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Antihypertensive & Anti-diabetic Activity of Simvastatin & Reserpine in Ang-II Induced Hypertension and STZ Induced Type-II Diabetes Mellitus

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Abstract

The aim of the present investigation is to study in-vivo anti-hypertensive activity of identified sEHIs using Angiotensin II induced hypertension and anti-diabetic activity. Male wistar rats (200-250 g) were obtained from Veterinary and Animal Sciences, India. The experiments were performed strictly according to guidelines provided by CPCSEA and approved by IAEC. In this study, osmotic mini- pumps (model 2002, Alzet, Cupertino, CA, USA) were implanted subcutaneously under anaesthesia (ketamine 80 mg/kg along with xylazine 10 mg/kg, intraperitoneally) for continuous administration of Ang II (150 ng/kg/min; n=6) for 2 weeks. The anti-diabetic effect of simvastatin and reserpine was investigated using earlier reported methods with slight modifications. Briefly, rats were randomly grouped in six groups. The acute toxicity study showed that oral administration of simvastatin and reserpine (2.5, 5, 10, 25, 50, 75, 100 mg/kg) caused dose dependent behavioral effects. The anti-hypertensive activity of simvastatin (10 mg/kg & 20 mg/kg) and reserpine (0.25mg/kg & 0.5 mg/kg) showed a significant dose dependent reduction in blood pressure and mean arterial pressure (P<0.0001) in Ang II induced hypertensive rats. Simvastatin (10 mg/kg and 20 mg/kg) and reserpine (0.25mg/kg & 0.5 mg/kg) treatment in diabetic rats significantly (P<0.0001) decreased blood glucose level in a dose dependent manner from day 1 to day 10 respectively when compared with diabetic control group. MS is a progressive and insidious disorder occurring in susceptible subgroups of population in response to adverse lifestyle and pathological conditions.

Key words: sEHIs, Angiotensin II, Hypertension, Anti-diabetic activity, Metabolic Syndrome (MS)

Introduction

Metabolic Syndrome (MS) refers to a cluster of concomitantly occurring cardio-metabolic abnormalities comprising hyperlipidemia, hypertension, insulin resistance, obesity, and atherosclerosis. It is characterized as including impaired glucose, LDL, HDL, high diastolic, and systolic pressure, raised triglyceride levels, and abnormal waist circumference. The concept of MS was introduced by Eskil Kylin, a Swedish physician when he advocated an association between gout, high blood pressure and hyperglycemia in the year 1920.Based on the IDF, abnormal waist circumference is needed along with two other factors to confirm the existence of MS. In addition, ATP III and NCEP mandate atleast three of the abovementioned components to confirm MS.

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Globally, MS is a growing public health concern with an increasing prevalence of approximately 25% (Belete et al., 2021; Sigit et al., 2020; Manaf et al., 2021).

MS is also known as the deadly quartet due to its association with hypertension, type II diabetes and hyperlipidemia. In view of this, one pill-multi-target approach provides a promising therapeutic strategy to offer efficient and safe drug therapy for the treatment of MS. The aim of the present investigation is to study in-vivo anti-hypertensive activity of identified sEHIs using Angiotensin II induced hypertension in Wistar rats and anti-diabetic activity of identified sEHIs using STZ+HFD induced diabetes in Wistar rats.

Material and Method

Animal

Male wistar rats (200-250 g) were obtained from Veterinary and Animal Sciences, India. The experiments were performed strictly according to guidelines provided by CPCSEA and approved by IAEC. Animals were kept in polypropylene cages (12/12h cycles) under standard condition of temperature (25 ± 2 °C) and humidity (45–55%). Rats were allowed to have *ad libitum* access to water and food and were housed in noiseless environment.

Acute toxicity studies

In view of low therapeutic index, we aimed to determine and re-validate safe and toxic doses of simvastatin and reserpine respectively employing acute toxicity studies using 423 OECD guideline in rats (Organization for Economic Development, 2001) with certain modifications in doses range (2.5, 5, 10, 25, 50,75, 100 mg/kg). Further, they were observed for 15 min, 4h, and 6h for any sign of behavioural toxicity like changes in lethargy, excitation, sedation, diarrhoea, salivation, tremors, convulsion, motor activity, central nervous and autonomic systems, circulatory, and respiratory system. The animals were observed for total 2 weeks for the long-term possible lethal outcomes.

Anti-hypertensive studies in rats

Effect of simvastatin and reserpine in Ang II induced hypertensive rats

Ang II is a vasoconstrictive peptide responsible for regulating plasma volume and blood pressure in experimental rat models. In this study, osmotic mini- pumps (model 2002, Alzet, Cupertino, CA, USA) were implanted subcutaneously under anaesthesia (ketamine 80 mg/kg along with xylazine 10 mg/kg, intraperitoneally) for continuous administration of Ang II (150 ng/kg/min; n=6) for 2 weeks. In brief, upper back of rat hair was shaved, skin of the dorsal surface ofthe back was incised and a fine cut was made directly in mid region. A cavity was made for placing osmotic pump subcutaneously. After performing the ligation, muscular and skin layer was immediately sutured and closed using sterile thread. To avoid any infection, a topical antibiotic (povidone io-dine tincture) was applied thrice in a day at the site of surgery. A total of thirty-six rats were randomly divided into six groups [I] Sham control (5% DMSO); [II] Ang II infused hypertensive rats; [III] Infused with Ang II + simvastatin (10 mg/kg); [IV] Infused with Ang II + simvastatin (20 mg/kg); [V] Infused with Ang II + reserpine (0.25 mg/kg); [VI] Infused with Ang II + reserpine (0.50 mg/kg). Postoperatively, all the rats were injected with ibuprofen for analgesic purpose and allowed to recover from surgery in ventilated incubator at room temperature (Nunes F.C. and Braga V.A. 2011; Braga
V.A. 2011; Snijder P.M. et al., 2014; Mohan M et al., 2010). After recovery from surgery, rats received the simvastatin, reserpine or vehicle treatment twice a day for consecutive days till normal blood pressure was attained.

Effect of simvastatin and reserpine in STZ+HFD-induced diabetic rats

STZ is well-known for producing cytotoxicity selectively in pancreatic islet beta cells and has been extensively used to induced type I DM in experimental rat model. The anti-diabetic effect of simvastatin and reserpine was investigated using earlier reported methods with slight modifications (Azad AK and Sulaiman WMAW 2020). Briefly, rats were randomly grouped in six groups: [I] Group I (received normal diet and 5% DMSO as vehicle without STZ+HFD) was served as NCD; [II] STZ+HFD induced diabetic rats; [III] STZ+HFD induced diabetic rats + simvastatin (10 mg/kg); [IV] STZ+HFD induced diabetic rats + simvastatin (20 mg/kg); [V] STZ+HFD induced diabetic rats + reserpine (0.25 mg/kg); [VI]

STZ+HFD induced diabetic rats + reserpine (0.5 mg/kg). Diabetes was induced by oral administration of HFD (NCD, mixture of vanaspati ghee, 2% raw cholesterol and coconut oil) for 2 weeks. Two weeks after beginning experimental HFD, Group II-VI were fastened for 12 h (*ad libitum* access to water) followed by a single intraperitoneal injection of STZ (35 mg/kg) dissolved in freshly prepared ice-cold sodium citrate buffer (0.1 M, pH 4.5). Overnight fasted rats with blood glucose ≥ 300 mg/dl on 3rd day after STZ administration were considered diabetic and selected for subsequent experiments. The vehicle, simvastatin or reserpine was given orally twice a day for consecutive days till normal blood glucose level was attained. After overnight fasting on 0th days (before start of experiment), blood glucose levels were analyzed from tail vein of rats using glucometer and strips.

Statistical Analysis

Data were expressed as a mean ± SD for six rats in each group, and analyzed using the Graph pad software for Windows Version 8.0.2.0. Statistical differences were computed using two-way and one-way ANOVA followed by Sidak's test. Data were considered statistically significant at ****P<0.0001, ***P<0.001, **P<0.01, P> 0.05.

Results and Discussion

Acute toxicity studies

The acute toxicity study showed that oral administration of simvastatin and reserpine (2.5, 5, 10, 25, 50, 75, 100 mg/kg) caused dose dependent behavioral effects. Simvastatin and reserpine didn't change the skin, fur colors, eyes, mucous membrane, the occurrence of secretions, excretions, jumping, licking, and sedation. However, mortality was observed after oral administration of simvastatin (75 mg/kg) and reserpine (100 mg/kg). The lips, limbs, and tails of the dead rats were cyanotic. In a study, simvastatin (80mg/kg) was orally administered to evaluate broader application of drug induced toxicity assessments using non- invasive approach. Okudan N and Belviranli M. 2020 showed that high dose of simvastatin (80 mg/kg) impairs cognitive and motor functions via decreasing brain-derived neurotrophic factor and irisin levels in hippocampus (Okudan N and Belviranli M., 2020). Simvastatin given in higher doses increases the risk of muscle toxicity associated with increased serum concentration (Butterweck V et al., 2009). Despite the fact that the earlier data on reserpine showed its LD50 at 420 mg/kg in rats, we found it to be 100 mg/kg. The effects that reserpine was reported to show above 5 mg/kg, intraperitoneally were not fatal but produced symptoms of apparent signs of severity (Glow PH et al., 1959; Cayman Chemicals OSHA HCS). Another study showed that single dose (24 mg/kg; orally and 0.5 mg/kg; intraperitoneally) administration of reserpine led to slight gastric mucosal lesions. They found that reserpine induced gastric lesions were highly dependent on duration, route and dose of administration as well as on individuals' gastric status. Notably, a forty-eight-fold difference exists between the two routes of administration for reserpine in terms of physiological effect. In addition, reserpine in controlled hypotensive tablets did not induce lesions during treatment of hypertension. Further, the dose required to induce gastric mucosal lesions was thirty times higher than necessary to reduce blood pressure. Reserpine was found to exhibit no other effect except reduction hypertension at doses 0.45 mg/kg/day which was equivalent of 31.5 mg per day in a 70 kg man.

Effect of simvastatin and reserpine in Ang II-induced hypertensive rat The anti-hypertensive activity of simvastatin (10 mg/kg & 20 mg/kg) and reserpine (0.25mg/kg & 0.5 mg/kg) showed a significant dose dependent reduction in blood pressure and mean arterial pressure (P<0.0001) in Ang II induced hypertensive rats. Treatment with simvastatin and reserpine significantly decreased systolic pressure, diastolic pressure and MAP. It is noteworthy that at simvastatin (20 mg/kg) and reserpine (0.5 mg/kg) showed normal blood pressure on day 7 and reserpine on day 12 as shown. A study evidenced similar results that simvastatin (10 mg/kg, oral) reduced the blood pressure by 30 to 50 mm of Hg in rats through normalizing the up regulation of ClC-3. Simvastatin (3 mg/kg) in combination with losartan (1 mg/kg) exerts anti-hypertensive effect in hypercholesterolemic hypertensive Wistar rats and patients. The effects are imparted by reducing cholesterol, ameliorate endothelial dysfunction,

reduce oxidative stress, increase nitric oxide and reduce vascular inflammation (Abdel et al., 2017). Similarly, reserpine (0.45 mg/kg/day) administered in drinking water was reported to exhibit anti-hypertensive effect. Further, these findings are in agreement with previously published studies in Ang II-induced hypertension that showed blood pressure lowering effect of sEHI. The mechanism for reducing blood pressure appears to be dependent on increasing urinary sodium excretion or attenuating vascular resistance. Such changes are in line with vascular and renal actions attributed to EETs. Ang II causes systemic hypertension by upregulating sEH expression. Ang II infused rats receiving treatment with sEHI indeed showed raised levels of EETs with decrease in systolic blood pressure. The ability of sEHI to reduce blood pressure in hypertensive rodent models has been controversial. Anti-hypertensive effects of sEHI have also been monitored in other recognized models of hypertension including DOCA salt hypertension. On other hand, administration of sEHI does not reduce blood pressure in L-NAME induced hypertensive, stroke-prone hypertensive or spontaneously hypertensive rats (Imig JDet al., 2009; Imig JD et al., 2002). Thus, results demonstrated that administration of simvastatin and reserpine, attenuates the blood pressure and improves vascular function in Ang II infused hypertensive rats.

Table No. 1: Effect of simvastatin on systolic blood pressure in Ang II inducedhypertensive rats

Duration	Sham Control	Ang II (150 ng/kg/min)	Simvastatin (10mg/kg)	Simvastatin(20mg/kg)
day 0	122.00±1.11****	212.00±1.23	221.00±7.11****	218.00±6.33****
day 3	124.00±2.22****	224.00±2.45	198.00±6.45****	153.00±3.67****
day 5	123.00±1.03****	227.00±4.56	159.00±8.11****	147.00±2.82****
day 7	122.00±2.14****	231.00±7.71	142.00±2.19****	128.00±2.85****

Table No. 2: Effect of simvastatin on diastolic blood pressure in Ang II inducedhypertensive rats

Duration	Sham Control	Ang II (150 ng/kg/min)	Simvastatin (10mg/kg)	Simvastatin(20mg/kg)
day 0	82.00±2.19****	182.00±9.81	184.00±7.17****	189.00±1.42****
day 3	84.00±1.28****	184.00±3.44	155.00±9.83****	126.00±5.54****
day 5	83.00±3.33****	184.00±6.12	134.00±3.21****	118.00±6.31****
day 7	84.00±1.18****	186.00±4.71	116.00±2.53****	95.00±5.86****

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Table No. 3: Effect of simvastatin on mean arterial pressure in Ang II inducedhypertensive rats

Duration	Sham Control	Ang II (150 ng/kg/min)	Simvastatin (10mg/kg)	Simvastatin (20mg/kg)
day 0	91.00±1.23****	197.00±1.25	207.00±3.53****	205.00±7.17****
day 3	91.00±2.44****	204.00±2.12	178.00±14.46****	141.00±6.52****
day 5	93.00±1.21****	205.00±4.34	154.00±1.46****	130.00±9.85****
day 7	94.00±2.44****	208.00±7.67	123.00±2.51****	108.00±2.72****

Table No. 4: Effect of reserpine on systolic blood pressure in Ang II inducedhypertensive rats

Duration	Sham Control	Ang II (150 ng/kg/min)	Reserpine (0.25 mg/kg)	Reserpine (0.5 mg/kg)
day 0	122.00±1.33****	212.00±1.65	220.00±7.17****	218.00±6.32****
day 6	123.00±2.59****	228.00±7.27	176.00±2.28****	159.00±2.82****
day 12	121.00±1.15****	234.00±2.91	143.00±2.46****	121.00±1.36****

Table No. 5: Effect of reserpine on diastolic blood pressure in Ang II inducedhypertensive rats

Duration	Sham Control	Ang II (150 ng/kg/min)	Reserpine (0.25 mg/kg)	Reserpine (0.5 mg/kg)
day 0	82.00±2.29****	182.00±9.33	180.00±7.37****	181.00±1.41****
day 6	84.00±1.18****	181.00±4.12	152.00±2.21****	129.00±5.83****
day 12	85.00±2.36****	183.00±2.46	108.00±2.55****	82.00±2.35****

Table No. 6: Effect of reserpine on mean arterial pressure in Ang II inducedhypertensive rats

Duration	Sham Control	Ang II (150 ng/kg/min)	Reserpine (0.25 mg/kg)	Reserpine (0.5 mg/kg)
day 0	91.00±1.43****	197.00±1.11	211.00±3.55****	179.00±7.47****
day 6	94.00±3.23****	204.00±2.28	138.00±2.28****	110.00±2.15****
day 12	95.00±2.95****	207.00±2.11	89.00±1.92****	68.00±3.59****

Anti-diabetic studies in rats

Diabetes is one of the most prevailing components of MS which significantly reduces the quality of life in patient. Selective sEHI exhibits multiple pharmacological activities including anti-inflammatory, analgesic, anti-hypertensive and other effects which protect liver, heart and brain from pathological insult (Minaz N et al., 2019). So, we evaluated the protective effect of newly identified sEHI, simvastatin and reserpine in STZ+HFD induced diabetes in rats.

Effect of simvastatin and reserpine in STZ+HFD induced diabetic rat

Previous studies related to diabetic animal model, have shown that expression of COX is increased and the level of EET is reduced. There has been growing incidental evidence to suggest the involvement of sEH in glucose related abnormalities. The protein CYP2J responsible for generating EETs is highly expressed in rat and human pancreatic tissue where significant amount of endogenous EETs have been verified. Also, it has been reported that EETs are potent insulin release mediator in isolated rat islets (Iyer A et al., 2011). In a recent study the role of TUCB, an sEHI was assessed in insulin and glucose homeostasis in STZ treated mice using both inhibition and sEH knockout (Luo P et al., 2010). We in this study investigated whether the treatment with sEHI improves glucose by increasing EETs. Simvastatin (10 mg/kg and 20 mg/kg) and reserpine (0.25mg/kg & 0.5 mg/kg) treatment in diabetic rats significantly (P<0.0001) decreased blood glucose level in a dose dependent manner from day 1 to day 10 respectively when compared with diabetic control group as shown in Table. A recent study supports our hypothesis, by showing that sEHI restored insulin signaling and glucose homeostasis together with increased pancreatic islet size in STZ induced diabetic model (Ajiboye BO et al., 2021; Minaz N et al., 2019). These might be the same mechanism through which simvastatin and reserpine improved glucose abnormalities in our study.

Table No. 7: Effect of simvastatin and reserpine on blood glucose level inSTZ+HFD induced diabetic rats

Groups	Day 0	Day 10
Normal Control	102.50±4.11	103.00±2.15
STZ+HFD	494.50±2.47	505.50±4.51
Simvastatin (10 mg/kg)	501.30±3.31	144.00±3.66****
Simvastatin (20 mg/kg)	497.00±4.29	121.00±6.32****
Reserpine (0.25 mg/kg)	534.00±4.11****	132.00±1.69****
Reserpine (0.5 mg/kg)	521.00±3.50****	108.00±3.28****

Conclusion

MS is a progressive and insidious disorder occurring in susceptible subgroups of population in response to adverse lifestyle and pathological conditions. Treatment of MS requires polypharmacy therapy, though associated risk factors are poorly controlled. Owing to narrow therapeutic index of reserpine and selection of experimental doses in rats, we performed acute toxicity study of both test compounds with slight modification in the doses. Results of acute toxicity studies indicated that simvastatin and reserpine at 75 mg/kg and 100 mg/kg causes mortality in rat, respectively.

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