Experimental research

Rosuvastatin accelerates the healing process of partial-thickness burn wounds in rats by reducing TNF-α levels

Alexandra Marneri[1], Francesk Mulita[2,3], Vasileios Leivaditis[4], Serafeim – Chrysovalantis Kotoulas[1], Ioannis Gkoutziotis[5], Stavrati Kalliopi[6], Konstantinos Tasios[2], Levan Tchabashvili[3], Nikolaos Michalopoulos[7], Konstantinos Mpallas[5]

[1]ICU, Hippokration General Hospital, Aristotle University of Thessaloniki, Thessaloniki, Greece
[2]Department of Surgery, General University Hospital of Patras, Patras, Greece
[3]Department of Surgery, General Hospital of Eastern Achaia, Unit of Aigio, Aigio, Greece
[4]Department of Cardiothoracic and Vascular Surgery, Westpfalz Klinikum, Kaiserslautern, Germany
[5]5th Department of Surgery, Aristotle University of Thessaloniki, Thessaloniki, Greece
[6]Second Department of Propaedeutic Surgery, Aristotle University of Thessaloniki, Thessaloniki, Greece
[7]First Propaedeutic Department of Surgery, Hippocration General Hospital, National and Kapodistrian University of Athens, School of Medicine, Athens, Greece

Corresponding author:
Francesk Mulita
General University
Hospital of Patras
Patras, Greece
E-mail: oknarfmulita@hotmail.com

Abstract

Introduction: Burn wound healing is a complex, dynamic process that involves a coordinated cascade of cellular responses and phases. Inflammation, proliferation and remodeling are the main phases of tissue repair, while tumor necrosis factor α (TNF-α) and procalcitonin (PCT) seem to be important mediators affecting the inflammatory state. Our aim was to assess the effect of rosuvastatin on tissue repair after partial thickness burn injury in healthy animals.

Material and methods: In this randomized prospective experimental study, 36 male rats were randomly divided into two groups: placebo-treated (PG) and topical rosuvastatin-treated (SG). Under anesthesia, a partial-thickness burn trauma was induced in the dorsal region of the rats using an iron seal. Tissue samples were collected for histopathological examination as well.

Results: Variables of TNF-α, procalcitonin and macroscopic assessment were normally distributed between the two groups on all studied days. The expression of TNF-α was found to be lower in burn injuries treated with topical rosuvastatin in comparison with placebo-treated animals on days 3, 6 and 9. PCT values in rosuvastatin-treated subgroups were statistically significantly lower than in placebo subgroups. Upon macroscopic examination, a significantly smaller burnt area in the statin-treated group was detected compared to the non-statin group on all days, except for day 3. Histopathological examination demonstrated higher levels of mean neutrophil infiltration in the placebo group (day 3). Finally, fibroblast proliferation, angiogenesis and re-epithelization levels were noted to be higher after the topical application of rosuvastatin.

Conclusions: Rosuvastatin accelerated wound healing and down-regulated TNF-α and PCT levels.

Key words: rats, tumor necrosis factor α, wound healing, experimental study, rosuvastatin, statins, burn wounds.

ATHEROSCLEROTIC DISEASES AMS

Introduction

The skin, encompassing the epidermis, dermis, and subcutaneous tissue, serves vital functions in protection, thermoregulation, and sensory perception [1, 2]. Burn injuries, caused by heat, chemicals, electricity, or radiation, exhibit histological zones of coagulative necrosis, stasis, and hyperemia [3, 4]. The depth determines burn classification (1st to 4th degree), impacting healing and scarring [5, 6]. Although burns have a higher degree of systemic inflammatory response and show higher generalized capillary permeability than other wounds [7], their healing follows the traditional wound healing model [7, 8]. This particular model is characterized by three phases: inflammation, multiplication, and maturation [9]. The inflammatory phase involves tumor necrosis factor α (TNF-α), cytokines, and growth factors, triggering hemostasis and inflammation stages. Platelet activation, coagulation cascade, and subsequent angiogenesis characterize this phase [10]. The multiplication phase includes epithelium formation, capillary growth, granulomatous tissue development, and wound shrinkage, influenced by VEGF, PDGF, and bFGF [11, 12]. The remodeling phase, lasting weeks to years, sees collagen type III replaced by type I, forming scars. Burn depth influences healing speed and esthetic recovery, with deeper burns resulting in longer healing periods and potential hypertrophic scarring [13]. Understanding the role of TNF-α, cytokines, and growth factors is crucial for effective burn care, as they play pivotal roles in inflammation, tissue repair, and scar formation [9, 14, 15].

Statins are widely used for the treatment of hyperlipidemia, and their main pharmacological action is served by the inhibition of 3-hydroxy-3-menthyl glutamate coenzyme A (HMG-CoA) reductase, a key enzyme in cholesterol biosynthesis, which converts HMG-CoA to mevalonic acid [16]. However, apart from their lipid-lowering action, recent studies have proved that statins, thanks to their pleiotropic effects, present anti-inflammatory and anti-oxidant activity, thereby amplifying the processes of angiogenesis, re-epithelization, and fibroblast proliferation [17]. Rosuvastatin, a synthetic hydrophilic statin, effectively lowers low-density lipoprotein and triglyceride levels while increasing high-density lipoprotein levels [18]. Notably, research has revealed that rosuvastatin specifically targets reactive oxygen species-induced oxidative stress and inflammation, making it a potent intervention for managing wound and burn healing [19].

The purpose of this experimental study was to evaluate the action of rosuvastatin on the pathophysiological disorders of burn injury. The evaluation was done by reproducing partial-thickness burns in rats. The experiment then attempted to study the processes observed in the body as a whole, using inflammatory markers, and on the skin locally, by histopathological examination of the affected tissues. In addition, the action of rosuvastatin in the above processes was also examined.

The current evidence endorsing the potential positive impact of rosuvastatin on wound healing is predominantly derived from animal studies characterized by limited sample sizes and short-term observation periods. Furthermore, the specific role of rosuvastatin in the context of burn wounds remains relatively unexplored.

Material and methods

A randomized prospective experimental study was carried out in male Wistar Albino rats with intervention and control groups. The study was a clinical trial, that is, a prospective randomized open-label study in which a comparison was made with a negative control group (placebo).

This was an experimental study of the anti-inflammatory action of rosuvastatin on burns of partial thickness. The application of this type of statin concerning the healing of a burn injury has not been studied previously.

Experimentation should therefore be carried out in groups of animals and not in groups of patients, with all the limitations and variations expected to occur. However, it may be a starting point for subsequent studies, once the hypotheses to be tested are confirmed. The experimental part of the study was conducted at the Laboratory of Scientific Research and Experimental Surgery of the Second Propedeutical Department of Surgery of the Aristotle University of Thessaloniki (Hippocrates Hospital).

Animals

Thirty-six male Wistar rats were used as animal models. The classification of the animal model is: Class: Mammalia, Order: Rodentia, Suborder: Sciurognathi, Family: Muridae, Genus: Rattus, Species: norvegicus, Stem: Wistar.

The rats were 12 to 16 weeks old with a body weight of approximately 300 g. Hosting of all animal models during the study took place in plexiglass cages, at a temperature between 20°C and 22°C, with a relative humidity between 55 and 65%, and a light cycle of 12 h of light and 12 h of darkness. Animal models had free access to water and food (ad libitum), which is specific to rats (blight no. 510, ELVIZ). Regarding the genetic standardization of animal models, there was a randomized series of experimental animals, while in the case of microbiological standardization there were standard (conventional) experimental animals.

Alexandra Marneri, Francesk Mulita, Vasileios Leivaditis, Serafeim – Chrysovalantis Kotoulas, Ioannis Gkoutziotis, Stavrati Kalliopi, Konstantinos Tasios, Levan Tchabashvili, Nikolaos Michalopoulos, Konstantinos Mpallas

Figure 1. Shaving of the dorsal lesion

Preparation of animals

All procedures were approved by the Committee for Bioethics and Ethics of Aristotle University of Thessaloniki and were conducted in accordance with its established guidelines. Animals were obtained from the laboratory of experimental surgery of the 2nd Propedeutical Department of Surgery of Aristotle University.

The operation began with the induction of anesthesia after immobilization of the animal with a special glove and intraperitoneal administration of ketamine hydrochloride (Ketalar amp, 50 mg/ml, Parke-Davis) at a dose of 70 mg/kg BA and xylazine (20 amp, mg/ml, Bayer), at a dose of 10 mg/kg BA. The dorsum of the rat was shaved. The shaver was held at a 45-degree angle to the
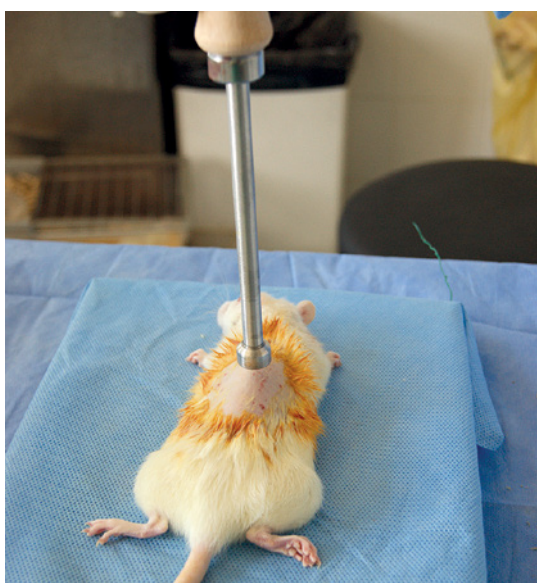
skin and a maximum of two crosses was allowed, in order to minimize skin trauma. Subsequently, the rat was immobilized on the surgical table and preparation of the surgical field was conducted. After anesthesia and under aseptic conditions, the animal's hair was removed in the area of the back, on a surface of at least 5 cm^2 (Figure 1).

Preparation of rosuvastatin and placebo cream

For the preparation of the mixture for the study group, 20 g of Eucerin cream base and 20 rosuvastatin 20 mg tablets were used, mixed, and homogenized. For the preparation of the mixture that was used in the placebo group, 20 g of Eucerin cream base was used.

Wound infection model

To cause the burn of the partial thickness of skin, a special metal iron cylindrical bar 2 cm in diameter, 2.5 mm thick, and weighing 500 g was used. These dimensions ensured efficient heat transfer [20]. The seal is composed of stainless steel and features a wooden handle. Thermal burns were performed on the back of 36 white Wistar rats. The metal seal was heated to 85°C. The thermal burn per rat was caused by placing the metal seal on the skin of the back for 10 s. This method induced a deep burn of the partial thickness of the skin [20]. After removing the seal, the wound was washed with saline, a photograph of the burn wounds was taken, and a record of the burnt surface of each animal was made. The animals were then placed in their cages and monitored until anesthesia had elapsed. The eschar was removed on the first post-burn day with surgical scissors. Subsequently, Eucerin cream or a mixture of Eucerin with rosuvastatin was applied daily, depending on the group of each rat. The day of burn removal is defined as day 0 and the first application of Eucerin cream and Eucerin cream mixed with rosuvastatin took place on that day. Wounds were covered with Tegaderm dressing (Figures 2, 3).

Figure 2. Burn injury, stainless steel seal

Figure 3. Application of topical rosuvastatin cream

Animal grouping

Thirty-six male rats were randomly divided into two main groups, the study group, and the placebo group, depending on whether the rosuvastatin cream excipient was used or not. These groups were in turn divided into 3 subgroups of 6 rats depending on the day that the animal was sacrificed. The investigation of the healing effect of the solution was assessed on the 3rd, 6th, and 9th burn days. Once the burn was induced, the animals were divided according to the days their burn wound was going to be studied.

Study group (SG)

1. The rats in this group were burned to a partial thickness using a metal signet ring and rosuvastatin cream excipient was applied to the burnt surface.
   1 a) Study group 1a – sacrifice on the 3rd post-burn day
   This group consisted of 6 rats that were sacrificed on the 3rd post-traumatic day.
   1 b) Study group 1b – sacrifice on the 6th post-traumatic day
   This group consisted of 6 rats that were sacrificed on the 6th post-traumatic day.
   1 c) Study group 1c – sacrifice on the 9th post-traumatic day
   This group consisted of 6 rats that were sacrificed on the 9th post-traumatic day.

Placebo group (PG)

2. For rats of this group, once partial skin burn had been caused using a metal signet ring, Eucerin cream was applied to the burnt surface.
   2 a) Placebo group – sacrifice on the 3rd post-traumatic day
   This group consisted of 6 rats that were sacrificed on the 3rd post-traumatic day.
   2 b) Placebo group – sacrifice on the 6th post-traumatic day
   This group consisted of 6 rats that were sacrificed on the 6th post-traumatic day.
   2 c) Placebo group – sacrifice on the 9th post-traumatic day
   This group consisted of 6 rats that were sacrificed on the 9th post-traumatic day.

Determination of plasma inflammatory mediator levels, TNF-α, and procalcitonin levels

After a macroscopic estimation, the values of the following parameters were determined as well:
– Procalcitonin (PCT) levels. Procalcitonin is a polypeptide of 116 amino acids. After a severe burn, high serum PCT levels are released, enforcing the systemic inflammatory response syndrome (SIRS). Consequently, high PCT plasma levels are correlated with delayed wound healing, septic complications, and clinical consequences (systemic inflammatory response index) [21–23].
– Tumor necrosis factor (TNF-α), a potent chemotactic agent that promotes local or systemic inflammation (TNF-α is a potent pyrogen) and stimulates the acute phase response [24, 25].

Subsequently, the values of the above parameters were determined on the 3rd, 6th, and 9th postburn day.

Finally, the tissue sections from the above tissues were sent for histopathological examination with an optical microscope.

The levels of TNF-α tumor necrosis factor were determined using the ELISA method. The antigen was added to the wells for the first incubation. After incubation, the wells were washed and a monoclonal antibody that is specific to TNF-α was added and incubated for an hour. A second-detector antibody and finally, after washing, a substrate solution was added, to produce a colored reaction product. The levels of PCT were measured by a chemiluminescent detection system and an immunoluminometric method [26]. For histopathological examination, the preparations were embedded in paraffin blocks, cut into 3 μm thick sections, and stained with hematoxylin-eosin staining, as well as immunohistochemical staining.

Monitoring of wound healing

In all rats, the lesion was macroscopically evaluated, and photographed, and the burnt surface was measured. The burning surface size was measured digitally using ImageJ Software, version 1.45 (National Institutes of Health [NIH], USA). Burn lesions were used to calculate wound healing based on the following formula: wound healing rate = [burn area (day X)/burn area (day 0)] × 100, planimetry method.

Statistical analysis

Statistical data processing was conducted using the R Studio software. The significance level was set at 0.05. If the data were normally distributed, the results were described by calculating the mean and the standard deviation. Comparison between values was done by Student's t-test. If the data did not follow a normal distribution, the results were described by calculating the median and range of values. The comparison between the groups was done by the Mann-Whitney test. If there was a statistically significant difference using eta squared, we could find out what percentage of this difference was due to the intervention. The measured values were expressed as the arithmetic mean ±

Alexandra Marneri, Francesk Mulita, Vasileios Leivaditis, Serafeim – Chrysovalantis Kotoulas, Ioannis Gkoutziotis, Stavrati Kalliopi, Konstantinos Tasios, Levan Tchabashvili, Nikolaos Michalopoulos, Konstantinos Mpallas

Table I. Summary measures for TNF-α levels in study group – 3rd day

Measure of central tendency	Measure of variation
Mean = 1201	IQR = 195
Median = 1079	Standard deviation = 302.36

Table II. Summary measures for TNF-α levels in placebo group – 3rd day

Measure of central tendency	Measure of variation
Mean = 2128	IQR = 97
Median = 2112.5	Standard deviation = 129.4321

Table III. Summary measures for TNF-α levels in study group – 6th day

Measure of central tendency	Measure of variation
Mean = 821.75	IQR = 57.75
Median = 824.5	Standard deviation = 38.17831

Table IV. Summary measures for TNF-α levels in placebo group – 6th day

Measure of central tendency	Measure of variation
Mean = 1866.75	IQR = 73.25
Median = 1860	Standard deviation = 95.78927

Table V. Summary measures for TNF-α levels in study group – 9th day

Measure of central tendency	Measure of variation
Mean = 628	IQR = 23
Median = 623	Standard deviation = 30.8545

Table VI. Summary measures for TNF-α levels in placebo group – 9th day

Measure of central tendency	Measure of variation
Mean = 1642.5	IQR = 97.5
Median = 1625	Standard deviation = 102.1029

standard deviation. For the comparison of the variables in the groups of animals under study, the normality test was used, the *t*-test for independent samples if there was a normal distribution, or the non-parametric Mann-Whitney test if there was no normal distribution, these being tests used to compare averages. Due to the small number of animals per group, the Levene test for equality of variances was applied within the *t*-test.

Outcome measures

The primary outcome was the confirmation of the hypothesis of the anti-inflammatory activity

of rosuvastatin in the healing of partial-thickness burn wounds. This was done by recording reduced values of the measured parameters in the experimental group. Our initial hypothesis was supported by the histological examination, the reduced levels of pro-inflammatory cytokines, and the faster macroscopic healing of the trauma compared to the control group.

Results

Effect of rosuvastatin on burn-induced TNF-α and procalcitonin levels

Burn-injured mice showed significant increases in TNF-α levels in the plasma. Both rosuvastatin- and placebo-treated rats showed maximum levels of TNF-α on the 3rd post-burn day and both groups demonstrated a decrease of the above-mentioned levels on the 6th and 9th post-trauma days. However, we observed reduced expression of TNF-α in all rosuvastatin-treated rats on days 3, 6, and 9 compared to the placebo group ($p < 0.05$) (Tables I–VI).

Based on our statistical analysis, as regards the variable of TNF-α levels of the 3rd, 6th, and 9th-day levels, we can state that:

1. The shape of histograms looks like a bell, the Q-Q plot is linear and the boxplot is symmetrical.
2. The mean, median, and mode are proximal to each other.

Consequently, we concluded that the TNF-α levels of the placebo and study group are normally distributed. The Shapiro-Wilk test was performed. According to the Shapiro-Wilk normality test, *p*-value > 0.05 for the 3rd, 6th, and 9th days of both placebo- and rosuvastatin-treated groups. Since the p-value is larger than 0.05, the TNF-α levels are normally distributed.

Association between TNF-α levels in placebo- and rosuvastatin-treated group

To describe the basic characteristics of the two groups, we investigated the measures of central tendency and variability (Table VII).

Graphical assessment

The tests of normality suggest that the data for TNF-α levels in placebo- and rosuvastatin-treated groups are normally distributed ($p > 0.05$). If we combine the data extracted from graphs, measures of central tendency, and normality tests, the overall decision is in favor of normality for TNF-α levels (Figures 4–6).

We performed Levene's test in order to determine whether the variance is homogeneous between rosuvastatin and placebo-treated rats. We tested our null hypothesis through a two-sample test. The *p*-value was lower than 0.05. Conse-

Table VII. Summary measures of TNF-α levels in placebo- and rosuvastatin-treated rats

| The following results for TNF-α levels in placebo- and rosuvastatin-treated groups are depicted in the console:													
3rd day													
Group: placebo													
	vars	n	mean	sd	median	trimmed	mad	min	max	range	skew	kurtosis	SE	IQR
X1	1	4	2128	129.43	2112.5	2128	102.3	1987	2300	313	0.26	−1.87	64.72	97
Group: rosuvastatin													
	vars	n	mean	sd	median	trimmed	mad	min	max	range	skew	kurtosis	se	IQR
X1	1	4	1201	302.37	1079	1201	77.1	996	1650	654	0.7	−1.72	151.18	195
6th day													
Group: placebo													
	vars	n	mean	sd	median	trimmed	mad	min	max	range	skew	kurtosis	se	IQR
X1	1	4	1866.75	95.79	1860	1866.75	83.77	1757	1990	233	0.16	−1.88	47.89	73.25
Group: rosuvastatin													
	vars	n	mean	sd	median	trimmed	mad	min	max	range	skew	kurtosis	se	IQR
X1	1	4	821.75	38.18	824.5	821.75	43.74	780	858	78	−0.06	−2.33	19.09	57.75
9th day													
Group: placebo													
	vars	n	mean	sd	median	trimmed	mad	min	max	range	skew	kurtosis	se	IQR
X1	1	4	1642.5	102.1	1625	1642.5	81.54	1540	1780	240	0.34	−1.92	51.05	97.5
Group: rosuvastatin													
	vars	n	mean	sd	median	trimmed	mad	min	max	range	skew	kurtosis	se	IQR
X1	1	4	628	30.85	623	628	22.24	596	670	74	0.35	−1.85	15.43	23
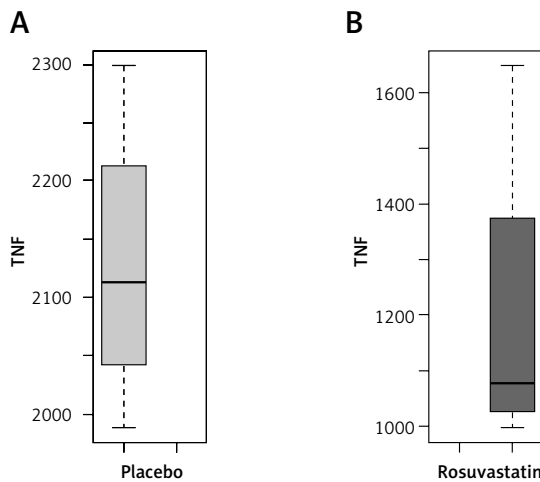
Figure 4. Boxplots of TNF-α levels for placebo- (**A**) and rosuvastatin-treated (**B**) rats on day 3
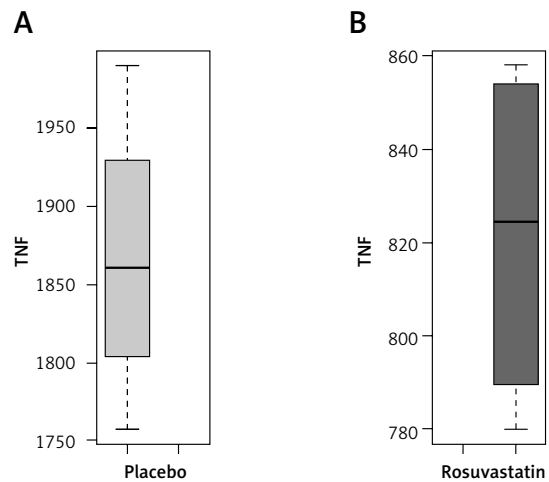

Figure 5. Boxplots of TNF-α levels for placebo- (**A**) and rosuvastatin-treated (**B**) rats on day 6

quently, there was a statistically significant difference in mean TNF-α levels between the two groups, on the 3rd, 6th, and 9th day.

Based on Figure 7, burn-wounded rats expressed significant increases in serum TNF-α levels. We can also note that TNF-α serum levels rise after the first post-burn hours and approach their maximum values on the third day and their minimum on day 9, not only for the study but for control groups as well. Moreover, the curve that represents each group seems to be similar. However, the concentration of TNF-α is statistically significantly different ($p < 0.05$) between rosuvastatin- and non-rosuvastatin-treated rats, concerning all post-wound studied points.

Procalcitonin

The mean values of PCT serum levels are presented in Figure 8. Based on our figure, we can

Alexandra Marneri, Francesk Mulita, Vasileios Leivaditis, Serafeim – Chrysovalantis Kotoulas, Ioannis Gkoutziotis, Stavrati Kalliopi, Konstantinos Tasios, Levan Tchabashvili, Nikolaos Michalopoulos, Konstantinos Mpallas

note that mean PCT serum levels are increased after the first post-traumatic hours and reach their maximum levels on day 3 and the minimum on day 9, for both the control and study group. These levels are statistically significantly different between the day of the burn (day 0: day of burn removal) and the 3rd day in the two groups. When PG and SG are compared, it appears that both

Figure 6. Boxplots of TNF-α levels for placebo- (**A**) and rosuvastatin-treated (**B**) rats on day 9

Figure 7. Line chart of TNF-α levels for placebo- and rosuvastatin-treated rats on days 3, 6 and 9

Figure 8. Line chart of PCT levels for placebo- and rosuvastatin-treated rats on days 0, 3, 6 and 9

treated animals follow a similar curve. However, there is a statistically significant difference in the concentration of PCT (pg/ml) between the two groups not only on day 3 but also on days 6 and 9.

Macroscopic assessment – planimetry

Rosuvastatin accelerates wound healing

Based on our statistical analysis, to define the main characteristics of the two groups we have to investigate the measures of central tendency and variability (Table VIII).

Graphical assessment

As we described before, the tests of normality we performed suggest that the data for the wound size in placebo- and rosuvastatin-treated groups are normally distributed ($p > 0.05$). Combining the information extracted from graphs, mean-median, and normality tests, the overall decision is in favor of normality for macroscopic assessment (Figures 9–11).

We performed Levene's test in order to determine whether there is homogeneity of variance between the two groups, and finally we tested our null hypothesis through a two-sample t-test. The p-value was lower than 0.05. Consequently, there was a statistically significant difference in mean diameter between the two groups, on the 6th and 9th day.

All the burn lesions were photographed and then macroscopically evaluated. Changes in wound diameter until the 9th day are expressed as a percentage of the initial burn area. Wound healing rate = [burn area (day X)/burn area (day 0)] × 100, planimetry method.

On day 3, rosuvastatin-treated groups presented almost 12.5% wound closure, whereas placebo-treated rats presented 8.5%, with no statistically significant difference. Rosuvastatin treatment resulted in significant differences in epithelization after day 6. Precisely, for placebo and rosuvastatin groups, 16.2% and 30.9% tissue repair was noted on day 6, and 28.7% and 45% on day 9, respectively (Figure 12).

Histopathological assessments

According to the Table IX, on the 3rd day, the average neutrophil infiltration was lower in the rosuvastatin group (average 40.5) than the placebo group (average 60). Moreover, as regards fibroblast proliferation, angiogenesis and re-epithelization, rosuvastatin-treated rats demonstrated higher levels, which led to up-regulation of the above-mentioned processes. Furthermore, on days 6 and 9, we can note that rosuvastatin stimulated both angiogenesis and re-epithelization.
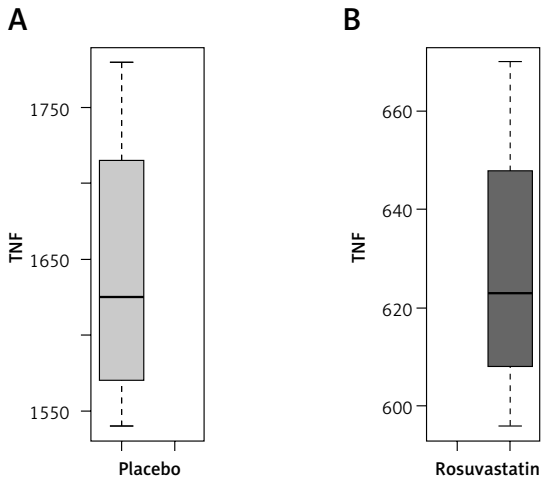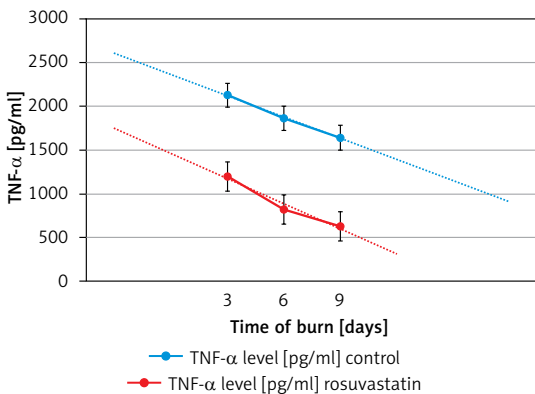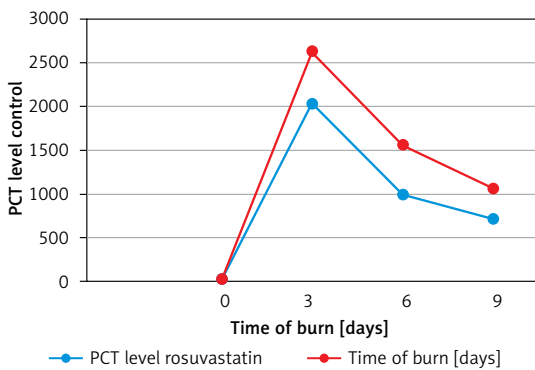
Table VIII. Summary measures of wound size (mm) in placebo- and rosuvastatin-treated rats

The following results for wound size diameter of placebo- or non-placebo-treated rats are presented in the console:													
3rd day													
Group: placebo													
	vars	n	mean	sd	median	trimmed	mad	min	max	range	skew	kurtosis	se	IQR
X1	1	4	1831.5	60.3	1830	1831.5	71.91	1768	1898	130	0.03	−2.27	30.15	85
Group: rosuvastatin													
	vars	n	mean	sd	median	trimmed	mad	min	max	range	skew	kurtosis	se	IQR
X1	1	4	1750.25	42.94	1761.5	1750.25	22.98	1689	1789	100	−0.53	−1.8	21.47	30.25
6th day													
Group: placebo													
	vars	n	mean	sd	median	trimmed	mad	min	max	range	skew	kurtosis	se	IQR
X1	1	4	1676.75	83.66	1660	1676.75	65.23	1597	1790	193	0.37	−1.94	41.83	85.75
Group: rosuvastatin													
	vars	n	mean	sd	median	trimmed	mad	min	max	range	skew	kurtosis	se	IQR
X1	1	4	1382.5	85.11	1377	1382.5	82.28	1286	1490	204	0.13	−1.95	42.55	81
9th day													
Group: placebo													
	vars	n	mean	sd	median	trimmed	mad	min	max	range	skew	kurtosis	se	IQR
X1	1	4	1426.25	110.41	1384	1426.25	37.06	1348	1589	241	0.67	−1.74	55.21	81.25
Group: rosuvastatin													
	vars	n	mean	sd	median	trimmed	mad	min	max	range	skew	kurtosis	se	IQR
X1	1	4	1128.5	76.62	1139	1128.5	71.16	1030	1206	176	−0.24	−2.04 38.31 87.5		
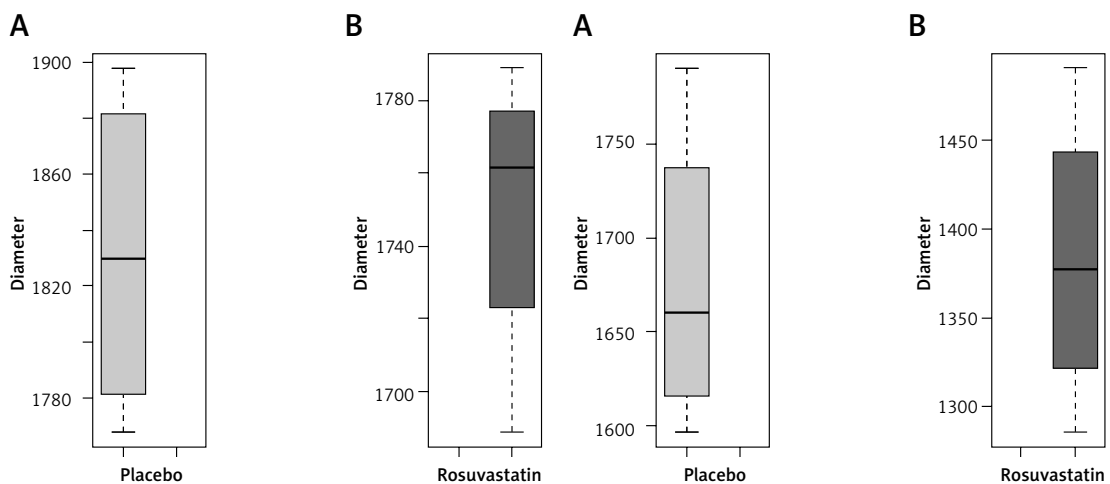
Figure 9. Boxplots of diameter (mm) for placebo- (**A**) and rosuvastatin-treated (**B**) rats on day 3

Figure 10. Boxplots of diameter for placebo- (**A**) and rosuvastatin-treated (**B**) rats on day 6

Discussion

Statins: mechanism of action

Cutaneous wound healing is a dynamic biological process, which involves the phases of inflammation, proliferation, and remodeling. Treatments that accelerate the skin repair procedure seem to be strong therapeutic weapons. Statins, primarily known for their hypolipidemic effects, exert their main pharmacological action by inhibiting 3-hydroxy-3-menthyl glutarate coenzyme A (HMG-CoA) reductase, a pivotal enzyme in cholesterol biosynthesis [27]. This inhibition reduces endogenous cholesterol synthesis in the liver, leading to increased expression of LDL receptors, enhanced circulation of LDL cholesterol, and sub-
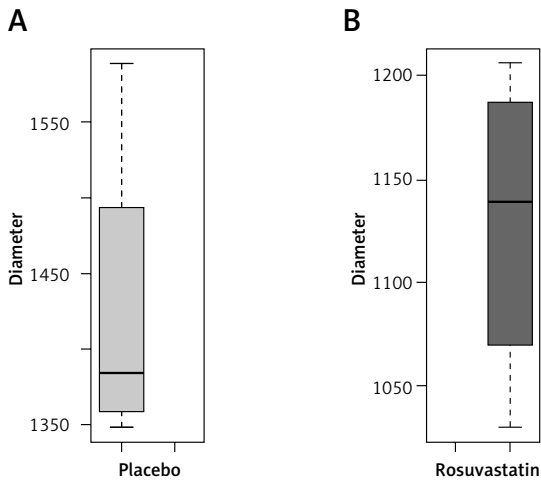
Alexandra Marneri, Francesk Mulita, Vasileios Leivaditis, Serafeim – Chrysovalantis Kotoulas, Ioannis Gkoutziotis, Stavrati Kalliopi, Konstantinos Tasios, Levan Tchabashvili, Nikolaos Michalopoulos, Konstantinos Mpallas

A

B

Figure 11. Boxplots of diameter for placebo- (**A**) and rosuvastatin-treated (**B**) rats on day 9

sequent lipid-lowering effects [28]. Beyond their hypolipidemic properties, statins exhibit pleiotropic actions [29], including antithrombotic effects, improvement of endothelial function, antioxidant properties, immunomodulation, and impact on various physiological systems such as the central nervous system, glucose metabolism, bones, and kidneys [28, 30–32]. These pleiotropic effects are thought to be lipid-independent and contribute to the broader therapeutic benefits of statins in addition to cholesterol reduction.

One of the significant pleiotropic effects of statins is their anti-inflammatory action. They reduce the expression of adhesion molecules [33], inhibit the binding of inflammatory cells to the endothelium, and decrease the secretion of pro-inflammatory cytokines IL-6, IL-1b, TNF-α, IL-8 and MCP-1 [34]. Additionally, they reduce C-reactive protein (CRP) levels, suppress Th17-mediated neutrophilic inflammation and inhibit macrophage and T cell activation [35]. Statins also affect the
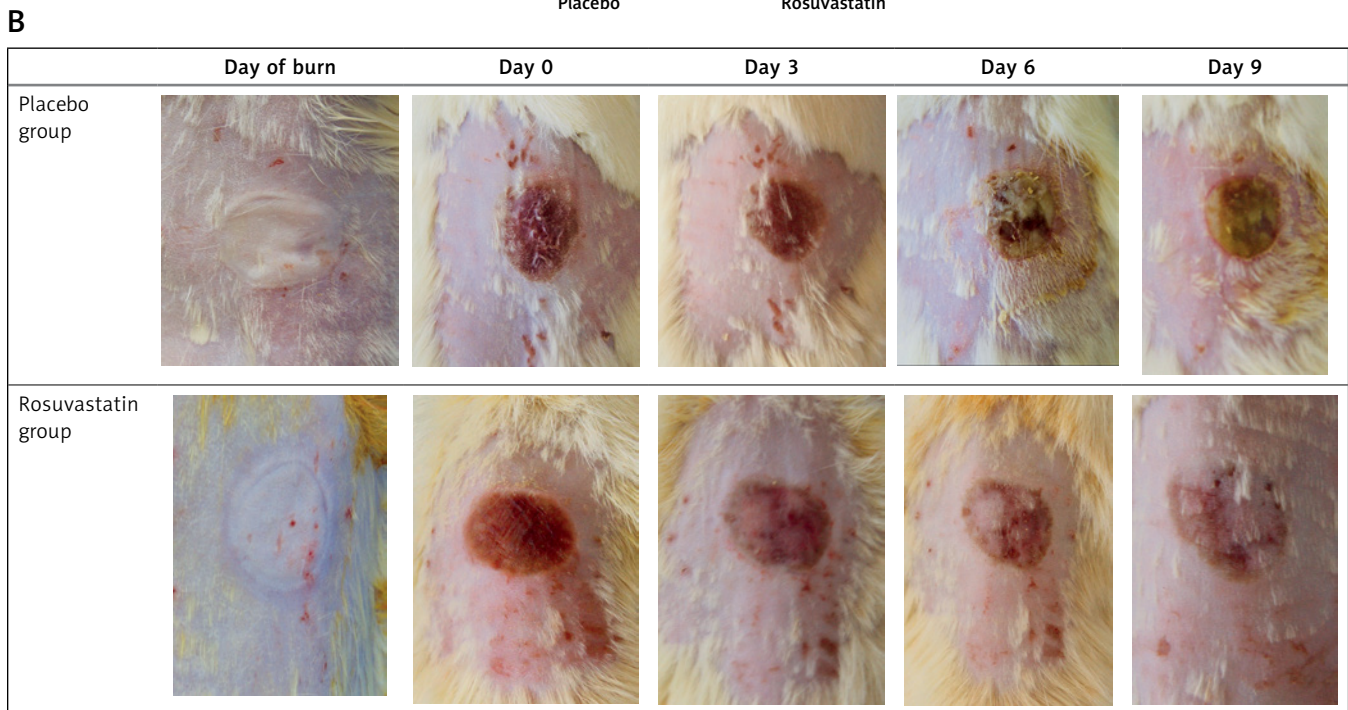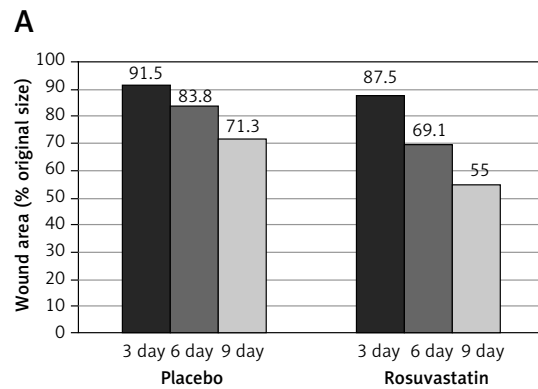
A

B

Figure 12. 3D clustered column of wound size for placebo- and rosuvastatin-treated rats on days 3, 6, and 9

Table IX. Summary of average levels of subepithelial neutrophils, fibroblast proliferation, angiogenesis, and re-epithelization on days 3, 6 and 9 for placebo and rosuvastatin groups

Variable	Day	Placebo group	Rosuvastatin group
Subepithelial neutrophils	3	60 ±14.8	40.5 ±12.2
	6	–	–
	9	–	–
Fibroblast proliferation	3	–	–
	6	42.1 ±19.1	64.5 ±20.2
	9	36.5 ±15.1	22.5 ±12.5
Angiogenesis	3	–	–
	6	24 ±15.2	48 ±10.6
	9	35.4 ±14.5	15.5 ±7.2
Re-epithelization	3	–	–
	6	6 ±9.6	34 ±22.2
	9	32 ±23.5	85.2 ±7.5

isoprenylation of proteins, including Rho, Ras, and Rac proteins, which play crucial roles in cell signaling pathways [36]. By inhibiting the isoprenylation process, statins disrupt important transduction pathways, influencing the concentration of adhesion molecules on endothelial cell surfaces. Additionally, statins reduce the transcriptional activity of NF-κB in inflammatory cells, suppressing the expression of cytokines and adhesives [37]. This anti-inflammatory effect extends beyond the vascular level, impacting systemic inflammation by lowering CRP levels [38].

Pleiotropic effects of statins on burn-wound healing

Based on statins' anti-inflammatory effects, studies indicate that topical application of simvastatin enhances angiogenesis and collagen deposition, contributing to the amelioration of second-degree burn wounds [39]. Combining simvastatin with bone marrow mesenchymal stromal cells (BMSCs) further promotes healing by positively influencing angiogenesis through the SDF-1α/CXCR4 pathway, showing improvements in wound closure percentage, epithelial thickness, collagen remodeling, and up-regulation of relevant biomarkers [40]. Additionally, the use of a self-nanoemulsion drug delivery system incorporating coconut oil loaded with simvastatin demonstrates synergistic benefits in managing burn wounds, emphasizing reduced wound diameter, decreased IL-6 levels, and intensified antimicrobial effects [41]. Furthermore, research on mice suggests that simvastatin protects against liver damage in burn injuries by inhibiting the TNF-α signaling pathway [42].

In another line of investigation, atorvastatin has been explored for its lipophilic characteristics and tissue diffusion capacity. Administration of atorvastatin during hospitalization for burn injuries demonstrated safety, with no adverse events or notable laboratory abnormalities reported [43]. Moreover, in a porcine burn model, atorvastatin administration exhibited enhanced graft take, demonstrating improved re-epithelialization of full-thickness burns, reduced inflammation, enhanced vascularization, and accelerated resolution of myofibroblasts [44].

Another study investigated the effect of atorvastatin on tissue repair in mice following skin trauma to the spine. In this study, administration of atorvastatin led to a decrease in the expression of IL-6 and TNF-α, thereby contributing to the faster wound healing [45]. Further data demonstrated that administration of pravastatin for wound healing in Sprague Dawley diabetic mice enhanced wound stability, hydroxyproline accumulation, and expression of endothelial nitric oxide synthase [46]. In a similar study, low-dose, pre-traumatic oral therapy with simvastatin increased angiogenesis, reduced inflammation, and improved wound healing in diabetic mice [47]. Simvastatin is, perhaps, the only statin whose role has been investigated in the healing of burn injuries, and studies have shown some positive effects.

Interestingly, a study involving the administration of simvastatin to mice demonstrated notable enhancement in the viability of free flaps by exerting effective anti-inflammatory, vasodilatory, and anticoagulant effects and by increasing the expression of endothelial thrombomodulin in the flap zone, which is also a zone of marginal viability [48]. These findings underscore the potential of statins, such as simvastatin and atorvastatin, as therapeutic agents for promoting burn wound healing through various mechanisms, including angiogenesis, anti-inflammatory effects, and tissue regeneration.

Alexandra Marneri, Francesk Mulita, Vasileios Leivaditis, Serafeim – Chrysovalantis Kotoulas, Ioannis Gkoutziotis, Stavrati Kalliopi, Konstantinos Tasios, Levan Tchabashvili, Nikolaos Michalopoulos, Konstantinos Mpallas

Rosuvastatin

Rosuvastatin, a synthetic hydrophilic statin, stands out within its class due to its exceptional pharmacokinetic and pharmacodynamic characteristics. It boasts the longest half-life among statins, attributed to its molecular structure allowing effective binding to HMG-CoA reductase, resulting in potent enzyme inhibition at lower doses [49]. The statin's liver selectivity minimizes bioavailability to non-hepatic tissues, reducing the risk of myotoxicity. Its metabolism, primarily by the 2C9 enzyme with minimal involvement of 3A4, contributes to fewer drug interactions involving cytochrome P450 [50].

The hydrophilic nature of rosuvastatin, in combination with the aforementioned selectivity of this statin to hepatocytes, results in its passive diffusion through the cell membranes being minimal, and it is almost completely absorbed by the liver [51]. As a result, the hypolipidemic effect of this statin is superior to that of simvastatin and pravastatin [52, 53].

Despite concerns about its pleiotropic effects in other tissues due to low uptake, studies affirm its pleiotropic actions, particularly through the inhibition of isoprenylation of Rho and Ras family proteins, critical for intracellular signal transmission. This inhibition results from the suppression of mevalonic acid synthesis, highlighting rosuvastatin's multifaceted impact beyond cholesterol reduction [54].

Pleiotropic effects of rosuvastatin

The pleiotropic effects of rosuvastatin are increasingly evident, with growing evidence pointing towards its beneficial impact on various neurological and myocardial function outcomes. Precisely, Qiu *et al.* observed significant enhancement in 7-day survival with rosuvastatin treatment. During the post-cardiac arrest phase, rosuvastatin demonstrated the ability to promptly enhance and stabilize circulatory function while mitigating cerebral injury. This effect was linked to the reduction of apoptosis in both myocardial and neural tissues [55].

The increasing evidence of rosuvastatin's effectiveness in neurological disorders, including epilepsy, Alzheimer's, Parkinson's, spinal cord injury, and cerebral ischemia, suggests its broad therapeutic potential. Rosuvastatin's neuroprotective effects are likely attributed to its anti-inflammatory, antioxidant, and anti-apoptotic activities, as demonstrated in recent research studies [56].

Another study aimed to examine how rosuvastatin influences angiotensin-converting enzyme 2 (ACE2) during neointimal formation following vascular balloon injury in rats and proved that rosuvastatin's ability to inhibit intimal thickening is associated with the elevation of ACE2, augmentation of Ang-(1-7), suppression of AT1, and activation of the P-ERK pathway [57].

Moreover, numerous viral pathogens contain components that undergo prenylation through the mammalian mevalonate pathway. In certain viral infections, rosuvastatin exhibits antiviral activity by inhibiting HMG-CoA reductase, thereby reducing the expression of prenylated proteins [58].

Boczar *et al.* attempted to assess both systemic markers and imaging indicators of inflammation in the ascending aorta, bone marrow, and spleen using 18F-FDG PET/CT in HIV-positive patients before and after rosuvastatin therapy. At baseline, HIV-positive patients exhibited significant markers of systemic inflammation, including monocyte activation, leading to the conclusion that treatment with a low dose of rosuvastatin in this HIV-positive cohort leads to a notable reduction in FDG uptake in the bone marrow, spleen, and thoracic aorta [59]. A study which led to a similar conclusion suggested that the lipid-lowering and anti-inflammatory properties of statins could potentially aid in preventing cardiovascular disease in individuals with human immunodeficiency virus (HIV) experiencing chronic inflammation, through elevation in proportions of CD16+ monocyte subsets [60].

Another investigation explored the impact of short-term administration of low- and high-dose rosuvastatin on the expression of ABCA1 and ABCG1 in human atherosclerotic plaques. Interestingly, high-dose rosuvastatin was found to elevate macrophage ABCA1 protein levels within atherosclerotic plaques, despite a decrease in mRNA, and this effect was unrelated to reductions in plasma cholesterol [61].

Finally, data indicated that the collective genetic potential of the gut microbiome to transport and metabolize metabolites along the phosphatidylcholine/carnitine-TMA-TMAO pathway was reduced in rosuvastatin-treated patients, with a corresponding increase of several metabolites in plasma. Consequently, these findings suggest that statins could have a clinically relevant impact on the gut microbiome [62].

Pleiotropic effects of rosuvastatin on wound healing

Experimental evidence suggests that rosuvastatin plays a significant role in wound healing by counteracting wound-healing inhibitors, such as farnesyl pyrophosphate (FPP), and enhancing endothelial and microvascular functions [63]. Additionally, it demonstrates antimicrobial properties against bacteria, including MRSA, and reduces

inflammation by suppressing the expression of inflammatory cytokines [64]. Studies also highlight its potential in managing chronic periodontitis by improving the repair process through the suppression of associated inflammation [65]. Researchers have explored various delivery systems, such as nanocubic vesicles with silver particles and transethosomal formulations, to enhance rosuvastatin's efficacy in wound management [66]. Despite challenges related to solubility and bioavailability, innovative approaches such as self-nanoemulsifying drug delivery systems (SNEDDS) and nanoemulsions are being investigated to overcome these constraints and improve the topical delivery of rosuvastatin for wound healing [67].

As we have mentioned, the existing support for the potential beneficial effects of rosuvastatin on wound healing primarily stems from animal studies, which are characterized by small sample sizes and relatively short observation periods. Additionally, the precise contribution of rosuvastatin in the context of burn wounds remains largely uninvestigated. Although the findings outlined above suggest that rosuvastatin may present a promising alternative for the treatment of burn wounds, it is crucial to note that further substantiation is required. In future, these outcomes necessitate validation through rigorous controlled clinical trials of high quality before any consideration for routine clinical application.

In our randomized prospective experimental study, we found that topical treatment of a partial-thickness burn with rosuvastatin ameliorates tissue repair. Through evaluation of the activity of rosuvastatin and procalcitonin levels in serum of burn-induced rats, we noted that the topical use of rosuvastatin reduced the above-mentioned levels. Specifically, topical rosuvastatin causes down-regulation of TNF-α and procalcitonin levels, modulates the inflammation process and improves burn outcomes.

In this experimental study we used 36 male rats that were randomly divided into two main groups. A partial-thickness burn trauma was induced on the back of the rats and 3rd, 6th and 9th post-burn days were examined. Half of the animals were treated with Eucerin cream and 50% with a mixture of Eucerin and rosuvastatin cream. Regarding our variables, we concluded that TNF-α, procalcitonin and wound size were normally distributed and we presented the mean and median values. Subsequently, we tried to identify the association between TNF-α and procalcitonin levels and the rate of wound closure on days 3, 6 and 9 for rosuvastatin and non-rosuvastatin treatment groups. Precisely, we proved that the data of TNF-α, procalcitonin and macroscopic assessment were normally distributed among the two groups on all studied days, and based on Student's t-test we found that there was a statistically significant difference in mean TNF-α and procalcitonin on day 9 between the two groups. Concerning the rate of wound closure, a statistically significant difference was not found for day 3 between the two groups, whereas tissue repair showed significant differences for the other days, in favor of rosuvastatin-treated rats.

Strengths

The effect of rosuvastatin on partial-thickness burn trauma has not been studied previously. We believe the study is of particular interest since rosuvastatin, as a younger generation statin, is characterized by fewer side effects and increased activity. Hence, the study may be considered original because it is the first study investigating the effect of rosuvastatin on an experimental model of partial-thickness burn injury in rats. Moreover, in this study we included an updated summary of evidence on the pleiotropic effects of rosuvastatin and the impact of statins on wound repair. Our results are generally in line with the previous experimental studies. Further points of our study include a thorough literature search both of major electronic databases and of grey literature.

Limitations

Finally, some limitations have to be acknowledged. Although in vivo burn models offer a detailed understanding of pathophysiological processes correlated with burn wounds, the use of larger animals seems to be one of the main limitations due to ethical or financial barriers. Despite their popularity, rat burn models present some wound healing differences and show reduced tissue repair time compared to those of humans [68]. Hence, it needs to be explored whether the conclusions in rats can be extrapolated to human burn models. Moreover, despite our thorough literature search, we identified only a few eligible studies and no meta-analyses that correlate the anti-inflammatory activity of statins with burn injury. The overall sample size was limited, leading to wide confidence intervals in our summary results.

In conclusion, our experimental study proved that the use of rosuvastatin through topical application treatment can modulate certain aspects of the inflammatory cascade, leading to improved clinical burn outcomes. In the absence of adequate evidence, further evidence-based studies seem to be crucial. Data should be collected to thoroughly assess the most effective statin drug, the appropriate doses, and the administration choices. Moreover, the majority of disposable data that detect the potential beneficial effect

Alexandra Marneri, Francesk Mulita, Vasileios Leivaditis, Serafeim – Chrysovalantis Kotoulas, Ioannis Gkoutziotis, Stavrati Kalliopi, Konstantinos Tasios, Levan Tchabashvili, Nikolaos Michalopoulos, Konstantinos Mpallas

of statins on skin tissue repair are based on animal-rat studies with limited overall sample sizes. Hence, large-scale clinical trials are needed to further prove the potential correlation of statins with an enhanced wound healing procedure. According to the World Health Organization (WHO), almost 265,000 deaths are caused by burns each year, most of which occur at home or in the workplace [69]. In addition, it is a global public health problem that affects people from all countries and of all ages. Nevertheless, the therapeutic landscape remains cloudy. Every day, new products are introduced to the market, aiming to heal the burn more effectively and esthetically. Focus is placed on the use of statins, which has a growing role in the treatment of burns and seems to be a promising, locally applicable, and low-cost solution [70]. Rosuvastatin is potential drug that, through topical application, is able to ameliorate tissue repair outcomes.

Funding

Ethical approval

Approval number: 11525-12/05/2023.

Conflict of interest

The authors declare no conflict of interest.

References

1. Tomic-Canic M, Burgess JL, O'Neill KE, Strbo N, Pastar I. Skin microbiota and its interplay with wound healing. Am J Clin Dermatol 2020; 21 (Suppl 1): 36-43.
2. Trejos M, Aristizabal Y, Aragón-Muriel A, Oñate-Garzón J, Liscano Y. Characterization and classification in silico of peptides with dual activity (antimicrobial and wound healing). Int J Mol Sci 2023; 24: 13091.
3. Walter AS, Volkmer E, Gauglitz G, Böcker W, Saller MM. Systematic review of molecular pathways in burn wound healing. Burns 2023; 49: 1525-33.
4. Wu J, Zhang F, Liu J, Yao H, Wang Y. Effect of silver-containing hydrofiber dressing on burn wound healing: a meta-analysis and systematic review. J Cosmet Dermatol 2023; 22: 1685-91.
5. Deana NF, Zaror C, del Sol M, Bagnato VS, Alves N. Wound contraction rate in excised and unexcised burn wounds with laser photobiomodulation: systematic review and meta-analysis of preclinical studies. Burns 2023; 49: 261-74.
6. Fijany AJ, Givechian KB, Zago I, et al. Tranexamic acid in burn surgery: a systematic review and meta-analysis. Burns 2023; 49: 1249-59.
7. Sharda D, Ghosh S, Kaur P, Basu B, Choudhury D. Chitosan-insulin nano-formulations as critical modulators of inflammatory cytokines and Nrf-2 pathway to accelerate burn wound healing. Discover Nano 2023; 18: 154.
8. Dong Y, Wang Z. ROS-scavenging materials for skin wound healing: advancements and applications. Front Bioeng Biotechnol 2023; 11: 1304835.
9. Irilouzadian R, Khalaji A, Baghsheikhi H, et al. The clinical outcomes of xenografts in the treatment of burn patients: a systematic review and meta-analysis. Eur J Med Res 2023; 28: 524.
10. Qiao Y, Zhang Q, Peng Y, et al. Effect of stem cell treatment on burn wounds: a systemic review and a meta-analysis. Int Wound J 2023; 20: 8-17.
11. Maita KC, Avila FR, Torres-Guzman RA, et al. Local anti-inflammatory effect and immunomodulatory activity of chitosan-based dressing in skin wound healing: a systematic review. J Clin Transl Res 2022; 8: 488-98.
12. Qian H, Shan Y, Gong R, et al. Fibroblasts in scar formation: biology and clinical translation. Oxid Med Cell Longev 2022; 2022: 4586569.
13. Wu J, Zhang F, Liu J, Yao H, Wang Y. Effect of silver-containing hydrofiber dressing on burn wound healing: a meta-analysis and systematic review. J Cosmet Dermatol 2023; 22: 1685-91.
14. Qiao Z, Yang H, Jin L, Li S, Wang X. The efficacy and safety of botulinum toxin injections in preventing postoperative scars and improving scar quality: a systematic review and meta-analysis. Aesthetic Plast Surg 2021; 45: 2350-62.
15. Correa-Araujo L, Prieto-Abello L, Lara-Bertrand A, et al. Bioengineered skin constructs based on mesenchymal stromal cells and acellular dermal matrix exposed to inflammatory microenvironment releasing growth factors involved in skin repair. Stem Cell Res Ther 2023; 14: 306.
16. Jaam M, Al-Naimi HN, Haddad MM, Abushanab D, Al-Badriyeh D. Comparative efficacy and safety among high-intensity statins. Systematic review and meta-analysis. J Comp Eff Res 2023; 12: e220163.
17. Pertzov B, Eliakim-Raz N, Atamna H, Trestioreanu AZ, Yahav D, Leibovici L. Hydroxymethylglutaryl-CoA reductase inhibitors (statins) for the treatment of sepsis in adults – a systematic review and meta-analysis. Clin Microbiol Infect 2019; 25: 280-9.
18. Lehtisalo M, Taskinen S, Tarkiainen EK, et al. A comprehensive pharmacogenomic study indicates roles for SLCO1B1, ABCG2 and SLCO2B1 in rosuvastatin pharmacokinetics. Br J Clin Pharmacol 2023; 89: 242-52.
19. Salem HF, Nafady MM, Ewees MGED, Hassan H, Khallaf RA. Rosuvastatin calcium-based novel nanocubic vesicles capped with silver nanoparticles-loaded hydrogel for wound healing management: optimization employing Box–Behnken design: in vitro and in vivo assessment. J Liposome Res 2022; 32: 45-61.
20. Friedrich EE, Sun LT, Natesan S, Zamora DO, Christy RJ, Washburn NR. Effects of hyaluronic acid conjugation on anti-TNF-α inhibition of inflammation in burns. J Biomed Mater Res A 2014; 102: 1527-36.
21. Hassan J, Khan S, Zahra R, et al. Role of procalcitonin and c-reactive protein as predictors of sepsis and in managing sepsis in postoperative patients: a systematic review. Cureus 2022; 14: e31067.
22. Huang YH, Chen CJ, Shao SC, et al. Comparison of the diagnostic accuracies of monocyte distribution width, procalcitonin, and C-reactive protein for sepsis: a systematic review and meta-analysis. Crit Care Med 2023; 51: e106-14.
23. Molano-Franco D, Arevalo-Rodriguez I, Muriel A, et al. Basal procalcitonin, C-reactive protein, interleukin-6, and presepsin for prediction of mortality in critically ill septic patients: a systematic review and meta-analysis. Diagn Progn Res 2023; 7: 15.
24. Yaseen HS, Asif M, Saadullah M, et al. Methanolic extract of Ephedra ciliata promotes wound healing and

arrests inflammatory cascade in vivo through down-regulation of TNF-α. Inflammopharmacology 2020; 28: 1691-704.

25. Verras GI, Mulita F. Butyrylcholinesterase levels correlate with surgical site infection risk and severity after colorectal surgery: a prospective single-center study. Front Surg 2024; 11: 1379410.

26. Zhu S, Zeng C, Zou Y, Hu Y, Tang C, Liu C. The clinical diagnostic values of SAA, PCT, CRP, and IL-6 in children with bacterial, viral, or co-infections. Int J Gen Med 2021; 14: 7107-13.

27. De Filippo O, D'Ascenzo F, Iannaccone M, et al. Safety and efficacy of bempedoic acid: a systematic review and meta-analysis of randomised controlled trials. Cardiovasc Diabetol 2023; 22: 324.

28. Stefanou MI, Palaiodimou L, Katsanos AH, et al. The effects of HMG-CoA reductase inhibitors on disease activity in multiple sclerosis: a systematic review and meta-analysis. Mult Scler Relat Disord 2022; 58: 103395.

29. Jamialahmadi T, Abbasifard M, Reiner Ž, Rizzo M, Eid AH, Sahebkar A. The effects of statin treatment on serum ferritin levels: a systematic review and meta-analysis. J Clin Med 2022; 11: 5251.

30. Di Spirito F, Schiavo L, Pilone V, Lanza A, Sbordone L, D'Ambrosio F. Periodontal and peri-implant diseases and systemically administered statins: a systematic review. Dent J (Basel) 2021; 9: 100.

31. Acton EK, Khazaal O, Willis AW, et al. Statins for the prevention of post-stroke seizure and epilepsy development: a systematic review and meta-analysis. J Stroke Cerebrovasc Dis 2021; 30: 106024.

32. Vahedian-Azimi A, Heidari Beni F, Fras Z, et al. Effects of statins on the incidence and outcomes of acute kidney injury in critically ill patients: a systematic review and meta-analysis. Arch Med Scie 2023; 19: 952-64.

33. Sorg H, Tilkorn DJ, Hager S, Hauser J, Mirastschijski U. Skin wound healing: an update on the current knowledge and concepts. Eur Surg Res 2017; 58: 81-94.

34. Liberale L, Carbone F, Camici GG, Montecucco F. IL-1β and statin treatment in patients with myocardial infarction and diabetic cardiomyopathy. J Clin Med 2019; 8: 1764.

35. Chen YR, Xiang XD, Sun F, et al. Simvastatin reduces NE-Tosis to attenuate severe asthma by inhibiting PAD4 expression. Oxid Med Cell Longev 2023; 2023: 1493684.

36. Beales ILP, Ogunwobi OO. Leptin activates Akt in oesophageal cancer cells via multiple atorvastatin-sensitive small GTPases. Mol Cell Biochem 2021; 476: 2307-16.

37. González-Herrera F, Clayton NS, Guzmán-Rivera D, et al. Statins change the cytokine profile in Trypanosoma cruzi-infected U937 macrophages and murine cardiac tissue through Rho-associated kinases inhibition. Front Immunol 2023; 13: 1035589.

38. Bedi O, Dhawan V, Sharma PL, Kumar P. Pleiotropic effects of statins: new therapeutic targets in drug design. Naunyn Schmiedebergs Arch Pharmacol 2016; 389: 695-712.

39. Ramhormozi P, Ansari JM, Simorgh S, et al. Simvastatin accelerates the healing process of burn wound in Wistar rats through Akt/mTOR signaling pathway. Ann Anatomy 2021; 236: 151652.

40. Ansari JM, Ramhormozi P, Shabani R, et al. Simvastatin combined with bone marrow mesenchymal stromal cells (BMSCs) improve burn wound healing by ameliorating angiogenesis through SDF-1α/CXCR4 pathway. Iran J Basic Med Sci 2020; 23: 751-9.

41. Hosny KM, Alhakamy NA, Sindi AM, Khallaf RA. Coconut oil nanoemulsion loaded with a statin hypolipidemic drug for management of burns: formulation and in vivo evaluation. Pharmaceutics 2020; 12: 11-6.

42. Zhao G, Yu YM, Kaneki M, Tompkins RG, Fischman AJ. Simvastatin protects hepatocytes from apoptosis by suppressing the TNF-α/caspase-3 signaling pathway in mice with burn injury. Ann Surg 2013; 257: 1129-36.

43. Boyko T, Marin C, Furnari G, Flynn W, Lukan JK. Safety profile of atorvastatin in the role of burn wound injury conversion. Am J Surg 2020; 220: 1323-6.

44. Akershoek JJ, Brouwer KM, Vlig M, et al. Differential effects of Losartan and Atorvastatin in partial and full thickness burn wounds. PLoS One 2017; 12: e0179350.

45. Suzuki-Banhesse VF, Azevedo FF, Araujo EP, et al. Effect of atorvastatin on wound healing in rats. Biol Res Nurs 2015; 17: 159-68.

46. Laing T, Hanson R, Chan F, Bouchier-Hayes D. Effect of pravastatin on experimental diabetic wound healing. J Surg Res 2010; 161: 336-40.

47. Sukpat S, Israsena N, Patumraj S. Pleiotropic effects of simvastatin on wound healing in diabetic mice. J Med Assoc Thai 2016; 99: 213-9.

48. Abdelfattah U, Elbanoby T, Kim EN, Park EJ, Suh HP, Hong JPJ. Effect of simvastatin use in free tissue transfer: an experimental study in a rat epigastric free flap model. J Reconstr Microsurg 2020; 36: 281-8.

49. Wang L, Luan Y, Jia C, et al. Pharmacokinetic interactions and tolerability of rosuvastatin and ezetimibe: a randomized, phase 1, crossover study in healthy Chinese participants. Eur J Drug Metab Pharmacokinet 2023; 48: 51-62.

50. Vrzal R, Zenata O, Bachleda P, Dvorak Z. The effects of drugs with immunosuppressive or immunomodulatory activities on xenobiotics-metabolizing enzymes expression in primary human hepatocytes. Toxicol Vitro 2015; 29: 1088-99.

51. Climent E, Benaiges D, Pedro-Botet J. Hydrophilic or lipophilic statins? Front Cardiovasc Med 2021; 8: 687585.

52. Bener A, Dogan M, Barakat L, Al-Hamaq AOAA. Comparison of cost-effectiveness, safety, and efficacy of rosuvastatin versus atorvastatin, pravastatin, and simvastatin in dyslipidemic diabetic patients with or without metabolic syndrome. J Prim Care Community Health 2014; 5: 180-7.

53. Sivkov A, Chernus N, Gorenkov R, Sivkov S, Sivkova S, Savina T. Relationship between genetic polymorphism of drug transporters and the efficacy of Rosuvastatin, atorvastatin and simvastatin in patients with hyperlipidemia. Lipids Health Dis 2021; 20: 157.

54. Kavalipati N, Shah J, Ramakrishan A, Vasnawala H. Pleiotropic effects of statins. Indian J Endocrinol Metab 2015; 19: 554.

55. Qiu Y, Wu Y, Meng M, et al. Rosuvastatin improves myocardial and neurological outcomes after asphyxial cardiac arrest and cardiopulmonary resuscitation in rats. Biomed Pharmacother 2017; 87: 503-8.

56. Husain I, Khan S, Khan S, Madaan T, Kumar S, Najmi AK. Unfolding the pleiotropic facades of rosuvastatin in therapeutic intervention of myriads of neurodegenerative disorders. Clin Exp Pharmacol Physiol 2019; 46: 283-91.

57. Li YH, Wang QX, Zhou JW, et al. Effects of rosuvastatin on expression of angiotensin-converting enzyme 2 after vascular balloon injury in rats. J Geriatr Cardiol 2013; 10: 151-8.

58. Bifulco M, Gazzerro P. Statins in coronavirus outbreak: it's time for experimental and clinical studies. Pharmacol Res 2020; 156: 104803.

Alexandra Marneri, Francesk Mulita, Vasileios Leivaditis, Serafeim – Chrysovalantis Kotoulas, Ioannis Gkoutziotis, Stavrati Kalliopi, Konstantinos Tasios, Levan Tchabashvili, Nikolaos Michalopoulos, Konstantinos Mpallas

59. Boczar KE, Faller E, Zeng W, et al. Anti-inflammatory effect of rosuvastatin in patients with HIV infection: an FDG-PET pilot study. J Nucl Cardiol 2022; 29: 3057-68.

60. Hearps AC, Angelovich TA, Trevillyan JM, et al. Effect of rosuvastatin therapy on biomarkers of inflammation and immune activation in people with human immunodeficiency virus at intermediate cardiovascular risk. J Infect Dis 2021; 224: 667-72.

61. Santovito D, Marcantonio P, Mastroiacovo D, et al. High dose rosuvastatin increases ABCA1 transporter in human atherosclerotic plaques in a cholesterol-independent fashion. Int J Cardiol 2020; 299: 249-53.

62. Kummen M, Solberg OG, Storm-Larsen C, et al. Rosuvastatin alters the genetic composition of the human gut microbiome. Sci Rep 2020; 10: 5397.

63. Tanaka S, Fukumoto Y, Nochioka K, et al. Statins exert the pleiotropic effects through small GTP-binding protein dissociation stimulator upregulation with a resultant Rac1 degradation. Arterioscler Thromb Vasc Biol 2013; 33: 1591-600.

64. Thangamani S, Mohammad H, Abushahba MFN, et al. Exploring simvastatin, an antihyperlipidemic drug, as a potential topical antibacterial agent. Sci Rep 2015; 5: 16407.

65. Pradeep AR, Karvekar S, Nagpal K, Patnaik K, Raju A, Singh P. Rosuvastatin 1.2 mg in situ gel combined with 1:1 mixture of autologous platelet-rich fibrin and porous hydroxyapatite bone graft in surgical treatment of mandibular class II furcation defects: a randomized clinical control Trial. J Periodontol 2016; 87: 5-13.

66. Salem HF, Nafady MM, Ewees MGED, Hassan H, Khallaf RA. Rosuvastatin calcium-based novel nanocubic vesicles capped with silver nanoparticles-loaded hydrogel for wound healing management: optimization employing Box–Behnken design: in vitro and in vivo assessment. J Liposome Res 2022; 32: 45-61.

67. Balakumar K, Raghavan CV, Selvan NT, Prasad RH, Abdu S. Self nanoemulsifying drug delivery system (SNEDDS) of Rosuvastatin calcium: design, formulation, bioavailability and pharmacokinetic evaluation. Colloids Surf B Biointerfaces 2013; 112: 337-43.

68. Abdullahi A, Amini-Nik S, Jeschke MG. Animal models in burn research. Cell Mol Life Sci 2014; 71: 3241-55.

69. Munteanu A, Florescu IP, Nitescu C. A modern method of treatment: the role of silver dressings in promoting healing and preventing pathological scarring in patients with burn wounds. J Med Life 2016; 9: 306-15.

70. Synodinou D, Savoie-White FH, Sangone A, et al. Health utilities in burn injury survivors: a systematic review. Burns 2022; 48: 13-22.