European Review for Medical and Pharmacological Sciences 2022; 26: 2794-2801

Anti-diabetic efficacy of combination treatment with the nitric oxide metabolite nitrite and the endogenous antioxidant glutathione in diabetic mice: glutathione combination does not hamper the anti-diabetic effect of nitrite

Y.-S. LEE, S.-O. JEONG, H.-O. PAE

Department of Microbiology and Immunology, Wonkwang University School of Medicine, Iksan, Republic of Korea

Abstract. – OBJECTIVE: The nitric oxide (NO) metabolite nitrite has been shown to attenuate hyperglycemia via its increase in insulin sensitivity and glucose uptake. However, the oral use of nitrite is limited due to its potential formation of the carcinogenic N-nitrosamines *via* reaction of acidic nitrite and the secondary amines. We investigated the anti-diabetic effect of sodium nitrite (SN) combined with glutathione (GSH) in streptozotocin (STZ)-induced diabetic mice for potential use of GSH as a protective agent in future nitrite therapy.

MATERIALS AND METHODS: STZ-induced diabetic mice were orally treated for 5 weeks with vehicle, SN, GSH or SN + GSH. Oral glucose tolerance test and the measurement of fasting blood glucose (FBG) and glycosylated hemoglobin (HbA1c) levels were carried out to evaluate anti-diabetic effects of SN and SN + GSH. Plasma levels of total NO metabolites (NO_x) were measured to confirm nitrite absorption.

RESULTS: SN and SN + GSH significantly improved the glucose tolerance ($p < 0.05$), but GSH alone did not. The efficacy of combination treatment with SN and GSH in improving the glucose tolerance was higher than that of SN alone. Oral treatment with SN or SN + GSH significant reduced FBG and HbA1c levels ($p < 0.05$). Interestingly, daily oral administration of SN + GSH was more effective in reducing FBG and HbA1c levels than that of SN alone. Administration of SN or SN + GSH significantly increased plasma NO_x levels ($p < 0.05$), and combination treatment with SN + GSH was more effective in increasing plasma NO_x levels than that with SN alone.

CONCLUSIONS: Combination treatment with SN and GSH is more effective in controlling hyperglycemia and increasing the plasma NO_x levels in an experimental mouse model of diabetes. Since oral administration of GSH has been shown to be non-toxic in humans, the combination of SN and GSH may be important in potential future nitrite therapy.

Key Words:
Nitric oxide, Nitrite, Glutathione, Combination treatment, Anti-diabetic effect, Nitrite therapy, Anti-carcinogenic activity.

Introduction

Nitric oxide (NO) is endogenously generated from the amino acid *L*-arginine in oxidative reactions catalyzed by NO synthases (NOSs), which is referred to the classical *L*-arginine-NOS-NO pathway[1]. Also, this free radical is generated from the NO metabolites (i.e., nitrate and nitrite) in reductive reactions catalyzed by nitrite reductases, which is referred to the alternative nitrate-nitrite-NO pathway[2]. It is generally acceptable that the nitrate-nitrite-NO pathway serves as a backup system to ensure NO generation when the *L*-arginine-NOS-NO pathway is inactivated or malfunctioning[3]. Hence, NO bioavailability has been shown to be mutually influenced and complementally controlled by these two pathways.

Decreased NO bioavailability, which could be due to the impairment of the *L*-arginine-NOS-NO pathway and/or the nitrate-nitrite-NO pathway, has been shown to be involved in development of type 2 diabetes and hypertension[4,5]. Increased NO bioavailability by using the NO precursors, including *L*-arginine and nitrate/nitrite, has been suggested to be a complementary and alternative therapy for type 2 diabetes and hypertension[4,5]. However, due to undesirable side effects of

Corresponding Author: Hyun-Ock Pae, Ph.D; e-mail: hopae@wku.ac.kr

long-term supplementation with *L*-arginine (i.e., increased arginase activity, decreased endothelial NOS expression, and increased cellular oxidative stress), nitrate/nitrite administration has been suggested as a better treatment for NO boosting[6-9]. Moreover, recent evidence from *in vitro* and *in vivo* studies[5,10] indicates that nitrate/nitrite, especially when nitrite is orally ingested, could attenuate hyperglycemia *via* its increase in insulin sensitivity and glucose uptake. However, the use of a high dose of nitrite is limited due to its potential toxicity[9,11,12]. For example, under acidic conditions, such as those found in the stomach, nitrite may react with the secondary amines to form the carcinogenic *N*-nitrosamines[11-14].

The tripeptide glutathione (GSH) is endogenously synthesized in animal, plant, and microbial cells[15]. In addition to its antioxidant defense, GSH plays pivotal roles in different physiological processes, including DNA synthesis, protein synthesis, and xenobiotic detoxification[15,16]. It is noteworthy that GSH has been found to be effective in counteracting the nitrite toxicity[17-22]. For example, GSH is capable of preventing the formation of *N*-nitrosamines by scavenging the active nitrite[18,19,23]. Thus, a combination strategy of nitrite with GSH may overcome the formation of *N*-nitrosamines under acidic conditions.

The present study was performed to investigate the anti-diabetic effect of nitrite combined with GSH in streptozotocin (STZ)-induced diabetic mice for potential use of GSH as a protective agent in future nitrite therapy.

Materials and Methods

Chemicals and Reagents

Sodium nitrite (SN), GSH, STZ, glucose and other chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA).

UV-Visible Spectroscopy

Absorption spectra were scanned at room temperature on a V-730 UV-visible spectrophotometer (JASCO International Co. Ltd., Tokyo, Japan). GSH was diluted in 0.5 M HCl solution and placed in both reference and sample cuvette. After correcting the baseline, SN was added to the sample cuvette and an equivalent volume of water, then, was added to the reference cuvette.

Experimental Animals

Specific pathogen-free male C57BL/6J mice (8 weeks old) were purchased from Samtako Biokorea (Osan, Republic of Korea), and housed in cages at room temperature (25 ± 2°C) on 12-h light-dark cycle, along with free access to standard mouse chow and water. The experimental animal protocol was reviewed and approved by the Animal Ethics Committee of Wonkwang University (No. WKU18-40). All efforts were made not only to minimize animal suffering but also to reduce the number of animals used.

Induction of Diabetes in the Experimental Mice

After 1-week adapted feeding, the mice were fasted for 12 h but given drinking water. Then, the mice were intraperitoneally injected with freshly prepared STZ dissolved in an ice-cold 0.1 M citrate buffer (pH 4.5) at a dose of 100 mg/kg body weight. After 4 h, STZ-injected mice were orally administrated with 0.2 mL of 5% glucose solution to prevent STZ-induced hypoglycemic mortality. 72 h after the STZ injection, the levels of blood glucose were measured from caudal vein blood samples by using a glucose meter, and the mice with fasting glucose levels above 11.1 mmol/L were used as the diabetic mice for our further experiments.

Experimental Design

Mice were randomly assigned to one of five groups: (1) normal mice (n = 5), (2) diabetic control mice (n = 5), (3) SN-treated diabetic mice (n = 5), (4) GSH-treated diabetic mice (n = 5), and (5) SN + GSH-treated diabetic mice (n = 5). Normal and diabetic control mice received regular drinking water. SN-treated mice and GSH-treated mice orally received water supplemented with 1 mM SN and 1 mM GSH for 5 weeks, respectively. SN + GSH-treated mice orally received water supplemented with a mixture (1 mM SN and 1 mM GSH) for 5 weeks. Fresh SN-/GSH-/SN + GSH-supplemented water was replaced every other day. All mice that were housed in individual cages in a temperature- and humidity-controlled room were allowed standard chow *ad libitum*.

Oral Glucose Tolerance Test

Following treatment of diabetic mice with each agent for 1-week, oral glucose tolerance test (OGTT) was carried out to confirm the anti-diabetic effects of SN, GSH and SN + GSH. Fol-

lowing overnight fast, diabetic mice were orally administered 20% glucose solution (1.5 g/kg body weight). The levels of blood glucose were measured from tail vein before and 30, 60, 90, 120 min after glucose administration.

Determination of Fasting Blood Glucose Levels and Body Weight

The levels of fasting blood glucose were determined in all experimental mice once a week throughout the study period. Blood was obtained from tail vein, and the levels of blood glucose were determined by using a glucose meter. Body weight was measured every 3 to 4 days for all the groups of experimental mice.

Determination of HbA1c Levels

At the end of the study, all mice were anesthetized with 5% isoflurane, and the whole blood was collected *via* intracardiac puncture with a heparinized 26G needle (Sigma-Aldrich, St. Louis, MO, USA). The levels of glycosylated hemoglobin (HbA1c) in a small aliquot (20 mL) of whole blood were determined by using an Enzyme-Linked Immunosorbent Assay kit (Calbiotech, Spring Valley, CA, USA). Plasma was separated from the remaining blood by centrifugation at 3000 rpm for 15 min at room temperature and stored at -70°C until required.

Determination of Plasma NO_x Levels

The plasma levels of the total nitrogen oxide NO_x (nitrate + nitrite) were determined at the end of the study by using nitrate/nitrite colorimetric assay kit (Cayman Chemical, Ann Arbor, MI, USA) according to manufacturer's instructions. Briefly, the method for NO_x analysis consists of a two-step process. In the first step, nitrate in plasma was converted into nitrite by utilizing the nitrate reductase. In the second step, nitrite was converted into purple azo compound by adding Greiss reagents. To determine the concentration of total nitrite, the absorbance of the azo compound was measured at the wavelength 540 nm.

Statistical Analysis

All data are expressed as mean ± standard error of mean (SEM). Differences between groups were tested for statistical significance using student *t*-test and one-way analysis of variance followed by Bonferroni correction using GraphPad Prism (GraphPad Software Inc., La Jolla, CA, USA). A level of $p < 0.05$ was considered to be significant.

Results

Confirmation of GSNO Formation by Reaction of SN and GSH Under Acidic Condition

Initially, we confirmed whether GSH could react spontaneously with SN under acidic condition. In spectrophotometric absorption spectra shown in Figure 1, the product absorption band (GSNO; λ_{max} = 334 nm) was clearly observed together with a complete disappearance of the reactant absorption band (SN; λ_{max} = 354 nm), when SN was immediately mixed with GSH under acidic condition. This indicates that the reaction of SN with GSH under acidic condition results in spontaneous formation of GSNO; however, it should be noted that at neutral pH, GSH is not essentially oxidized by SN for GSNO formation.

Effects of SN and SN + GSH on Oral Glucose Tolerance in Diabetic Mice

OGTT was performed to assess glucose absorption from the bloodstream in diabetic mice. At 1-week experimental point, a 20% glucose solution was orally administrated to fasting diabetic mice and the levels of blood glucose were measured at various time points. While in the diabetic control mice, blood glucose levels remained elevated beyond 120 minutes of oral glucose load, daily administration of SN or SN + GSH significantly improved the glucose tolerance

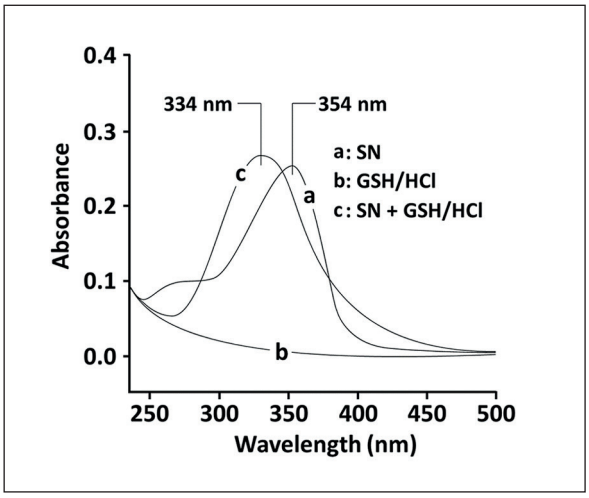

Figure 1. UV-visible absorption spectra of SN, GSH and SN + GSH/HCl. After addition of 0.3 mM SN to the solution containing 0.3 mM GSH in 0.5 M HCl, absorption spectra were immediately scanned against a blank containing 0.3 mM GSH in 0.5 M HCl. Experimental procedures are detailed in the section of Materials and Methods.

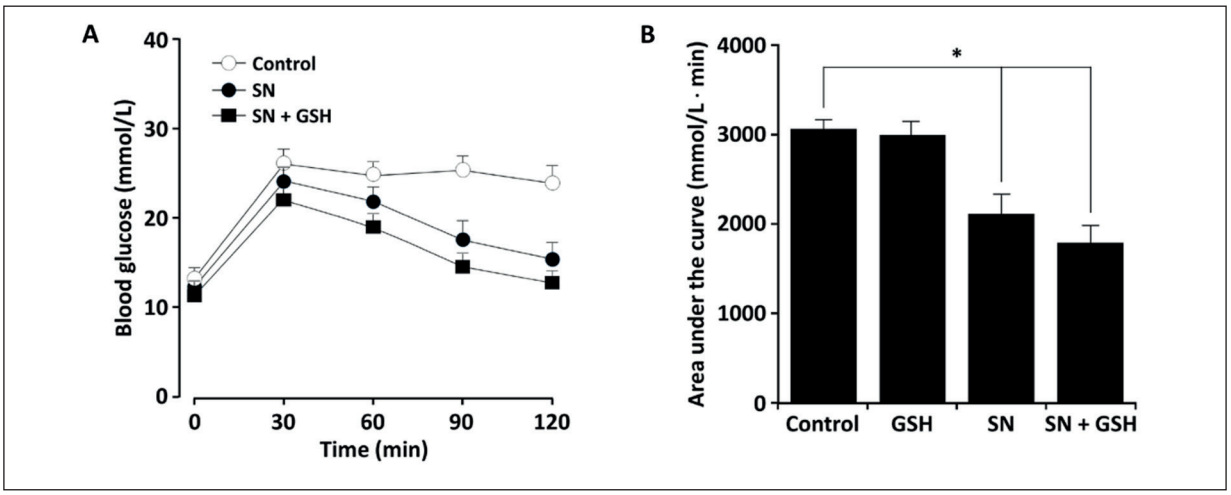

Figure 2. Inhibitory effects of SN and SN + GSH on blood glucose during OGTT in STZ-induced diabetic mice. **A)** Changes in levels of blood glucose during OGTT. **B)** Corresponding area under the curve of OGTT. Experimental procedures are detailed in the section of Materials and Methods. Results are presented as mean ± SEM ($n = 5$). *$p < 0.05$.

of the diabetic mice within 120 minutes of oral glucose load (Figure 2A). However, the improvement of the glucose tolerance of the diabetic mice was superior with SN + GSH, as compared to that with SN alone. Daily oral administration of GSH alone had no significant effect on the glucose tolerance (Figure 2B).

Effects of SN and SN + GSH on Fasting Blood Glucose Levels in Diabetic Mice

Effects of SN and SN + GSH on fasting blood glucose levels are shown in Figure 3. During the treatment period and at the end of the treatment, we observed a continuous increase in the levels of fasting blood glucose in untreated diabetic control mice. However, in diabetic mice treated with SN or SN + GSH, a significant decrease in the levels of fasting blood glucose over the period of five weeks was observed. Interestingly, daily oral administration of SN combined with GSH was more effective in reducing the levels of fasting blood glucose than that of SN alone. Daily oral treatment of diabetic mice with GSH alone had no significant effect on the levels of fasting blood glucose.

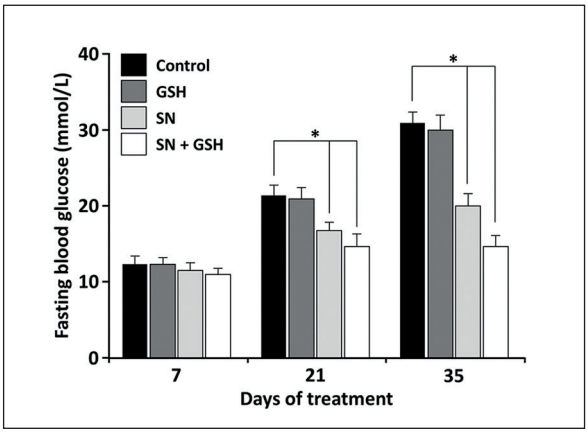

Figure 3. Anti-diabetic effects of SN and SN + GSH on fasting blood glucose for 5 weeks in STZ-induced diabetic mice. Experimental procedures are detailed in the section of Materials and Methods. Results are presented as mean ± SEM ($n = 5$). *$p < 0.05$.

Effects of SN and SN + GSH on Glycosylated Hemoglobin Levels of Diabetic Mice

The total glycosylated hemoglobin (HbA1c) levels of the experimental groups of mice are shown in Figure 4. HbA1c levels of diabetic control mice were significantly higher than those of normal mice. However, daily oral treatment of diabetic mice with SN for five weeks significantly decreased the levels of HbA1c. Treatment with SN + GSH also resulted in improvement in the HbA1c levels of diabetic mice, but the improvement is higher than that with SN alone. No significant change in HbA1c levels was observed in diabetic mice treated with GSH alone.

Effects of SN and SN + GSH on Body-Weight Loss in Diabetic Mice

In the present study, the initial body weights of all experimental groups showed no evident

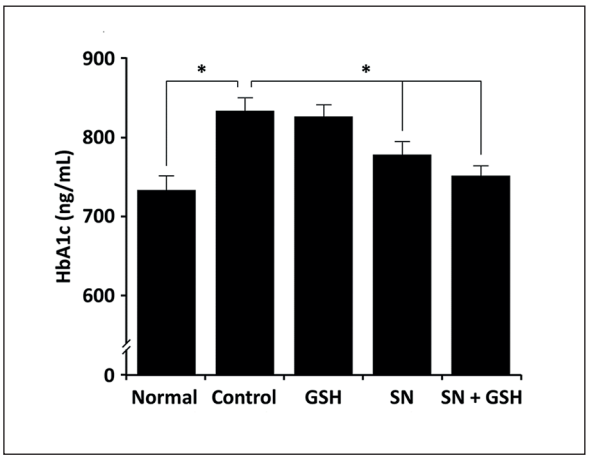

Figure 4. Improving effects of SN and SN + GSH on glycated hemoglobin (HbA1c) from whole blood in STZ-induced diabetic mice. Experimental procedures are detailed in the section of Materials and Methods. Results are presented as mean ± SEM ($n = 5$). *$p < 0.05$.

difference between the groups. At the end of the experiment (day 35), the diabetic control mice expanded less body weight than the non-diabetic normal mice (Figure 5). The body-weight loss of diabetic mice was partly reversed by daily oral treatment with both SN and SN + GSH (Figure 5). There was no significant difference in the body weight between the groups treated with SN and SN + GSH (Figure 5).

Effects of SN and SN + GSH on Plasma NO_x Levels in Diabetic Mice

Hyperglycemia decreased plasma NO_x (nitrate + nitrite) levels as revealed by lower plasma NO_x levels in the diabetic control mice, as compared with the non-diabetic normal mice (Figure 6). However, daily oral treatment of diabetic mice with SN or SN + GSH significantly increased plasma NO_x levels, as compared to untreated diabetic control mice. Interestingly, combination treatment with SN and GSH was more effective in increasing plasma NO_x levels than that with SN alone. There was no significant difference in the plasma NO_x levels between diabetic control mice and diabetic mice treated with GSH alone.

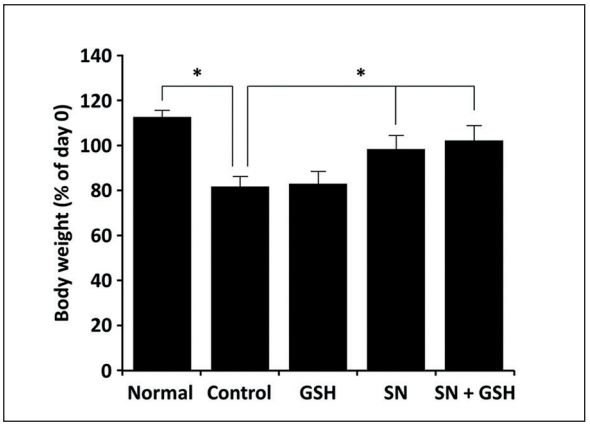

Figure 5. Effects of SN and SN + GSH on body-weight loss in STZ-induced diabetic mice. Experimental procedures are detailed in the section of Materials and Methods. Results are presented as mean ± SEM ($n = 5$). *$p < 0.05$.

Discussion

Although the mechanisms underlying anti-diabetic effect of the NO metabolite nitrite are poorly understood, scholars[5] highlight the therapeutic effect of nitrite, especially, on type 2 diabetes. In the present study, we sought to determine the efficacy of nitrite combined with glutathione (GSH), an endogenous antioxidant capable of scavenging the reactive nitrite, in controlling hyperglycemia in STZ-induced diabetic mice. Our results clearly show that the anti-diabetic activity of the combination of nitrite and GSH was greater than that of nitrite alone; the anti-diabetic efficacy of nitrite was not hampered by combined GSH. In addition, we observed nitrite combined with GSH to be more effective in increasing plasma NO_x than nitrite alone.

Exogenous administration of nitrite has been shown to exert a beneficial effect on prevention of

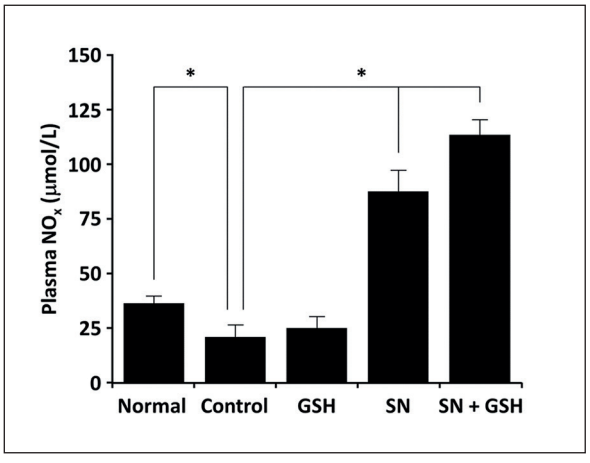

Figure 6. Effects of SN and SN + GSH on plasma levels of total NO metabolites (NO_x) in STZ-induced diabetic mice. Experimental procedures are detailed in the section of Materials and Methods. Results are presented as mean ± SEM ($n = 5$). *$p < 0.05$.

type 2 diabetes and insulin resistance[24-28]. However, under acidic conditions, such as those found in the stomach, nitrite may be capable of reacting with a secondary amine (e.g., dimethylamine, diethylamine, or ethylmethylamine), which may arise through the degradation of food proteins or by unwanted contamination of the secondary amines, eventually leading to the formation of the carcinogenic *N*-nitrosamine[29,30]. Fortunately, it has been suggested that the antioxidant polyphenols, such as ascorbic acid, and the thiol compounds, such as GSH, are capable of scavenging the reactive nitrite in the stomach, thereby preventing the *N*-nitrosamine formation[19,21,23]. At neutral pH, GSH is not essentially oxidized by nitrite, whereas at acidic pH, *S*-nitrosoglutathione (GSNO) is spontaneously formed by multiple interaction of GSH with nitrite[31], as spectrophotometrically shown in Figure 1. Importantly, the formation of GSNO is kinetically more favored than that of *N*-nitrosamine[23], implying that the *N*-nitrosamine formation can be inhibited or prevented by concomitant GSH ingestion. Given that GSH has its own ability to scavenge nitrite by forming the above-mentioned GSNO, one may ask whether the anti-diabetic effect of nitrite would be affected by combination treatment with nitrite and GSH. We, therefore, tested the efficacy of oral combination treatment with nitrite and GSH for hyperglycemic control in diabetic mice.

In the oral glucose tolerance test, sodium nitrite (SN) significantly improved the glucose tolerance. The results suggest that the improvement in glucose tolerance by SN could be due to the improved insulin signaling pathway and/or glucose uptake[25-27]. Combination of SN and GSH also improved the glucose tolerance. However, the efficacy of SN + GSH in improving the glucose tolerance was higher than that of SN alone. An abnormal increase in fasting blood glucose levels is a key characteristic feature of STZ-induced diabetic mice[32]. In our study, SN significantly reduced the fasting glucose levels in diabetic mice, and the reducing effect of SN was enhanced by concomitant GSH treatment. In addition, oral administration of SN significantly reduced the glycosylated HbA1c levels, as compared to untreated diabetic control mice. It could be due to improved glycemic control after SN treatment. Furthermore, the efficacy of SN + GSH in reducing the HbA1c levels was higher than that of SN alone.

Despite scavenging the reactive nitrite by concomitant GSH treatment, how could the anti-diabetic effect of nitrite be still observed in diabetic mice? As a matter of fact, GSNO is an endogenous compound produced by the oxidative reaction of NO with GSH in the animal body[33]. It is considered not only to be as an intracellular NO reservoir, but also to be as an NO vehicle throughout the cell[34]. It is most likely that GSNO, which has been formed by the chemical interaction between GSH and nitrite under acidic conditions, may decompose to release NO in the intestinal milieu. The cell-permeable NO is then capable of diffusing across the intestine, thereby resulting in NO/nitrite absorption. It is also possible that GSNO *per se* may be absorbed in the intestine through an unknown mechanism. In our study, plasma levels of the total NO metabolite NO_x (nitrate + nitrite) in diabetic mice treated with SN or SN + GSH were higher than those in untreated diabetic mice. It could be due to effective NO/nitrite absorption after oral administration of SN or SN + GSH. Notably, plasma NO_x levels were higher in diabetic mice treated with SN + GSH than those in diabetic mice treated with SN alone. These results may explain why the anti-diabetic effect of SN + GSH is better than that of SN; the more absorption of nitrite, the more effective anti-diabetic effect.

Limitations of the Study

The anti-diabetic effect of combination treatment with SN and GSH in STZ-induced diabetic mice was investigated in this study, but future studies are required to examine other beneficial effects of this combination treatment in animal disease models. The STZ-induced mouse model of diabetes is most extensively used to investigate human disease. From an animal welfare perspective for animal research, the long-term effects of combination treatment with SN and GSH was unable to be evaluated in STZ-induced diabetic model, due to a severe body-weight loss in diabetic control mice. Future evaluation of the long-term effects of the combination treatment is needed using other clinically relevant models.

Conclusions

Herein, our results demonstrate that in an experimental model of diabetes, combination treatment with nitrite and glutathione is more effective in controlling hyperglycemia than that with nitrite alone. In addition, this study clearly demonstrates that the oral supplementation of nitrite combined with glutathione exerts an enhanc-

ing effect on plasma NO metabolites. Since oral administration of glutathione has been shown to be non-toxic in humans, the combination of nitrite and glutathione may be important in potential future nitrite therapy.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Acknowledgements

This study was supported by Wonkwang University in 2021.

Authors' Contribution

All authors participated in the design and interpretation of the studies and in analysis of the data and wrote the draft manuscript and reviewed the final manuscript.

References

1) Bondonno CP, Croft KD, Hodgson JM. Dietary nitrate, nitric oxide, and cardiovascular health. Crit Rev Food Sci Nutr 2016; 56: 2036-2052.
2) Lundberg JO, Weitzberg E, Gladwin MT. The nitrate-nitrite-nitric oxide pathway in physiology and therapeutics. Nat Rev Drug Discov 2008; 7: 156-167.
3) Tejero J, Shiva S, Gladwin MT. Sources of vascular nitric oxide and reactive oxygen species and their regulation. Physiol Rev 2019; 99: 311-379.
4) Omar SA, Webb AJ, Lundberg JO, Weitzberg E. Therapeutic effects of inorganic nitrate and nitrite in cardiovascular and metabolic diseases. J Intern Med 2016; 279: 315-336.
5) Ghasemi A, Jeddi S. Anti-obesity and anti-diabetic effects of nitrate and nitrite. Nitric Oxide 2017; 70: 9-24.
6) Luiking YC, Ten Have GA, Wolfe RR, Deutz NE. Arginine de novo and nitric oxide production in disease states. Am J Physiol Endocrinol Metab 2012; 303: E1177-E1189.
7) Wu Z, Hou Y, Hu S, Bazer FW, Meininger CJ, McNeal CJ, Wu G. Catabolism and safety of supplemental L-arginine in animals. Amino Acids 2016; 48: 1541-152.
8) DeMartino AW, Kim-Shapiro DB, Patel RP, Gladwin MT. Nitrite and nitrate chemical biology and signaling. Br J Pharmacol 2019; 176: 228-245.
9) Habermeyer M, Roth A, Guth S, Diel P, Engel KH, Epe B, Fürst P, Heinz V, Humpf HU, Joost HG, Knorr D, de Kok T, Kulling S, Lampen A, Marko D, Rechkemmer G, Rietjens I, Stadler RH, Vieths S, Vogel R, Steinberg P, Eisenbrand G. Nitrate and nitrite in the diet: how to assess their benefit and risk for human health. Mol Nutr Food Res 2015; 59:106-128.
10) Bahadoran Z, Ghasemi A, Mirmiran P, Azizi F, Hadaegh F. Beneficial effects of inorganic nitrate/nitrite in type 2 diabetes and its complications. Nutr Metab (Lond) 2015; 12: 16.
11) Karwowska M, Kononiuk A. Nitrates/nitrites in food-risk for nitrosative stress and benefits. Antioxidants (Basel) 2020; 9: 241.
12) Kalaycıoğlu Z, Erim FB. Nitrate and nitrites in foods: worldwide regional distribution in view of their risks and benefits. J Agric Food Chem 2019; 67: 7205-7222.
13) Bedale W, Sindelar JJ, Milkowski AL. Dietary nitrate and nitrite: benefits, risks, and evolving perceptions. Meat Sci 2016; 120: 85-92.
14) Sindelar JJ, Milkowski AL. Human safety controversies surrounding nitrate and nitrite in the diet. Nitric Oxide 2012; 26: 259-266.
15) Forman HJ, Zhang H, Rinna A. Glutathione: overview of its protective roles, measurement, and biosynthesis. Mol Aspects Med 2009; 30: 1-12.
16) Lu SC. Glutathione synthesis. Biochim Biophys Acta 2013; 1830: 3143-3153.
17) Gray JI, Dugan LR Jr. Inhibition of N-nitrosamine formation in model food systems. J Food Sci1975; 40: 981-984.
18) Rundlöf T, Olsson E, Wiernik A, Back S, Aune M, Johansson L, Wahlberg I. Potential nitrite scavengers as inhibitors of the formation of N-nitrosamines in solution and tobacco matrix systems. J Agric Food Chem 2000; 48: 4381-4388.
19) Shenoy NR, Choughuley AS. Inhibitory effect of diet related sulphydryl compounds on the formation of carcinogenic nitrosamines. Cancer Lett 1992; 65: 227-232.
20) Grisham MB, Ware K, Gilleland HE Jr, Gilleland LB, Abell CL, Yamada T. Neutrophil-mediated nitrosamine formation: role of nitric oxide in rats. Gastroenterology 1992; 103: 1260-1266.
21) Kako Y, Toyoda Y, Hatanaka Y, Suwa Y, Nukaya H, Nagao M. Inhibition of mutagenesis by p-aminobenzoic acid as a nitrite scavenger. Mutat Res 1992; 282: 119-125.
22) Kuo WN, Kocis JM, Nibbs J. Nitrosation of cysteine and reduced glutathione by nitrite at physiological pH. Front Biosci 2003; 8: a62-a69.
23) Caulfield JL, Singh SP, Wishnok JS, Deen WM, Tannenbaum SR. Bicarbonate inhibits N-nitrosation in oxygenated nitric oxide solutions. J Biol Chem 1996; 271: 25859-25863.
24) Gheibi S, Bakhtiarzadeh F, Jeddi S, Farrokhfall K, Zardooz H, Ghasemi A. Nitrite increases glucose-stimulated insulin secretion and islet insulin content in obese type 2 diabetic male rats. Nitric Oxide 2017; 64: 39-51.
25) Ohtake K, Nakano G, Ehara N, Sonoda K, Ito J, Uchida H, Kobayashi J. Dietary nitrite supple-

mentation improves insulin resistance in type 2 diabetic KKA(y) mice. Nitric Oxide 2015; 44: 31-38.

26) Liu Y, Croft KD, Hodgson JM, Mori T, Ward NC. Mechanisms of the protective effects of nitrate and nitrite in cardiovascular and metabolic diseases. Nitric Oxide 2020; 96: 35-43.

27) Jiang H, Torregrossa AC, Potts A, Pierini D, Aranke M, Garg HK, Bryan NS. Dietary nitrite improves insulin signaling through GLUT4 translocation. Free Radic Biol Med 2014; 67: 51-57.

28) Jeddi S, Gheibi S, Carlström M, Kashfi K, Ghasemi A. Long-term co-administration of sodium nitrite and sodium hydrosulfide inhibits hepatic gluconeogenesis in male type 2 diabetic rats: Role of PI3K-Akt-eNOS pathway. Life Sci 2021; 265: 118770.

29) de La Pomélie D, Santé-Lhoutellier V, Gatellier P. Mechanisms and kinetics of tryptophan N-nitrosation in a gastro-intestinal model. Food Chem 2017; 218: 487-495.

30) Sander J, Burkle G. Induction of malignant tumors in rats by simultaneous feeding of nitrite and secondary amines. Z Krebsforsch 1969; 73: 54-66.

31) Yap LP, Sancheti H, Ybanez MD, Garcia J, Cadenas E, Han D. Determination of GSH, GSSG, and GSNO using HPLC with electrochemical detection. Methods Enzymol 2010; 473: 137-147.

32) Liu J, Wu S, Cheng Y, Liu Q, Su L, Yang Y, Zhang X, Wu M, Choi JI, Tong H. Sargassum fusiforme alginate relieves hyperglycemia and modulates intestinal microbiota and metabolites in type 2 diabetic mice. Nutrients 202; 13: 2887.

33) Singh SP, Wishnok JS, Keshive M, Deen WM, Tannenbaum SR. The chemistry of the S-nitrosoglutathione/glutathione system. Proc Natl Acad Sci USA 1996; 93: 14428-14433.

34) Broniowska KA, Diers AR, Hogg N. S-nitrosoglutathione. Biochim Biophys Acta 2013; 1830: 3173-3181.