm 0.014	0.147 \pm 0.03	0.091 \pm 0.008	0.157 \pm 0.033	0.0011*	0.0002*	0.54	< 0.0001*

* achieved statistical significance

els did not differ significantly from SEP group. Directly comparing SV and RV groups showed significantly differences for all measurements. (Table 6) (Figure 6)

Discussions

The sepsis model

As we decided to use a previously described murine sepsis model (25), this study did not focus on proving septic state of the animals as required by present definition of sepsis (1). Nonetheless, we showed significant differences between the control group (C) who received the *sham* operation and the sepsis group (SEP) to whom *caecal ligation and puncture* technique was applied.

The SEP group had a higher white blood cell count (WBC) during the whole studied period. Also lymphocyte percentage (LYM%) was smaller for the SEP group but in the context of a higher total blood cell count we may safely assume that the WBC count rose on behalf of the polymorphonuclear cells (PMN) as a response to the infectious injury.

Procalcitonin (PCT) levels differed with a mean of 0.297 ng/ml for C group and 3.87 ng/ml for SEP group (values for day 1, $p < 0.05$) (Figure 3). The studied interleukin levels were also higher for the SEP group. Regarding IL-1 β and IL-6 we have noticed an increase in mean values for the SEP group in day 7 compared with previous determinations (from day 4) (Figure 4 and 5). This difference is less obvious for IL-1 β but of a greater magnitude for IL-6 levels. TNF- α and

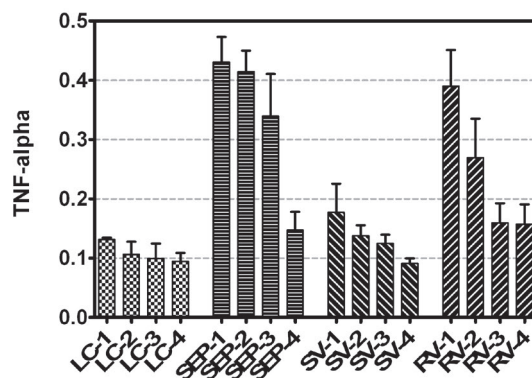


Figure 6. Tumour Necrosis Factor - alpha.
(mean and SD)

PCT did not showed such behaviour. The data we have collected suggest that between day 4 and 14 there is a new peak for IL-6 and IL-1 β blood levels. This only happens for the statin treated groups.

Statins effect on CLP-induced sepsis

In order to evaluate the effect of the administered statins, we compared the SEP group to the SV or RV one.

Regarding WBC count, we encountered slightly different effects for SV and RV. In the first day both statins had a lowering effect on WBC counts. In day 4 we found higher counts compared to day 1 (a rising in WBC number that was not present in the SEP group). By day 7 the number of WBC dropped significantly for SV but almost doubled for the RV group (Figure 1). In day 14 we found similar values for SV and RV, both lower than the SEP group. Lymphocyte

percentage did not differ across these groups (Figure 2) so we concluded that any significant difference in WBC count was caused by granulocytes.

Regarding PCT blood concentration, when compared with SEP, we observed lower values for the SV group ($p=0.0005$) from the first day but no difference for the RV group ($p=0.37$). PCT values in the SV group remained lower throughout the entire experiment whereas in the RV group the only difference observed was in day 7 when PCT mean value was the same as the one in SV group (and significantly lower than the SEP group) (Figure 3). In day 14 there was no difference between the three groups.

IL-1 β levels were lower in SV group (mean values were close to those observed in C group) but significant higher levels were recorded for the RV group. (Figure 4)

Both Simvastatin and Rosuvastatin had a clear effect on IL-6 levels compared to SEP group. Both SV and RV groups showed smaller levels in day 1. By day 4 there was no difference in the RV group but the SV group showed increased levels. In day 7 the rise in IL-6 levels observed in the SEP group was not matched in any of the other groups. By day 14 no difference was noted between the three groups.

Regarding TNF- α , Simvastatin showed a higher impact resulting in lower levels compared with SEP group. In the RV group we had no difference in day 1 but the levels started to drop by day 4. Even if this lowering effect was smaller than in SV group, the differences were significant ($p<0.0001$ for day 4 and 7). (Figure 6)

Which statin?

To assess the differences between the effect of the two statins (Simvastatin and Rosuvastatin) on our CLP-induced sepsis model we compared the mean values for all the studied parameters.

Regarding white blood cells we found *higher* counts in the SV group for the first 4 days

(in day 1 these differences were not statistically significant; $p=0.054$) (Figure 1) but in day 7 we observed a drastic increase in WBC for the RV group. This increase was not matched by the SV group (Figure 1).

No differences were found regarding lymphocyte percentage (LYM%). (Figure 2)

PCT had smaller values the first 4 days in SV group. By day 7 the recorded values for the two groups were similar. (Figure 3)

IL-1 β values differed significantly among the two groups. Consistent smaller values were recorded for the SV one for all four measurements. (Figure 4)

For IL-6 we showed smaller values for RV. The differences were significant for all but day 14. (Figure 5)

TNF- α levels were higher for the RV group with statistical significance for all measurements. (Figure 6)

Statins vs. corticosteroids

Both type of drugs have similar anti-inflammatory, immunomodulatory, anti-oxidant, anti-thrombotic and protective vascular endothelial effects with small differences among each action. What actually makes these two therapeutic approaches different is the time needed for the onset of their effect. The anti-inflammatory effects of corticosteroids can be observed within 24 hours, whereas statins achieved measurable anti-inflammatory effect only after 7 to 14 days of treatment as shown by some studies using human subjects (39,40). This delayed effect seem to make statins an unsuitable candidate for systemic inflammation suppression during the acute phase of sepsis (17,41). However the last guidelines offered by Sepsis Surviving Champaign (1) issued a strong recommendation that corticosteroids should not be used for the treatment of sepsis or septic shock (unless hemodynamic stability could not be achieved by the use of fluid resuscitation and vasopressors).

Summary

When looking at the whole array of data we noticed that PCT, IL-1 β and TNF- α levels were lower for the Simvastatin treated group. Also the level of IL-1 β for the Rosuvastatin treated group was higher than the one found in the not-treated sepsis group (SEP). TNF- α level after treatment with Rosuvastatin was similar with the one found in SEP group. All this suggests that Simvastatin has a greater anti-inflammatory effect compared to Rosuvastatin. Moreover, Rosuvastatin could induce a paradoxical pro-inflammatory effect (IL-1 β level higher in RV group compared to SEP). Even so, the level of IL-6 in day 1 was lower for RV group, dropped significantly by day 4 and remained at this level. In contrast the Simvastatin treated group showed higher initial levels that remained the same at least for the first seven days. IL-6 is considered a major marker of sepsis and a prognosis factor (28,41). For this study the animals were monitored for 14 days. During this time no subject died so mortality could not be evaluated.

Limitations

The major limitation of the study consists in the relative wide period of time between measurements. This only made available a few snapshots of the actual immunologic activity during sepsis with the possibility of missing some of the rapid modifications that would take place in between measurements. Also the number of subjects used caused a high degree of heterogeneity which resulted in a variation coefficient over 30% for some of the measurements. Because of the high complexity of the immunological response future research should monitor a larger number of inflammation markers.

Conclusion

We conclude that both drugs showed anti-inflammatory effects on the murine CLP-induced

sepsis model. Between the two, Simvastatin had a greater impact on lowering blood levels of established pro-inflammatory markers. Moreover, Rosuvastatin seem to have a paradoxical pro-inflammatory effect suggested by the increased levels of IL-1 β (RV vs. SEP). Even if both Simvastatin and Rosuvastatin caused lower levels of IL-6 compared with non-treated septic subjects, relatively higher levels of IL-6 were detected in SV group compared with RV.

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