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Anti-inflammatory Effect of Simvastatin-Aspirin Combination

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ABSTRACT
The aim of this study is to investigate the anti-inflammatory effects of simvastatin alone and in combination with aspirin, the most widely used analgesic, antipyretic and anti-inflammatory agent, and to evaluate the effect of using them in combination which may produce synergistic effect and lower the dose required for each agent. 72 Male Wistar albino rats suffering from air pouch granuloma were used. They were divided into 12 groups each comprising 6 rats, they received either simvastatin (20 mg/kg/day) or aspirin (25 mg/kg/day) or combined therapy (simvastatin & aspirin) for either 3 or 6 executive days. The control groups received the solvents only for the same periods. Biochemical markers of inflammation as serum tumor necrosis factor-α, interleukin-6 and interleukin-4 were measured by an Elisa method. Antioxidant activity was calorimetrically assessed by measuring serum nitric oxide concentration. Results indicated that treatment with simvastatin alone had no significant difference from treatment with aspirin alone which give solid ground for the predicted anti-inflammatory effects of simvastatin. Furthermore, our study demonstrated that treatment with the combined therapy reduced the extent of inflammation as compared to treatment with simvastatin alone or aspirin alone indicating that aspirin-simvastatin combination represents a synergistic combination concerning the immune response to the inflammatory challenge. The combination treatment with agents that inhibit different aspects of the signal transduction pathways will be transformational and have better efficacy with fewer side effects.

Keywords: Simvastatin; tumor necrosis factor-α (TNF-α); interleukin-6 (IL-6); interleukin-4 (IL-4).

INTRODUCTION

The inflammatory response is an attempt by the body to restore and maintain homeostasis after injury or infection and is an integral part of body defense. For the survival of the host, inflammation is a necessary and beneficial process but chronic inflammation can cause harm. [1] Soluble mediators such as nitric oxide (NO), tumor necrosis factor-α (TNF-α), and interleukins usually play a role in controlling important functions such as the regulation of blood pressure, platelet aggregation, and body temperature. [2] Under pathologically inflammatory conditions, however, the production of these molecules promotes events ranging from increased leukocyte infiltration and vascular permeability to organ failure. [3] The selective inhibition of these and other inflammatory activities remains an important goal for the effective treatment of inflammation.

The aim of this study is to investigate the possible predicted anti-inflammatory effects of simvastatin comparing it with Aspirin; the popular and effective drug that inhibit inflammatory reactions and platelet aggregation and to evaluate the possibility of combination of statins and aspirin to improve inflammation and in turn reduce unwanted side effects possibly caused by high dose single agent administration.

Simvastatin is a hypolipidimic drug belonging to the class of pharmaceuticals called statins. [4] Statins, competitive inhibitors of 3-hydroxy-3-methylglutaryl–coenzyme A (HMG-CoA) reductase, the rate limiting enzyme in cholesterol synthesis, are widely prescribed for the treatment of hypercholesterolemia [5] and are the principal therapy for more than 25 million people at risk of cardiovascular disease worldwide. [6] McCarey *et al.* reported that Statins appear to have therapeutic benefits in diseases that are unrelated to elevated serum cholesterol levels, such as rheumatologic diseases and ischemic stroke. [7] Statins might exert beneficial effects beyond cholesterol reduction; include improving endothelial function, decreasing vascular inflammation, inhibiting smooth-muscle proliferation and immunomodulation. Most of these effects are mediated through inhibition of isoprenoid synthesis, with subsequent effects on multiple downstream signaling pathways. [6]

Acetyl salicylic acid (Aspirin) is the most widely used drug in the world because it possesses profound analgesic, anti-thrombotic and anti-inflammatory properties. [8] Aspirin causes irreversible inhibition by selectively and rapidly

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acetylating a serine residue (Ser 530) near the C-terminus of the COX family of enzymes. [9] There are at least two different types of cyclooxygenase: COX-1 and COX-2. Aspirin irreversibly inhibits COX-1 and modifies the enzymatic activity of COX-2. Normally COX produces prostaglandins, most of which are pro-inflammatory, and thromboxanes, which promote clotting. Aspirin-modified COX-2 produces lipoxins, most of which are anti-inflammatory. [10]

MATERIAL AND METHODS

Induction of inflammation

After a period of adaptation, induction of inflammation was done by air pouch method in which animals were anaesthetized with ether, and then 5 ml of air were injected under the skin on their backs. [11-12] After 3 days, the pouches were reinjected with 3 ml of air. On day 6, 1 ml of 1% carrageenan (Fluka, Switzerland) in saline was injected into the pouches. [13] At the day of injection, the animals (72 male Wistar albino rats) where divided into 12 groups each comprising 6 rats, they received the anti-inflammatory treatment for either 3 or 6 executive days.

Doses

Simvastatin-treated rats received a daily intraperitoneal (i.p) injection of simvastatin (Julphar, UAE) at a dose of (20 mg/kg/day). [14] The final concentration used was 8mg/ml; this dose was chosen based on findings in mice of maximal stroke protection. [15] Simvastatin was prepared by being dissolved in 0.5% carboxy methyl cellulose (CMC) [12] and the control groups were given an equal volume of vehicle.

Aspirin-treated rats received a daily I.P injection of aspirin (Aspegic-Amriya Pharm. Ind., Egypt) at dose of (25 mg/kg/day). [16-17] The final concentration used was 10 mg/ml; this dose was chosen based on findings that high dose of aspirin is used as antirheumatic therapy, abrogate COX-2 activity, suggesting that non selective doses of NSAIDs should be used with caution because they may deprive the heart of its innate defensive response. [16]

Combined therapy-treated rats received a daily i.p injection of both simvastatin (20 mg/kg/day) and aspirin (25 mg/kg/day); the control groups were given an equal volume of both vehicles.

Experimental design

Group I: received 0.5 ml 0.5% CMC i.p for 3 days and served as simvastatin control group for 3 days.

Group II: received (20 mg/kg/day) simvastatin in 0.5% CMC i.p for 3 days and served as simvastatin treated group for 3 days.

Group III: received 0.5 ml 0.5% CMC i.p for 6 days and served as simvastatin control group for 6 days.

Group IV: received (20 mg/kg/day) simvastatin in 0.5% CMC i.p for 6 days and served as simvastatin treated group for 6 days.

Group V: received 0.5 ml saline i.p for 3 days and served as aspirin control group for 3 days.

Group VI: received (25 mg/kg/day) aspirin in 0.5% saline i.p for 3 days and served as aspirin treated group for 3 days.

Group VII: received 0.5 ml saline i.p for 6 days and served as aspirin control group for 6 days.

Group VIII: received (25 mg/kg/day) aspirin in saline for 6 days and served as aspirin treated group for 6 days.

Group IX: received 0.5 ml CMC & 0.5 ml saline for 3 days and served as combined therapy control group.

Group X: received (20 mg/kg/day) simvastatin in 0.5% CMC and (25mg/kg/day) aspirin in saline for 3 days and served as combined therapy treated group.

Group XI: received 0.5 ml CMC and 0.5 ml saline for 6 days and served as combined therapy control group for 6 days.

Group XII: received (20 mg/kg/day) simvastatin in 0.5% CMC and (25 mg/kg/day) aspirin in saline for 6 days and served as combined therapy treated group for 6 days.

Samples: At the end of the experiment (after 3 or 6 days), animals were anaesthetized; blood was collected and allowed to clot, centrifuged at 2,000 r.p.m for 10 min. The serum was separated and used in the same day for the measurement of: Nitric oxide (NO)**.** The rest of the serum was kept deeply frozen at (-20^{o}C) for the measurement of: Tumor necrosis factor-α (TNF-α), Interleukin-6 (IL-6) and Interleukin-4 (IL-4).

Chemicals

Evaluation of antioxidant activity by measurement of nitric oxide concentrations colorimetrically using kits from Biodiagnostic Co, Egypt according to reported methods. [18] Investigation of tumor necrosis factor-α, quantitatively by an Elisa method using Kits from Bender MedSystems, Austria according to the reported methods, [19-20] interleukin-6 was quantitatively assayed by an Elisa method using kits from from Bender MedSystems, Austria, following the reported methods, [21-22] and interleukin-4 was also quantitatively measured by an Elisa method using Kits from Bender MedSystems, Austria according to reported procedures. [23-24]

Statistical analysis

Data from the control and treated animals in the different groups in the present study where characterized by their means and standard errors. Comparisons between results of samples of different groups were tested by ANOVA & Tukey-Kramer multiple comparison tests. Statistical analysis was done by using GraphPad prism program version 5 produced by GraphPad Software Inc. and an IBM PC/AT compatible computer. Statistical significance was set up at $p < 0.05$, $p < 0.01$ or < 0.001.

Table 1: Effect of i.p injection of simvastatin (20 mg/kg/day), aspirin (25 mg/kg/day) or their combination for 3 or 6 days on serum levels of nitric oxide (NO) in rats subcutaneously injected 1ml of (1%) carrageenan in saline into dorsal pouch

Groups	Serum levels of nitric oxide (µmol/L) (4$^{th.}$ day)	Serum levels of nitric oxide (µmol/L) (7$^{th.}$ day)
Simvastatin control group (n=6)	13.05±1.14	12.27±1.07
Simvastatin group (n=6)	31.26 ^{a} ±2.82	31.67 ^{a} ±2.64
Aspirin control group (n=6)	14.74±1.32	10.54±0.87
Aspirin group (n=6)	31.6 ^{a} ±2.89	34.49 ^{a} ±2.53
Combined Therapy control group (n=6)	15.11±0.98	12.36±1.02
Combined Therapy group (n=6)	34.58 ^{a} ±3.35	42.58 $^{a\,b\,c\,e}$ ±1.09

n: Number of rats

^{a} :Significance against corresponding control group at $p<0.001$

^{b} : Significance against simvastatin treated group 4th day at $p <0.05$

^{c} : Significance against aspirin treated group 4th day at $p <0.05$

^{e} : Significance against simvastatin treated group 7th day at $p <0.05$

Values are expressed as mean of 6 rats ± S.E.M. and compared using ANOVA & Tukey-Kramer multiple comparison tests.

RESULTS

Our results (Table 1, Fig. 1 & 2) showed obviously that i.p injection of simvastatin (20 mg/kg/day), aspirin (25 mg/kg/day) or their combination for 3 days or 6 days resulted in significant increase of serum levels of NO at ($p < 0.001$) as

compared to corresponding control groups. Moreover, (Fig. 3) showed significant increase in serum NO levels in the combined therapy treated group in the 7th day as compared to simvastatin and aspirin treated groups in the 4th day & against simvastatin treated group in the 7th day at ($p < 0.05$).

i.p injection of simvastatin (20 mg/kg/day), aspirin (25 mg/kg/day) or their combination for 3 days or 6 days (Table 2) resulted in dramatic decrease of serum levels of TNF-α below the detection limit.

Table 2: Effect of i.p injection of simvastatin (20 mg/kg/day), aspirin (25 mg/kg/day) or their combination for 3 or 6 days on serum levels of tumor necrosis factor-alpha (TNF-α) in rats subcutaneously injected 1 ml of (1%) carrageenan in saline into dorsal pouch

Groups	Serum levels of TNF-α (pg/ml) (4th day)	Serum levels of TNF-α (pg/ml) (7th day)
Simvastatin control group (n=6)	160.11±14.45	191.1±14.87
Simvastatin group (n=6)	< 39 [a]	< 39 [a]
Aspirin control group (n=6)	150.9±12.50	216.9±19.35
Aspirin group (n=6)	< 39 [a]	< 39 [a]
Combined Therapy control group (n=6)	175.7±13.76	196.7±9.89
Combined Therapy group (n=6)	< 39 [a]	< 39 [a]

n: Number of rats
[a] : Significance against corresponding control group at P < 0.001
Values were expressed as mean of 6 rats ± S.E.M. and compared using ANOVA & Tukey-Kramer multiple comparison tests.

Table 3: Effect of i.p injection of simvastatin (20 mg/kg/day), aspirin (25 mg/kg/day) or their combination for 3 or 6 days on serum levels of interlukin-6 (IL-6) in rats subcutaneously injected 1 ml of (1%) carrageenan in saline into dorsal pouch

Groups	Serum levels of IL-6 (pg/ml) (4th day)	Serum levels of IL-6 (pg/ml) (7th day)
Simvastatin control group (n=6)	534.5±8.90	556.60±14.68
Simvastatin group (n=6)	112.6[a] ±6.37	94.84[a] ±3.98
Aspirin control group (n=6)	547.60±12.55	570.80±15.04
Aspirin group (n=6)	106.70[a] ±4.64	91.65[ab] ±4.48
Combined Therapy control group (n=6)	557.2±12.37	541.50±16.01
Combined Therapy group (n=6)	93.59[a] ±3.90	67.70[abcdef] ±3.70

n: Number of rats
[a] : Significance against corresponding control group at P < 0.001
[b] : Significance against simvastatin treated group 4th day at p < 0.05
[c] : Significance against aspirin treated group 4th day at p < 0.05
[d] : Significance against combined therapy treated group 4th day at p < 0.05
[e] : Significance against simvastatin treated group 7th day at p < 0.05
[f] : Significance against aspirin treated group 7th day at p < 0.05
Values were expressed as mean of 6 rats ± S.E.M. and compared using ANOVA & Tukey-Kramer multiple comparison tests

The of i.p injection of simvastatin (20 mg/kg/day), aspirin (25 mg/kg/day) or their combination for 3 days or 6 days (Table 3, Fig. 4 & 5) resulted in dramatic significant decrease of IL-6 serum levels at ($p < 0.001$) as compared to corresponding control groups. Furthermore, (Fig. 5) showed that combined therapy treated group in the 7th day triggered significant decrease in serum IL-6 levels as compared to simvastatin, aspirin treated groups in the 7th day at ($p < 0.05$). Significant decrease in IL-6 serum levels was recorded in aspirin treated group (Table 3, Fig. 6) in the 7th day as compared to simvastatin treated group in the 4th day at ($p < 0.05$). In addition, I.P injection of combined therapy for 6 days caused significant decrease in serum IL-6 levels in the combined therapy treated group in the 7th day as compared to simvastatin, aspirin & combined therapy treated groups in the 4th day at ($p < 0.05$) and as compared to simvastatin and aspirin treated groups in the 7th day as well at ($p < 0.05$).

Our results shown in (Table 4, Fig. 7) demonstrated that serum concentration of IL-4 was highly increased after the i.p injection of simvastatin (20 mg/kg/day), aspirin (25 mg/kg/day) or their combination for 3 days or 6 days as compared to corresponding control groups which had serum levels below the detection limit. i.p injection of simvastatin (20 mg/kg/day) or aspirin or (25 mg/kg/day) for 6 days resulted in similar significant increase of serum levels of IL-4 at ($p < 0.05$) as compared to groups received either simvastatin or aspirin for 3 days. While i.p injection of combined therapy for 6 days caused higher significant increase in IL-4 serum levels at ($p < 0.05$) when compared to i.p injection of simvastatin, aspirin and combined therapy for 3 days and as compared to i.p injection of aspirin for 6 days.

Table 4: Effect of i.p injection of simvastatin (20 mg/kg), aspirin (25 mg/kg) or their combination for 3 or 6 days on serum levels of interlukin-4 (IL-4) in rats subcutaneously injected 1 ml of (1%) carrageenan in saline into dorsal pouch

Groups	Serum levels of IL-4 (pg/ml) (4th day)	Serum levels of IL-4 (pg/ml) (7th day)
Simvastatin control group (n=6)	<1.6	<1.6
Simvastatin group (n=6)	3.22 [a] ±0.32	6.24 [a b c] ±0.45
Aspirin control group (n=6)	<1.6	<1.6
Aspirin group (n=6)	3.26 [a] ±0.22	5.46 [a b c] ±0.45
Combined Therapy control group (n=6)	<1.6	<1.6
Combined Therapy group (n=6)	5.31 [a b c d f] ±0.56	7.77 [a] ±0.52

n: Number of rats
[a] : Significance against corresponding control group at P<0.001
[b] : Significance against simvastatin treated group 4th day at p<0.05
[c] : Significance against aspirin treated group 4th day at p<0.05
[d] : Significance against combined therapy treated group 4th day at p<0.05
[f] : Significance against aspirin treated group 7th day at p<0.01
Values were expressed as mean of 6 rats ± S.E.M. and compared using ANOVA & Tukey-Kramer multiple comparison tests.

DISCUSSION

Free oxygen radicals are general mediators of signal transduction pathways, which are able to induce cytokine production from various cell types. [25] Studies attesting the role of antioxidants, which potentially inhibit oxidant-mediated activation of transcription factors, reported that antioxidants also prevent the transcriptional activation of inflammatory cytokines. These studies suggest that antioxidants may play a role in decreasing the immune response by suppressing the oxidative stress. [26] Our work revealed that **i**.p injection of simvastatin resulted in significant increase of serum level of NO as compared to corresponding control groups. The increase of endothelial nitric oxide production may inhibit NF-κB *via* the induction and stabilization of inhibitory kappa B-alpha (IκB-α). [27] Laufs & Liao [28] mentioned that mevalonate destabilizes endogenous nitric oxide synthase (eNOS) mRNA and statins increase its half-life by blocking mevalonate synthesis i.e statins increase eNOS expression by prolonging eNOS mRNA half-life rather than by inducing eNOS gene transcription. Birnbaum *et al.* [29] assessed that statins are also anti-inflammatory because they trigger 15-epi-lipoxin A4 synthesis, endothelial NO synthase-derived NO, dampens proinflammatory cytokines and thereby inhibiting T cell activation. Wang *et al.* [6] concluded that the mechanism is owing to inhibition of RhoA geranylgeranylation, alteration

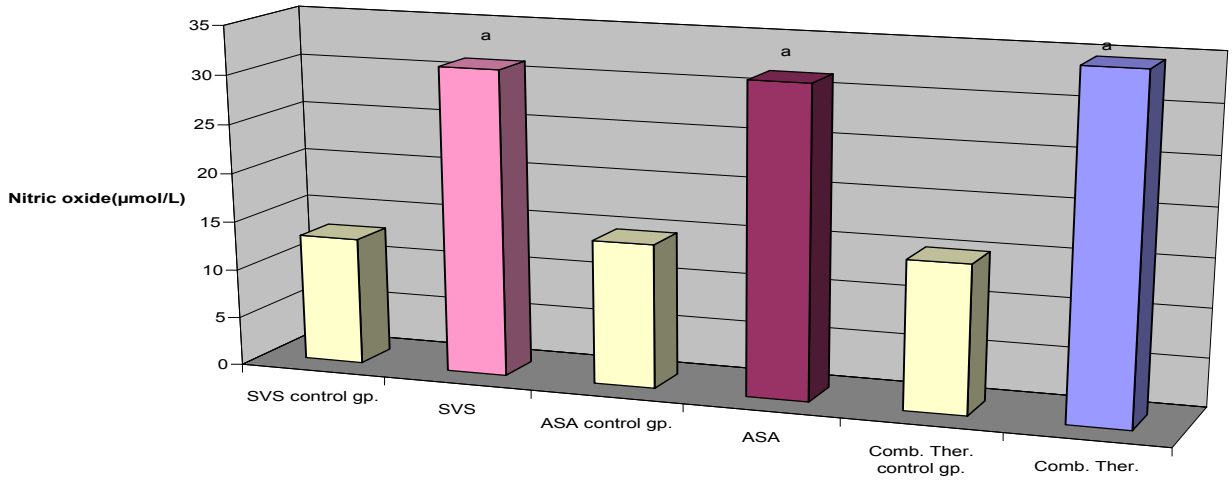

Fig 1: effect of I.P injection of (20mg/kg) SVS, (25mg/kg) ASA or their combination for 3 days on serum levels of nitric oxide(No) in rats compared to corresponding control groups.

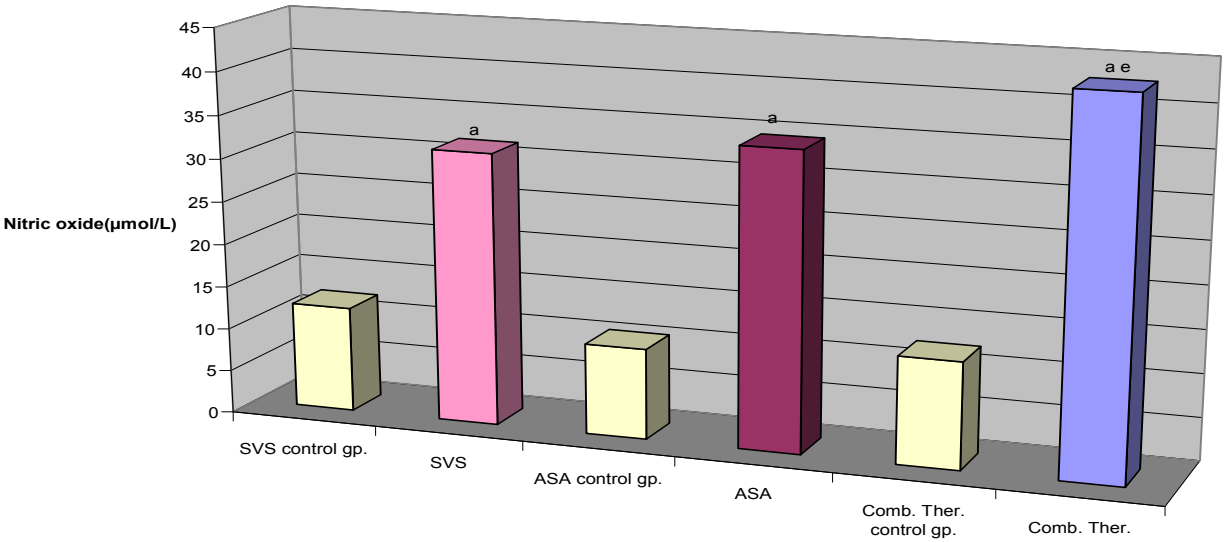

Fig. 2: effect of I.P injection of (20mg/kg) SVS, (25mg/kg) ASA or their combination for 6 days on serum levels of nitric oxide (No) in rats compared to corresponding control groups.

Values were expressed as mean of 6 rats ± S.E.M. and compared using ANOVA & Tukey-Kramer multiple comparison tests.

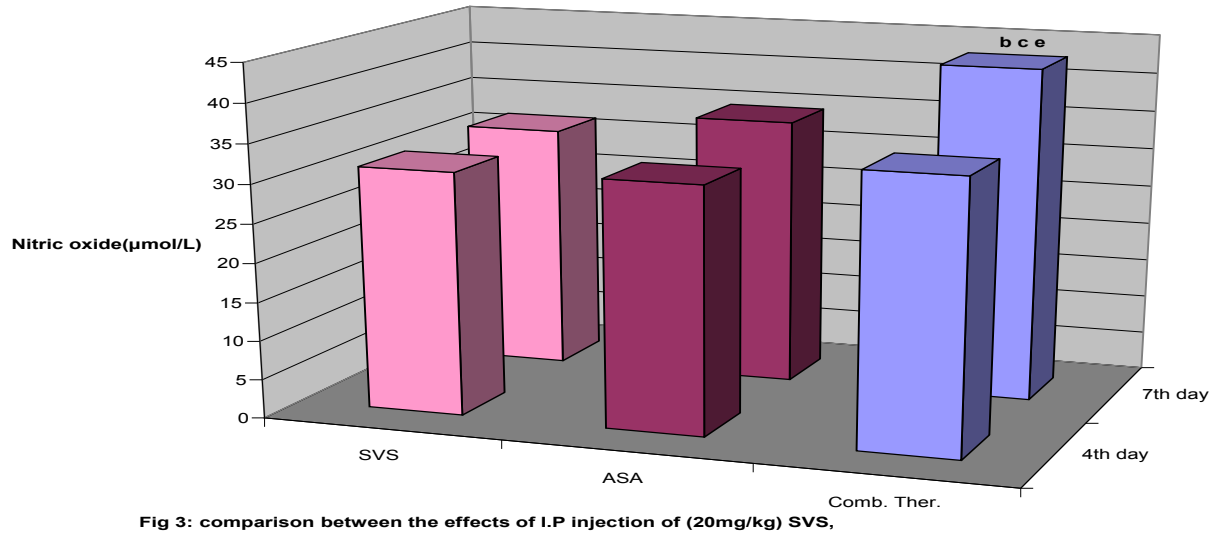

Fig 3: comparison between the effects of I.P injection of (20mg/kg) SVS, (25mg/kg) ASA or their combination for 3 & 6 days on serum levels of nitric oxide (No) in rats.

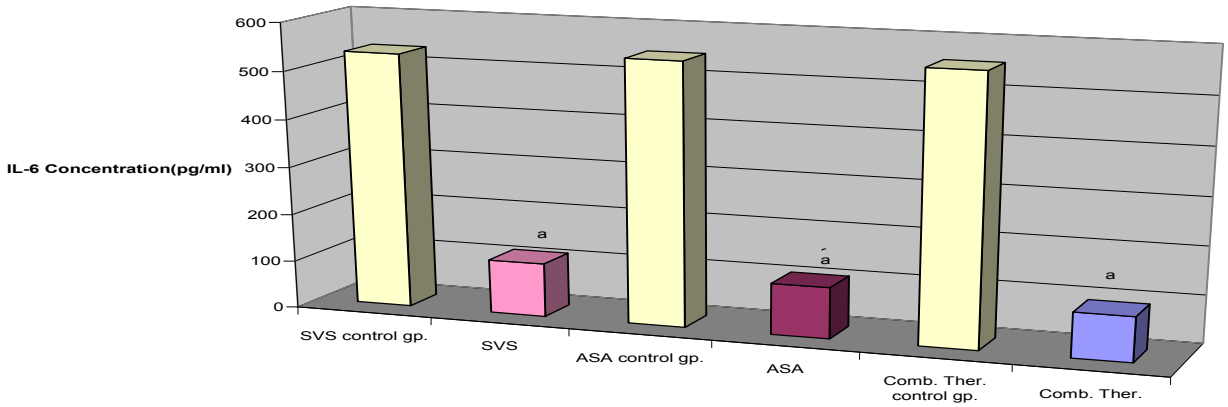

Fig. 4: effect of I.P injection of (20mg/kg) SVS, (25mg/kg) ASA or their combination for 3 days on serum levels of interlukin-6 (IL-6) in rats compared to corresponding control groups.

Values were expressed as mean of 6 rats ± S.E.M. and compared using ANOVA & Tukey-Kramer multiple comparison tests.

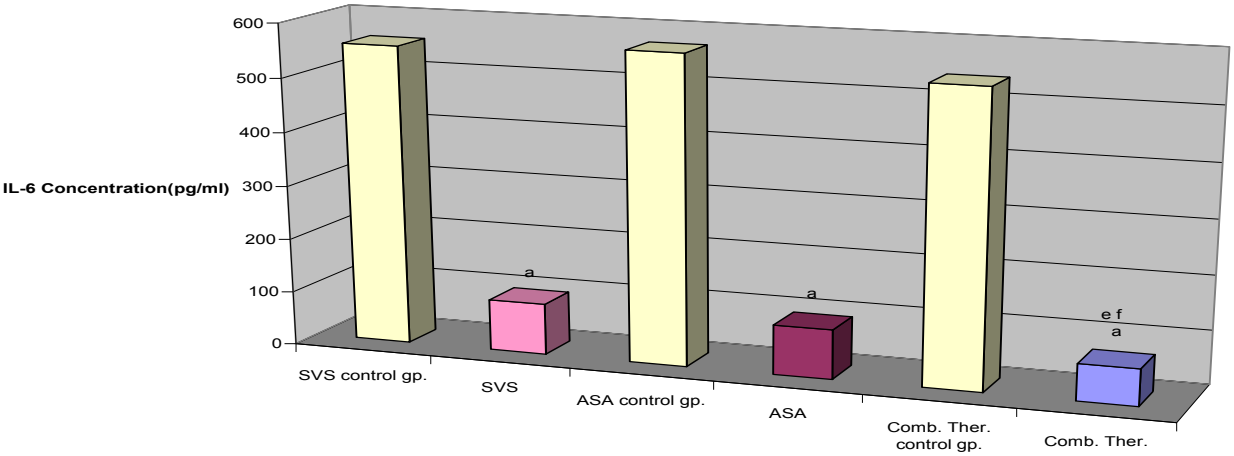

Fig. 5: effect of I.P injection of (20mg/kg) SVS, (25mg/kg) ASA or their combination for 6 days on serum levels of interlukin-6 (IL-6) in rats compared to corresponding control groups.

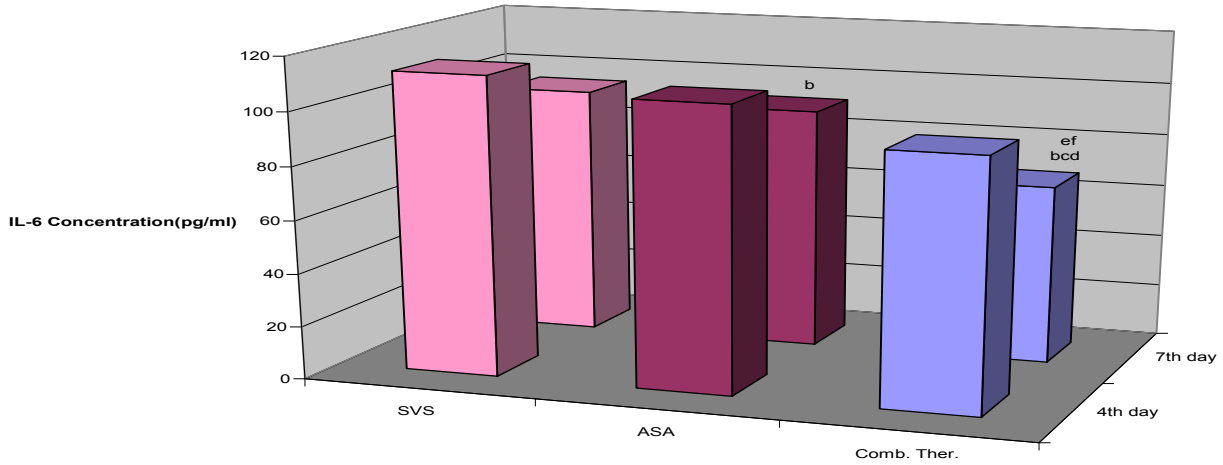

Fig. 6: comparison between the effects of I.P injection of either (20mg/kg) SVS, (25mg/kg) ASA or their combination for 3 & 6 days on serum levels of interlukin-6 (IL-6) in rats.

Values were expressed as mean of 6 rats ± S.E.M. and compared using ANOVA & Tukey-Kramer multiple comparison tests.

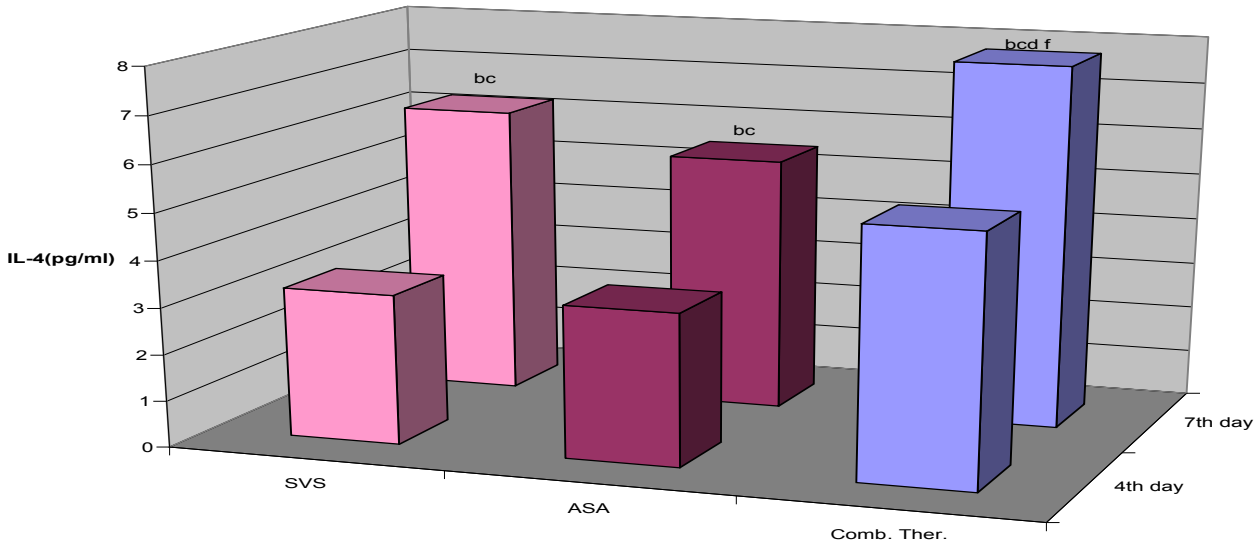

Fig. 7: comparison between the effects of I.P injection of (20mg/kg) SVS, (25mg/kg) ASA or their combination for 3 & 6 days on serum levels of interlukin-4 (IL-4) in rat.

Values were expressed as mean of 6 rats ± S.E.M. and compared using ANOVA & Tukey-Kramer multiple comparison tests.

of the cytoskeleton and localization of the eNOS mRNA. Alongside, this study showed that I.P injection of aspirin resulted in significant increase of serum level of NO as compared to corresponding control groups. Aspirin increases cGMP *via* NO and subsequent activation of soluble guanylyl cyclase which is clearly demonstrated by a series of experiments. [30] The enhanced activity of the NO/cGMP system under the influence of aspirin is attributable to a direct stimulatory effect of aspirin on eNOS. These findings confirm the role of the NO/cGMP pathway in this process and point specifically to cGMP as a causative mediator in antioxidant protection. [31]

In comparison, there was no significant difference in serum NO level after simvastatin therapy as compared to using aspirin therapy, which give evidence regarding the antioxidant activity of simvastatin which is comparable to that of aspirin. On the contrary, there was significant increase in serum NO levels in combined therapy treated group in the 7^{th} day as compared to simvastatin and aspirin treated groups in the 4^{th} day& against simvastatin treated group in the 7^{th} day which indicates that the antioxidant activity of combined therapy increased with longer time and the combination of the two drugs. This elevation may be due to potentially overlapping mechanisms of both aspirin and simvastatin.

The present study demonstrated that simvastatin had decreased serum level of TNF-α and IL-6 (below the detection limit in case of TNF-α) as compared to corresponding control groups. Simvastatin exerts direct anti-inflammatory effects via peroxisome proliferator–activated receptor alpha (PPARα), independent of its plasma cholesterol lowering activities. PPARα is a nuclear receptor that regulates gene expression; PPARα not only regulates lipid metabolism but also exerts pronounced anti-inflammatory activities by negatively interfering with proinflammatory signaling pathways including NFκB.[32] This molecular action is exemplified by the inhibition of inflammatory induction of genes, such as vascular cell adhesion molecule-1, IL-6, and TNF-α. PPARα clearly plays a functional role in the anti-inflammatory effects of simvastatin *in vitro* and *in vivo*. [32] The present work showed that I.P injection of aspirin had resulted in a dramatic decrease of serum levels of TNF-α and IL-6 as compared to corresponding control groups.

Yoo *et al* .[33] assessed that ASA inhibit proinflammatory cytokine production by blocking NF-κB activation. They also demonstrated that this inhibitory effect of ASA on NF-κB activation is secondary to the stabilization of inhibitory kappa B-alpha (IκB-α) by blocking the phosphorylation of IκB-α and its subsequent degradation. This blocking of IκB-α phosphorylation by ASA was due to the inhibition of inhibitory kappa B kinase (IKK) activity.

Injection of combined therapy (simvastatin and aspirin) had led to a significant decrease of serum levels of TNF-α and IL-6 compared to corresponding control groups. Furthermore, injection of combined therapy for 6 days caused significant decrease in serum levels of IL-6 as compared to simvastatin, aspirin and combined therapy treated groups in the 4th day and as compared to simvastatin, aspirin treated groups in the 7th day as well.

This decrease is due to modulation of signal transduction pathways caused by statins, as simvastatin negatively interfers with proinflammatory signaling pathways including NF-κB activation. Beside, aspirin has inhibitory effect on NF-κB activation so combination regiments with statins and aspirin significantly enhanced the efficacy of either type of agents administered alone. Such potentiated effect may provide a solid ground for the combined therapy.

IL-4 has properties that exemplify many of the characteristics of the set of immune recognition-induced lymphokines. It is made in response to immunologic recognition, principally, not exclusively, by $CD4^+$ T lymphocytes. It mediates much of its action in short range interactions between target cells and IL-producing T cells, and it has a wide range of function. [34]

In the present work, serum levels of IL-4 were highly increased after the i.p injection of simvastatin, for 3 or 6 days as compared to corresponding control groups which had levels below the detection limit. Moreover, i.p injection of simvastatin for 6 days resulted in significant increase of serum level of IL-4 as compared to groups received simvastatin or aspirin for 3 days.

The increase in the level of IL-4 after simvastatin treatment may be due to reduction of inflammatory infiltration, abrogated the T_H1 immune responses and diminished T-cell proliferation, probably via direct engagement of the T-cell receptor. [35] The type of immune response supported may also depend on the ability of statins to induce the release of the T_H2-promoting cytokines (e.g. IL-4 and IL-10), and diminish secretion of the T_H1 subtype (e.g. IL-2, IL-12, or IFNγ). [36]

This study revealed that, serum levels of IL-4 were highly increased after the i.p injection of aspirin, for 3 or 6 days as compared to corresponding control groups which had levels below the detection limit. Also, i.p injection of aspirin for 6 days resulted in significant increase in IL-4 serum levels as compared to groups received simvastatin or aspirin for 3 days. The increase in the level of IL-4 after aspirin treatment may be attributed to the biosynthesis of endogenous aspirin-triggered lipoxins (ATLs); ATLs can in turn act directly on polymorphonuclear leukocyte (PMN) and/or the appearance of IL-4. This represents that lipoxins induce upregulation of a potential anti-inflammatory cytokine such as IL-4. Hence, it is of particular interest that IL-4 inhibits PMN influx in acute antibody-mediated inflammation. [37]

Our study showed that serum levels of IL-4 were highly increased with combined therapy for 3 or 6 days as compared to corresponding control groups which had serum levels below the detection limit. Also I.P injection of combined therapy for 6 days caused significant increase in IL-4 serum level when compared to groups treated with mono therapy of either simvastatin, aspirin & combined therapy for 3 days and against the group treated with aspirin for 6 days.

We can summarize that injection of simvastatin resulted in a significant increase of serum level of NO which was accompanied by dramatic significant decrease of serum levels of TNF-α and IL-6. Also, serum levels of IL-4 were highly increased after injection of simvastatin. In comparison, there were no significant differences between simvastatin treated groups as compared to aspirin treated groups in serum levels of NO, aspirin also conferred dramatic significant decrease of serum levels of TNF-α below the detection limit which was comparable to that happened after simvastatin therapy, while aspirin caused more reduction of IL-6 level in serum as compared to group received simvastatin. Simvastatin or aspirin treatment yielded the same significant increase of serum levels of IL-4. Combined therapy possesses better antioxidant activity as it resulted in significant increase of serum level of NO as compared to simvastatin or aspirin therapy. This elevation may be due to that simvastatin and aspirin act by distinct but potentially overlapping mechanisms that give evidence that combination therapies might be even more effective than any single therapy alone. Treatment with combined therapy resulted in decrease of serum levels of TNF- α below the detection limit and caused better significant decrease of IL-6 as compared to either simvastatin or aspirin therapy alone. Contrarily, serum levels of IL-4 were highly increased after the injection of combined therapy compared with either simvastatin or aspirin therapy alone. This is because both simvastatin and aspirin dampens proinflammatory cytokines that affect immune response and account for appearance of IL-4 that inhibits PMN influx in inflammation.

CONCLUSION

We can conclude that treatment with simvastatin alone had no significant difference from treatment with aspirin alone which give solid ground for the predicted anti-inflammatory effects of simvastatin. Furthermore, our study demonstrated that treatment with the combined therapy reduced the extent of inflammation as compared to treatment of simvastatin alone or aspirin alone. So, the combinations of statins plus aspirin may produce synergy and have additive effects and a lot of benefits in immune response. The combination treatment with agents that inhibit different aspects of the signal transduction pathways will be transformational and have better efficacy with fewer side effects. Finally, therapies directed specifically against proinflammatory cytokines have to be tested in appropriate prospective clinical trials to investigate their potential in inflammation management. Strategies to diminish proinflammatory cytokine signals should target the mechanisms of immune activation, the intracellular pathways regulating cytokine production and/or the fate of cytokines once they have been released into the circulation.

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