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Research Article

SIMVASTATIN AND ROSUVASTATIN IN THE PROTECTION AGAINST NSAID INDUCED GASTRIC MUCOSAL INJURY IN RATS & ROLE OF THEIR ANTIOXIDANT ACTIVITY

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ABSTRACT
Statins , the most effective drugs for the treatment of hyperlipidemia also exhibit other effects unrelated to their lipid lowering effects among which are antioxidant and anti-inflammatory actions .To investigate the gastroprotective effects of simvastatin and rosuvastatin against NSAID induced gastric mucosal damage and also to evaluate the influence of the antioxidant activity of those two chemically different statins on their gastrotoprotective effects.The study was conducted on 60 adult male albino rats, divided into 6 groups, the first served as a control received the vehicle , the second received 60mg/kg indomethacin orally. The third and fourth groups were pretreated with either simvastatin or rosuvastatin respectively in a dose of 20mg/kg orally for 2 weeks and then received indomethacin 60mg/kg one hour before the last dose of the statin. The role of nitric oxide (NO) in the gastroprotective effects of statins was investigated by using 20mg/kg intraperitoneal N^G-L-Arginine Methyl Ester (L-NAME) a nitric oxide synthase inhibitor which was given 30 minutes prior to the last dose of either simvastatin or rosuvastatin and this served as fifth and sixth groups respectively. The rats were then sacrificed after 6 hours and their stomachs were isolated and submitted to macroscopical assessment and for the measurement of the gastric prostaglandinE2 (PGE2), and myeloperoxidase (MPO). Rosuvastatin and simvastatin produced significant gastroprotective effects. Their protective effects were associated with marked decrease in MPO activity and increase in gastric PGE2 levels. However, the gastroprotective and antioxidant effects of the flurophenyl containing rosuvastatin were significantly better than that of simvastatin. L-NAME pretreatment decreased the protective effects of both rosuvastatin and simvastatin and this indicates the role of NO in their gastroprotective effects.
KEY WORDS: Statins, antioxidant activity, NSAID gastropathy.

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) which are widely used in the treatment of pain, fever, and inflammation carry various side effects, especially those occurring in the gastrointestinal tract including gastric mucosal erosions and ulcerations[1]. The gastric ulcerogenic action of NSAIDs is attributed to their ability to inhibit gastric prostaglandins (PGs) [2] and nitric oxide production[3] which also plays an important role in mediating mucosal defense[4]. One other important cause of mucosal damage associated with NSAIDS for which much attention has recently been focused is the formation of reactive oxygen species (ROS) like superoxide and hydroxyl radicals resulting from neutrophil adherence to the vascular endothelium[4, 5]. This neutrophil infiltration into the gastric mucosal tissues is considered a critical process in the pathogenesis of NSAID gastropathy[6,7,8] and measurement of the free radicals scavenger activity (myeloperoxidase (MPO) activity) is now widely used as an index of this neutrophil infiltration[9,10].

Statins are a group of medications defined as 3 – Hydroxy 3-methyl glutaryl co enzyme A (HMG CoA) reductase inhibitors, have been recognized as the most effective therapeutic agents for reducing serum cholesterol levels[9].Interestingly these drugs have also been reported to exhibit other effects unrelated to their lipid lowering effect (pleotropic effect) among which are antioxidant (free radicals scavenger activity) and anti-inflammatory actions[10,11,12] .

The present study examines the gastroprotective effects of two structurally different statins ; type I statin (simvastatin) a statin substituted with a butyryl group and the second is type II statin (rosuvastatin) substituted with flurophenyl group[13] in the prevention against indomethacin induced gastric mucosal damage. It also evaluates the influence of the antioxidant activity of these two compounds as measured by the MPO activity on their gastroprotective effects as well as comparing the potential of their *in vitro* antioxidant effects. In addition their effect on gastric prostaglandin E2 (PGE2) levels and NO production was also studied.

MATERIALS AND METHODS

Indomethacin, simvastatin and rosuvastatin raw materials were obtained as a gift samples from the national center for drug researches and control. Other reagents were purchased from sigma -Aldrich company. This study was conducted on 60 adult male albino-Wister rats weighing (180-200g) and was initiated after seeking approval from the ethical and scientific committee in the Department of Pharmacology / College of Medicine /Baghdad University on April 2011. The animals were divided into experimental groups of ten animals each. Indomethacin and HMG –CoA reductase inhibitors simvastatin and rosuvastatin were freshly prepared before administration and suspended in a vehicle of 0.9% NaCl containing tween 80 and 1% carboxy methyl cellulose (CMC). A dose of 20mg/kg of each statin was administered by gavage once a day for a period of 2 weeks. On the day of the experiment and after 24 hour of starvation (during starvation, rats were kept in cages provided with a wide wire –mesh floor to avoid coprophagy but allowed free access to tap water which was with held two hours before the procedure) 60mg/kg indomethacin was given orally followed one hour later by the last doses of statins. L-NAME was dissolved in phosphate buffer saline (PH 7.2) at a concentration of 32.5 mg/ml for intraperitoneal (I.P) administration according to the method of Griffith and Kilbourn (1996) [14]. The animals were divided into six groups the first group served as a control received the vehicle, the second group received indomethacin orally of 60mg/kg. The third and fourth groups were pretreated orally for 2 weeks with 20mg/kg of either simvastatin or rosuvastatin

respectively. In order to study the role of NO in the protective effect of statins, intraperitoneal L-NAME 20mg/kg was administered 30 minutes before the last dose of simvastatin and rosuvastatin and served as the fifth group and sixth groups.

At the end of each experiment (6 hours following indomethacin administration) the rats were sacrificed and their stomachs were isolated .Stomachs were opened along the greater curvature and the lengths of ulcerative lesions were measured with a digital caliper and the stomach then quickly divided into two parts and each part was kept in suitable and special buffer and stored at -20^{o}C for biological assay.

Assessment of gastric mucosal damage: Gastric damage score (GDS) was calculated by the summation of the lengths of all linear erosions according to Santucci et al. (1994)[15].

Biological assays: Gastric mucosal samples were collected each in specific buffer and stored in freeze until evaluation of biological parameters:

A :prostaglandin E2 assay: The samples used for assay of PGE2 were kept in sodium phosphate buffer (10 mmol/l ; pH 7.4).At the time of the procedure, tissue was minced with scissors, placed in a shaking water bath at (37^{o}C) for 20 min, then samples were centrifuged at (9000 x g) for 1 min the concentration of PGE2 in the supernatant was determined by enzyme linked immunosorbent system (ELISA) using commercially available kit according to Wallace et al 2000[16]

B: Gastric MPO activity assay: The samples used to assay gastric MPO were kept in phosphate buffer saline (50 mmol/l ; pH 6). One hundred milligram of gastric tissue was homogenized in 2 ml of PBS (50mm) containing 0.5% hexadecyl trimethyl ammonium bromide (HTAB) (pH 6). Each sample was homogenized on ice bath for 2 minutes using a polytron homogenizer and then centrifuged at 2000 x g for 5 min. at 4^{o}C. MPO activity of supernatant was determined by adding 0.1 ml of the supernatant to 2.9 ml of 50mm phosphate buffer containing 0.167 mg/ml of O-diansidine HCl and 50 µl of 1% H2O2, the change in absorbance at 460 nm over a 3 minutes period was measured spectrophotometrically. One unit of MPO activity was defined as that which would convert 1 Mmol of H2O2 to water in 1 min. at 22^{o}C. The results were reported as the MPO unit /mg of tissue according to Bradley et al. (1982) [17]

Measurement of antioxidative activity *in vitro*

Antioxidative activities of simvastatin and rosuvastatin were determined by a redox –linked colorimetric method using iron (Fe (III)) as an easily reduced oxidant in stoichiometric excess[18]. Antioxidants could reduce Fe (III) to Fe (II), which subsequently reacted with 1, 10 – phenanthroline to form a colored complex [19]. The intensity of the absorbance at 510 nm reflected the reducing power as antioxidative activity. Each statins (10µmol/L) and 0.01 mol/L Fe (III) chloride were subsequently mixed with 0.5 mol/L acetic acid and 0.05mol/L 1,10 – phenanthroline and incubated for 30min at room temperature . The colored complex Fe (II) – 1,10 – phenanthroline was measured at absorbance of 510nm . Gallic acid was used as a positive control.

Statistical Analysis

Statistical analyses and graphics were performed using SPSS Ver. 13 software for Windows (Statistical Analysis for Social Sciences, Apache Software Foundation, USA). All data were expressed as mean ± standard error of mean (SEM). One-way analysis of variance (ANOVA–test) was used for comparison between several experimental groups. The level of statistical significance was set as *p value* < 0.05.

RESULTS

Indomethacin treated group: Intragastric instillation of 60 mg/Kg indomethacin on empty stomach, caused extensive multiple hemorrhagic lesions affecting mostly the glandular portion of the stomach in all animals, which was observed 6 hrs after indomethacin administration. The extent of the lesions were expressed by the gastric damage score (GDS) measuring (23.35±0.78mm) as depicted in fig 1. In addition indomethacin caused significant increase ($p<0.05$) in gastric MPO activity mean (24.59±0.74u/mg) versus (4.82±0.22/mg) in the control group as shown in figure 2. Also there was significant suppression ($p<0.05$) of gastric PGE2 mean (80±1.97ng/g) versus (275.50± 2.73ng/g) in the control group as shown in figure 3.

Simvastatin pretreated group: Simvastatin pretreatment caused significant reduction ($p< 0.05$) of GDS mean (8.72±0.28mm) compared to (23.35±0.78mm) in the indomethacin treated group as shown in figure 1. By evaluating the effect of simvastatin on MPO activity; there was significant decrease ($p< 0.05$) in MPO activity mean (13.64± 0.52u/mg) compared to (24.59± 0.74u/mg) in the indomethacin treated group as shown in figure 2. Gastric PGE2 level was significantly increased ($p< 0.05$) mean **(**135.50± 2.63ng/g**)** versus (80±1.97ng/g) in the indomethacin treated group as shown in figure 3.

L-NAME pretreatment caused significant decrease ($p< 0.05$) in the gastroprotective effect of simvastatin GDS (16.13±0.29mm) compared to (8.72±0.28mm) the simvastatin alone treated group as depicted in figure 1.

Rosuvastatin pretreated group: Rosuvastatin pretreatment demonstrated gastroprotective action against indomethacin induced gastropathy ; GDS (3.38± 0.27mm) versus (23.35± 0.78mm) in the indomethacin treated group ($p< 0.05$) as shown in figure 1 and which was significantly greater than that of simvastatin. This protection was associated with the ability of rosuvastatin to significantly decrease ($p< 0.05$) the gastric MPO activity mean (8.44±0.23u/mg) compared to (24.59±0.74u/mg) in the indomethacin treated group as shown in figure 2. This inhibition of MPO was significantly higher than that of simvastatin. In addition rosuvastatin upregulated ($p< 0.05$) gastric PGE2 level mean **(**135±2.98ng/g**)** versus (80±1.97ng/g) in the indomethacin treated group as shown in figure 3. This increase in the gastric PGE2 level was equal to that of simvastatin.

L-NAME pretreatment caused significant decrease ($p< 0.05$) in the gastroprotective effect of rosuvastatin GDS (12.38±0.32mm) versus (3.38±0.27mm) in the rosuvastatin alone treated group as depicted in figure 1. On the other hand, L-NAME pretreatment caused significant decrease ($p< 0.05$) in the gastroprotective effect of rosuvastatin compared to that of simvastatin and this may indicate that NO releasing effect in rosuvastatin was higher than that of simvastatin as depicted in figure 1.

Regarding the antioxidant activity of these two statins expressed by their reducing power in vitro. The reducing power of rosuvastatin was shown to be significantly stronger than simvastatin ($p< 0.001$) as depicted in table 1.

DISCUSSION

This study exploits the role of simvastatin and rosuvastatin in the prevention of mucosal damage induced by NSAIDs. Pretreatment with both statins produced significant reduction in GDS greater with rosuvastatin than with simvastatin. The variation in the extent of gastric protection achieved with each drug seems to be influenced by the antioxidant activity of the compound since both drugs upregulated PGE2 to the

same level. Rosuvastatin was shown in this study to possess a stronger reducing power than simvastatin, likely due to flurophenyl moiety in its structure which has been reported to possess powerful antioxidant effects[20]. In addition Rosuvastatin elicited a higher inhibitory effect on MPO activity which is a specific marker for oxyradical generation and neutrophil infiltration occurring early in the course of NSAIDs gastric injury [21] and is also an indicator of the degree of ulceration induced by NSAIDs [22]. Moreover, it has been shown that there is a complex interdependent relationship between MPO activity and NO levels, where NO serves as a substrate for MPO and hence peroxidase may function as a catalytic sink for NO increasing its consumption and influencing its bioavailability at sites of inflammation[23]. This has been indirectly demonstrated in this study where L-NAME abrogated the NO protective effects of rosuvastatin more than simvastatin due to the greater inhibitory effect of rosuvastatin on MPO.

In conclusion rosuvastatin and simvastatin produced significant gastroprotective effects and the variation in the extent of gastric protection achieved seems to be influenced by the antioxidant activity of the drug.

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REFERENCES

1. Mari Hagiwara, Keiko Kataoka , Hideki Arimochi , Tomomi Kuwahara, Haruyuki Nakayama , Yoshinari Ohnishi. Inhibitory effect of fluvastatin on ileal ulcer formation in rats induced by nonsteroidal anti-inflammatory drug. World J Gastroenterol 2005; 11(7): 1040-1043.
2. Vane JR, Botting RM. Mechanism of action of anti-inflammatory drugs. International Journal Tissue React. 1998;20:3–15.
3. Martin MJ , Jimene MD , Motilva V . New issues about nitric oxide and it is effects on the gastrointestinal tract .Curr. Pharm. Des . 2001; 7: 881-908.
4. Catalayud S , Barrachina D, Esplugues JV . Nitric oxide : relation to integrity , injury , and healing of the gastric mucosa . Microsc. Res . Tech.2001 ; 53: 325-335.
5. Ma L, Elliot SN , Cirino G, Buret A , Ignarro LJ, Wallace JL . Platelets modulate gastric ulcer healing: role of endostatin and vascular endothelial growth factor release . Proc. Natl Acad. Sci . USA 2001; 98 : 6470-6475.
6. Pan L, Tang Q , Fu Q, Hu B , Xrang J, Qran J .Role of nitric oxide in protective effect of berberine in ethanol –induced gastric ulcer in mice . Acta Pharmacol. Sin .2005; 26: 1334-1338.
7. Wallace JL, McCaferty D-M, Carter L et al. Tissue-selective inhibition of prostaglandin synthesis in rat by tepoxalin: anti-inflammatory without gastropathy. Gastroenterology 1993; 105: 1630-1636.
8. Vaananen PM, Meddings JB and Wallace JL. Role of oxygen-derived free radicals in indomethacin-induced gastric injury. American Journal of Physiology 1991; 261: 470- 475.
9. Robert W Mahley and Thomas P Bersot . Drug therapy for hypercholesterolemia and dyslipidemia. In: Goodman and Gilman's the pharmacological basis of therapeutics. (Laurence L Brunton. John S Lazo and Keith L. Parker.) 2006 , section V , ch:35. McGraw Hill: 948- 949.
10. Werner, N. , Nickenig G. , Laufs U: Pleotropic effects of HMG-CoA reductase inhibitors. Basic Res. Cardiol. 2002; 97 , 105-116.
11. Wainwright, C.L: Statins : is there no end to their usefulness ? Cardiovasc. Res. 2005; 65(2), 296-298.
12. Tamargo J. , Caballero R. , Go´mez R. . Nu´nez L. , Vaquero M. , Delpo´n E: Lipid lowering therapy with statins , a new approach to antiarrythmic therapy . Pharmacol. Therap. 2007; 114 (1), 107-126.
13. Istvan , Eva S , Deisenhofer , Johann . Structural mechanism for statin inhibition of HMG –Co A . Science Magazine 2001 ; 292(5519) : 1160-1164.
14. Griffith O.W, and Kilbourn R.G. Nitric oxide synthase inhibitors , amino acids. methods .J. enzymol. 1996; 268 :375-392.
15. Santucci L, Fiorucci S. & Giansanti M. Pentoxifylline prevents indomethacin-induced acute gastric mucosal damage in rats. Role of TNF-α, Gut. 1994; 35: 909-915.
16. Wallace J.L, McKnight W, Reuter B.K & Vergnolle N. NSAID-induced gastric damage in rats: requirement for inhibition of both COX & COX-2. J. Gastroenterol. 2000; 119:706-714.
17. Bradley P.P, Christensen R.D and Rothstein G. Cellular and extracellular myeloperoxidase in pyogenic inflammation, Blood , 1982 ;60: 618-622.
18. Benzie IF , Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power : The FRAP assay . Anal . Biochem. 1996; 239: 70-76.
19. Higashimoto M ,Akada Y , Sato M , Yamada Y , Kuwahara T ,Ohnishi Y . Inhibitory effects of herbal teas and herb extract on the mutagenicity of 1- methyl -1,2,3,4-tetrahydro-Beta-carboline –carboxylic acid upon treatment with nitrite in the presence of ethanol . Environ . Mutagen Res .2001; 23: 1-7.
20. Jin Dai , and Russell J. Mumper. Plant Phenolics: Extraction, Analysis and Their Antioxidant and Anticancer Properties. Molecules 2010; 15: 7313-7352.
21. Bayir Y, Odabasoglu F, Cakir A, Aslan A, Suleyman H, Halici M, et al. The inhibition of gastric mucosal lesion, oxidative stress and neutrophil-infiltration in rats by the lichen constituent diffractaic acid. Phytomedicine. 2006;13:584–590.
22. Odabasoglu F, Cakir A, Suleyman H, Aslan A, Bayir Y, Halici M, et al. Gastroprotective and antioxidant effects of usnic acid on indomethacin -induced gastric ulcer in rats. J Ethnopharmacol.2006;103:59–65.
23. Abu-Soud HM and Hazen SL . Nitric Oxide Is a Physiological Substrate for Mammalian Peroxidases. The Journal Of Biological Chemistry. 2000;275(48) :37524–37532 .

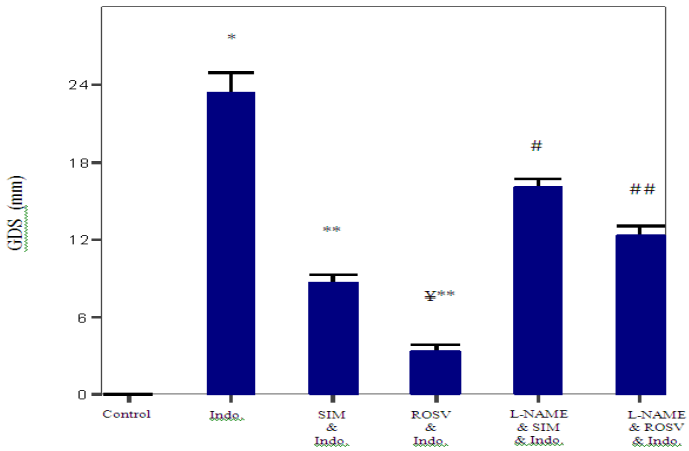

Figure 1: The effect of rosuvastatin versus simvastatin pretreatment on the gastric damage score induced by indomethacin and the effect of L-NAME.
The results are expressed as the mean ± SEM
* P < 0.05 when compared with control group .
** P < 0.05 when compared with indomethacin group.
¥ P < 0.05 when compared with simvastatin group..
# P < 0.05 when compared with L-NAME pretreated simvastatin group.
P < 0.05 when compared with L-NAME pretreated rosuvastatin group.

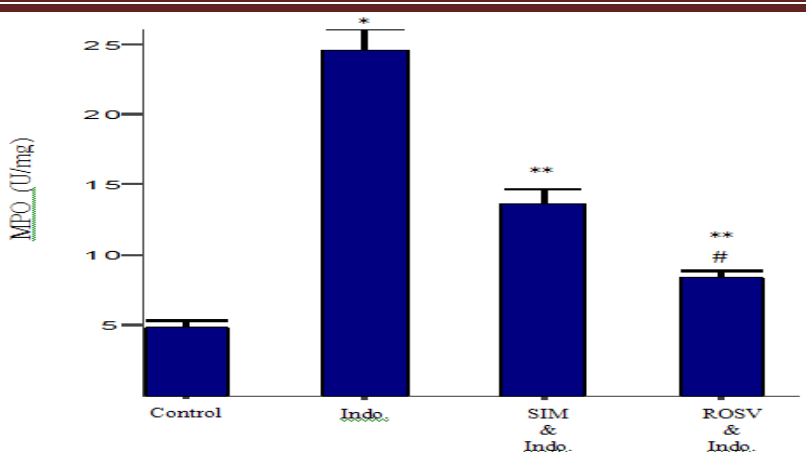

Fig 2: The effect of rosuvastatin versus simvastatin pretreatment on the increased gastric MPO activity induced by indomethacin .
The results are expressed as the mean ± SEM .
* P < 0.05 when compared with control group.
** P < 0.05 when compared with indomethacin .
#P < 0.05 when compared with simvastatin group

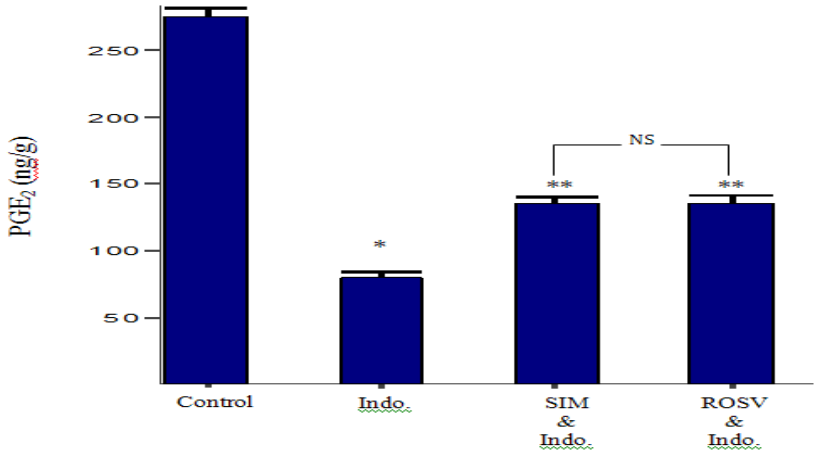

Fig. 3: The effect of rosuvastatin versus simvastatin pretreatment on the gastric PGE2 levels inhibited by indomethacin.
The results are expressed as the mean ± SEM.
*P< 0.05 when compared with control group.
** P < 0.05 when compared with indomethacin.
NS: not significant

Table 1 : Reducing power of HMG-CoA reductase inhibitors(mean ±SD).The intensity of the absorbance at 510nm reflected antioxidative activity as the reducing power .

HMG-Co A reductase inhibitors	Dose (mmol/L)	Absorbance at 510 nm (as the reducing power)
Vehicle	zero	0.18 ± 0.02
Simvastatin	0.01	0.32± 0.18[2]
Rosuvastatin	0.01	0.56± 0.016[2]
Gallic acid	0.01	0.72± 0.014[1]

[1] Gallic acid was not a HMG-Co A reductase inhibitor and used as a positive control of this test .
[2] Significantly different from the solvent at p< 0.001(ANOVA)

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