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Brief Report

The role of S-methylisothiourea hemisulfate as inducible nitric oxide synthase inhibitor against kidney iron deposition in iron overload rats

Maryam Maleki[1], Melika Samadi[1], Mehrangiz Khanmoradi[2], Mehdi Nematbakhsh[1,2,3], Ardeshir Talebi[1], Hamid Nasri[1]

[1]Water and Electrolytes Research Center, Isfahan University of Medical Sciences, [2]Department of Physiology, Isfahan University of Medical Sciences, [3]Isfahan MN Institute of Basic and Applied Sciences Research, Isfahan, Iran

Abstract

Background: Iron dextran is in common use to maintain iron stores. However, it is potentially toxic and may lead to iron deposition (ID) and impair functions of organs. Iron overload can regulate the expression of inducible nitric oxide synthase (iNOS) in some cells that has an important role in tissue destruction. S-methylisothiourea hemisulfate (SMT) is a direct inhibitor of iNOS, and this study was designed to investigate the effect of SMT against kidney ID in iron overload rats.

Materials and Methods: 24 Wistar rats (male and female) were randomly assigned to two groups. Iron overloading was performed by iron dextran 100 mg/kg/day every other day for 2 weeks. In addition, during the study, groups 1 and 2 received vehicle and SMT (10 mg/kg, ip), respectively. Finally, blood samples were obtained, and the kidneys were prepared for histopathological procedures.

Results: SMT significantly reduced the serum levels of creatinine and blood urea nitrogen. However, SMT did not alter the serum levels of iron and nitrite, and the kidney tissue level of nitrite. Co-administration of SMT with iron dextran did not attenuate the ID in the kidney.

Conclusion: SMT, as a specific iNOS inhibitor, could not protect the kidney from ID while it attenuated the serum levels of kidney function biomarkers.

Key Words: Iron deposition, kidney, nitric oxide synthase, S-methylisothiourea hemisulfate

Address for correspondence:
Prof. Mehdi Nematbakhsh, Water and Electrolytes Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.
E-mail: nematbakhsh@med.mui.ac.ir
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INTRODUCTION

Iron is essential for various processes in the human body such as oxygen transport, energy metabolism, and DNA synthesis.[1] Although iron is a vital mineral nutrient for living cells, it is potentially toxic.[2] The critical role of iron in transferring oxygen, metabolic reactions, cellular respiration, production of oxygen radicals, DNA synthesis, and repair is balanced toward iron harmful effects, which lead to iron deposition (ID), oxidative stress, and finally cellular damage.[3,4] It is

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essential to control iron homeostasis tightly to balance its role as a necessary element for survival versus its toxic effects.[3] Regular blood transfusion in thalassemia patients causes iron overload and hemosiderosis in many organs such as the liver, kidney, and heart,[5] because the body has no physiological mechanism to actively excrete excess iron.[6]

Almost all patients treated by erythropoiesis-stimulating agents should receive additional iron supplements.[7] Parenteral iron transfusion should be administered with exact attention to the total body iron storage or presence of active inflammation; otherwise, it results in iron overload, oxidative stress, and inflammation.[7,8] Regulation of iron metabolism is so important since both iron deficiency and iron overload can cause disease even in the brain, which is highly influenced by iron metabolism defects and consequently accumulation and ID.[9] Iron overload is one of the common iron-related toxicities. Excess iron has a central role in producing reactive oxygen species (ROS) and reactive nitrogen species (RNS), which can damage biological molecules such as lipids, proteins, and nucleic acids; resulting in oxidative stress and various diseases.[4] Furthermore, it is reported that iron overload can increase inducible nitric oxide (NO) synthase (iNOS) in some organs.[10,11] S-methylisothiourea hemisulfate (SMT); a nonamino acid analogue of L-arginine is one of the inhibitory agents that reduces iNOS effects. The agent is shown to be 10–30 times more effective and powerful than other iNOS inhibitors *in vitro*.[12] SMT inhibits NO production, which in turn reduces endogenous NO concentration.[13] Due to increase of iNOS in some organs[10,11] by iron overloading, it is hypothesized that inhibition of iNOS may attenuate iron overloading or at least it's side effects. Therefore, in the current study, we attempted to perform a moderate iron overload model in 2 weeks, and the role of SMT against iron overload was investigated.

MATERIALS AND METHODS

Animals

Twenty-four adult Wistar rats (12 males and 12 females; 169.83 ± 3.54 g) were used. The animals were fed on conventional diets and had free access to tap water. The rats were kept at the room temperature (23–25°C) and maintained under standard conditions of light (light/dark: 12:12 h). The experiments were carried out in accordance with the guidelines of the Ethics Committee of Isfahan University of Medical Sciences.

Experimental protocol

The animals were randomly divided into two experimental groups. Group 1 (six male and six female) received iron dextran (Vifor Inc., Switzerland) 100 mg/kg, ip every other day and saline as a vehicle for SMT every day for 2 weeks.

Group 2 (six male and six female) received iron dextran regimen the same as Group 1 and SMT (10 mg/kg, ip) every day for 2 weeks.

We used equal sample size of male and female rats in each group of experiments to include the possible gender effect on the results.

At the end of the experiment, the blood samples were obtained by heart puncture under chloral hydrate anesthetization. Then, the rats were sacrificed, and kidneys, uterus (in females), and testis (in males) were removed and weighted immediately. The kidneys were also prepared for histopathological procedures. The weight of animals was recorded on a daily basis.

Measurements

The levels of serum creatinine (Cr) and blood urea nitrogen (BUN) were determined using quantitative diagnostic kits (Pars Azmoon, Iran). The serum level of nitrite (stable NO metabolite) was measured using a colorimetric kit (Promega Corporation, USA). The serum level of iron was measured using a diagnostic kit with the photometric method.

Histopathological procedures

The removed kidneys were fixed in 10% neutral formalin solution to examine ID in the kidney. The kidney ID was evaluated by a pathologist who was blind to the study. The level of ID in the kidney tissue was scored on the basis of the intensity of tissue in the range of 0–100%, and 0 was assigned to normal kidney tissue without any ID.

Statistical analysis

Data are expressed as mean ± standard error of the mean. The serum levels of BUN, Cr, iron, and nitrite; kidney nitrite, and the percentage of ID in kidney tissue were compared between the groups using the Student's t-test. $P \leq 0.05$ was considered statistically significant.

RESULTS

Bodyweight change

No significant changes in body ($P = 0.45$), kidney ($P = 0.62$), testis (in male, $P = 0.18$), and uterus (in female, $P = 0.19$) weights were observed between the groups [Figure 1 and Table 1].

Effect of S-methylisothiourea hemisulfate on serum blood urea nitrogen, creatinine, iron, and nitrite levels

S-methylisothiourea hemisulfate significantly reduced the serum levels of Cr ($P = 0.053$) and BUN ($P = 0.00$)

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as kidney functional parameters [Figure 1]. However, SMT did not alter the serum levels of iron and nitrite. The kidney tissue level of nitrite also did not change by SMT [Figure 1, Table 1].

Iron deposition in kidney tissue

Histological investigations demonstrated ID in the kidney tissue of both groups. However, no statistical difference ($P = 0.72$) was detected between the groups [Figure 1]. This finding revealed that SMT as a selective iNOS inhibitor could not attenuate the ID in the kidney. The sample images of ID in kidney tissue are provided in Figure 2.

DISCUSSION

Chronic iron overload result from several conditions, including genetic defects of iron uptake and storage, frequent red blood cell transfusions, metabolic alterations, chronic liver disease, or excessive iron intake.[14] Considering the importance of chronic iron overload, in this study, the role of simultaneous use of SMT with iron against ID was investigated. SMT is an iNOS inhibitor, and some studies have shown the specificity of SMT for iNOS.[12,15] Nitrite is also considered as an indicator of NO production.[16] We found that simultaneous use of SMT and iron did not attenuate the intensity of ID in the kidney.

Nitric oxide plays an important role in many physiological and pathophysiological processes.[17,18] It is an inorganic radical produced by NO synthase (NOS).[18,19] Three isoforms of NOS; namely, neuronal NOS (nNOS, type 1), inducible NOS (iNOS, type 2), and endothelial NOS (eNOS, type 3), have been identified that produce NO from L-arginine.[18,19] The iNOS isoform produces large quantities of NO that can result in tissue damage or death.[20] Although plasma nitrite and nitrate are not significant biomarkers of oxidative stress, they reflect the presence of NO species in plasma.[21] In addition, nitrite more corresponds to NO produced by eNOS in vasculature under physiological conditions rather than iNOS or nNOS.[22] Accordingly, it could be assumed as the reason for insignificant difference in nitrite levels

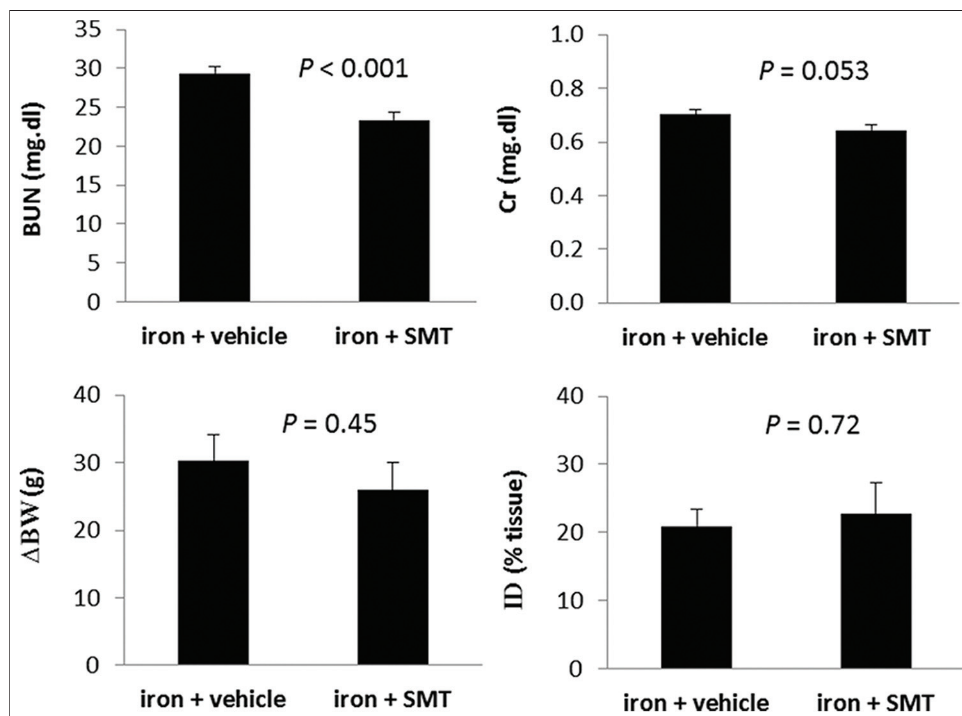

Figure 1: The serum level of blood urea nitrogen, creatinine, bodyweight change (ΔBW), and percentage of iron deposition (ID) in kidney tissue (ID) in two iron overload groups treated with vehicle and S-methylisothiourea hemisulfate. The *P* value obtained by the Student's *t*-test

Table 1: The serum levels of Fe and SN, KN, and KW/100 g BW, TW/100 g BW, and UW/100 g BW/100 g BW in two iron overload groups treated with vehicle and SMT

Group	Fe (mg/dl)	SN (μmol/L)	KN (μmol/L)	KW/100 g BW	TW/100 g BW	UW/100 g BW
Iron+vehicle	270.9±18.2	13.3±1.9	35.0±3.9	0.722±0.026	0.95±0.15	0.064±0.013
Iron+SMT	268.7±16.3	14.2±1.9	41.9±5.4	0.736±0.010	1.34±0.22	0.041±0.002
P	0.92	0.75	0.30	0.62	0.18	0.19

The *P* value obtained by the Student's *t*-test. SMT: S-methylisothiourea hemisulfate, Fe: Iron, SN: Nitrite, KN: Kidney level of nitrite, KW: Kidney weight, BW: Body weight, TW: Testis weight, UW: Uterus weight

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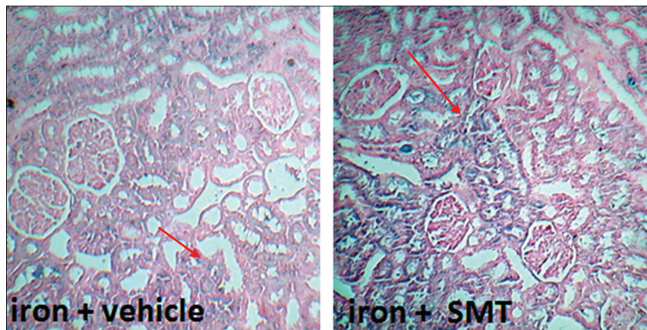

Figure 2: Sample images of iron deposition in two groups of experiment

between the two groups.[23,24] It was reported that chronic iron overload result in ID in the glomeruli and proximal tubules with various kidney injuries and evidence of augmentative ROS activity, increased ROS-mediated inactivation, sequestration of NO, and compensatory up-regulation of renal eNOS and iNOS expressions.[25] We used SMT that significantly inhibit iNOS activity without significant effects on constitutive NOS.[26,27] In the current study, the groups were insignificantly different in the serum level of nitrite, which is a marker of NO production from endothelium[28] and endothelium function.[29] The underlying reason for the difference could be up-regulation of renal eNOS expressions in chronic iron overload. We did not measure the serum nitrate level, another metabolite of NO because plasma nitrate level is influenced by some other NOS independent factors too, and it cannot be a good indicator of NOS activity.[29,30]

Testis and uterus weight respectively in male and female rats (per 100 g BW) in two iron overload groups treated with vehicle and SMT were insignificantly different. In another study with the same chronic iron overload condition, it was reported that no changes in bodyweights and testis weight were observed in iron overload group,[14] and this model of chronic iron overload produced a mild oxidative damage in rat testis.[14] It has been reported that iron overload cause a significant decrease in testis content of lipid soluble antioxidants, inversely correlated with tissue iron concentration, but the acute iron overload did not affect testis weight.[31] In addition, some *in vitro* studies have shown that NO is one of the inflammatory vasodilatory factors that can suppress steroidogenesis in Leydig cells even in the absence of inflammation. This is produced by one of the three NOS enzymes, especially iNOS, which is calcium independent and is expressed in normal testis. NOS inhibitors primarily reduce NO and increase testosterone levels. However, after few hours, testosterone level and spermatogenesis decline due to the low concentration of NO and consequently reduced blood flow, which affect Leydig and Sertoli cells. This complex role of iNOS and NO in testis is similar to the condition of inflammation and ROS production, which should be in an appropriate balance with NO. Therefore, iNOS inhibitors influence normal and inflamed testis similarly.[32,33]

It was reported that iron overload causes an increase in BUN and Cr.[15,34,35] Urea specifically and reversibly inhibits the inducible NO synthesis in macrophages.[36] Thus, possibly in our study simultaneous with loss of BUN, iNOS activity enhanced NO synthesis and therefore compensated the SMT function. SMT, a nonamino acid analogue of L-arginine, is a direct inhibitor of iNOS that inhibits the function of iNOS competitively (in a dose-dependent manner), so does not interfere with the production of iNOS.[15,37]

CONCLUSION

S-methylisothiourea hemisulfate as a specific iNOS inhibitor could not prevent ID in kidney while it attenuates the serum levels of kidney function biomarkers; BUN and Cr. However, the increase in iNOS by iron overload[10,11] may be prevented by other specific iNOS inhibitors.

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