DOI 10.5012/bkcs.2009.30.12.2879

Antihyperglycemic Effects of Methylenedisalicylic Acid

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Received October 17, 2009, Accepted November 2, 2009

Key Words: Methylenedisalicylic acid, Antihyperglycemic, Mouse, PTP1B, IKK-β

Diabetes and obesity have emerged as major health concerns in the modern society. The increasing prevalence of these disorders has been recognized as public threats and as such, there is an urgent need for safe and effective treatments.[1,2] Protein tyrosine phosphatase 1B (PTP1B) is a validated target enzyme for the development of small molecule inhibitors as treatment of both diabetes and obesity.[3] In an effort to develop PTP1B inhibitors, we recently synthesized diverse derivatives of methylenedisalicylic acid (MDSA).[4,5] Among those, **SA18** and **SA32**, proved to be potent PTP1B inhibitors (IC_{50} values: 20 ± 1 μM and 19 ± 1 μM, respectively) and suppressed diet-induced weight gain in a mouse model system.[4,5] After 8 wk on a high fat diet (HFD) to develop obesity, mice were fed HFD or HFD + **SA** compounds for a further 4 wk. During this period, weight gain was significantly suppressed in drug-fed mice groups ($p < 0.005$); the HFD-fed control group gained 5.5 g compared to 1.3 and 1.0 g in the HFD + **SA18** or HFD + **SA32** mice groups, respectively. No differences were observed in fasting glucose levels between the obese control and the test groups. Glucose tolerance was not examined.

Further studies revealed that **SA18** and **SA32** also inhibit inhibitor of κB kinase-β (IKK-β), a recently identified target for diabetes/obesity drug development.[6] Heterozygous deletion of IKK-β in mice resulted in suppression of diet-induced weight gain and improvement of glucose tolerance and fasting glucose levels.[7]

As the parent compound for the design of **SA18** and **SA32**, MDSA was previously examined for inhibitory activity on PTP1B and IKK-β and was not an effective inhibitor of either PTP1B (IC_{50} = 3600 ± 800 μM)[4,5] or IKK-β (no inhibition at 10 μM, unpublished result). Weak inhibitory activity toward these enzymes disqualified MDSA as a promising lead for additional study. However, literature search revealed MDSA use as an anionic counterpart of the peptide antibiotic bacitracin.[8] The MDSA salt of bacitracin is used as a veterinary food additive for poultry, swine, and cattle.[9] As an antibiotic effective against the organisms of the digestive tract, bacitracin helps to prevent disease and enhance growth in animals. As a counter ion of bacitracin, MDSA is fed to animals from weaning to slaughter. Over half a century ago, Radomski *et al.* examined the toxicity of bacitracin, MDSA, and a complex of the two.[10] No significant adverse effects or differences in growth performance associated with MDSA feeding were reported for the various animals.[9,10] Also reported was that MDSA decreased the average weight gain of rats nearly 10%, when supplied in the diet as a 0.5 or 1.0% mixture for a 3 month period.[10] Another study indicated that MDSA is well-absorbed orally and not metabolized before secretion.[11]

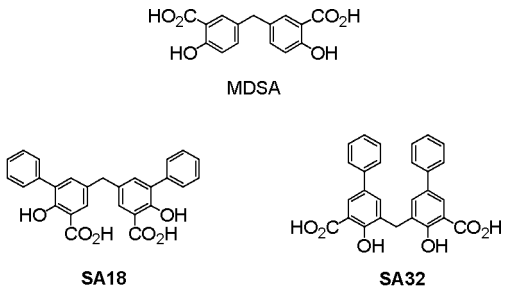

Figure 1. Compounds used or referred in this study.

Intrigued by these study results, we examined the anti-hyperglycemic and anti-obesity effects of MDSA in a diet-induced obese/diabetic mouse model system.[12] C57BL/6J mouse strain is genetically disposed to develop obesity and hyperglycemia if raised on a HFD.[13] After 8 wk on a HFD to induce obesity and hyperglycemia, the test group of mice was fed HFD + MDSA[14] for a further 4 wk. Obese/diabetic and lean control groups of mice were maintained on the HFD and LFD, respectively, throughout the whole 12 wk study period. The MDSA was administered as a mixture with the food (5.0 g MDSA per kg of diet). The daily uptake of MDSA was approximated 13 mg/day/mouse, which was equivalent to 370 mg/day/kg of mouse weight. Body weight was measured every 3 d throughout the study. In contrast to the results obtained by Radomski *et al.* with rats, no significant difference was observed between the HFD and HFD + MDSA groups (data not shown).

At the end of the 4 wks of drug-feeding period, the fasting glucose levels of the drug-fed group were significantly lower than the HFD control group (Fig. 2, 0 min). For the glucose tolerance test, mice were fasted for 6 h and glucose (1.0 g/kg of body weight) was injected intraperitoneally. Blood glucose levels were measured from tail bleeds at various time points after the glucose injection. The MDSA-fed group exhibited a significantly faster decrease in the blood glucose concentration compared to the HFD control group (Fig. 2).

After a 5 d recovery period on their own diet (the test group on HFD + MDSA), mice were fasted overnight and whole blood collected by cardiac puncture. The blood samples were analyzed for triglyceride, total cholesterol, and free fatty acids; no significant difference in these lipid parameters was observed between the HFD and HFD + MDSA groups (data not shown).

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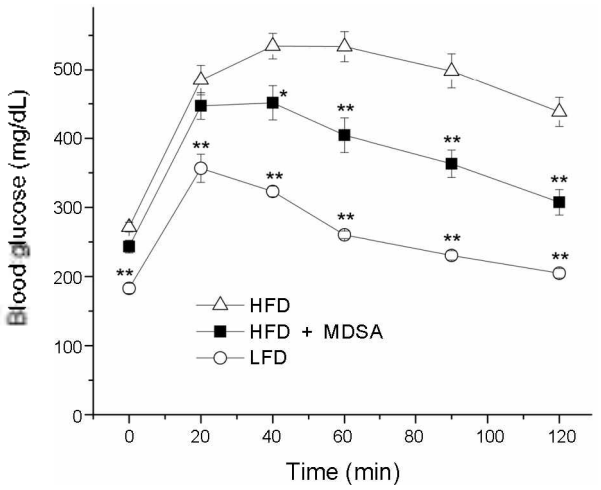

Figure 2. Intraperitoneal glucose tolerance test. Fourteen mice (C57BL/6J Jms Slc, male, 5-wk old) were fed HFD for 8 wk, and then divided into two groups (7 mice/group). The two groups were fed HFD (△) or HFD + MDSA (■) for 4 wk. The lean control group (○) was fed LFD all throughout the entire 12-wk period. The mice were then fasted for 6 h. At 0 time point, blood was withdrawn from the tip of the tail for the measurement of a baseline glucose level (fasting glucose level). After the intraperitoneal injection of glucose (1.0 g/kg body weight), the blood glucose level was measured at 20, 40, 60, 90, and 120 min time points. All values are mean values ± SEM; n = 7/group. Statistical significance was calculated between HFD and other mice groups using a 1-way ANOVA, where, *$p < 0.05$ and **$p < 0.005$.

In conclusion, MDSA improved glucose tolerance in mice without decreasing the fasting glucose level. Conversely, MDSA did not exhibit an antiobesity effect; this is inconsistent with the results by Radomski *et al.*[10] It is interesting to note the contradictory *in vivo* effects of MDSA and **SA18/32**, both sharing a common scaffold. The improvement of glucose tolerance by MDSA is apparently not due to an inhibition of PTP1B or IKK-β. An adequate explanation for the mechanism of these pharmacological effects requires further study.

Acknowledgments. This study was supported by Inha University. B. R. Bhattarai was a recipient of a BK21 fellowship.

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12. Mouse experiment: Twenty one mice (C57BL/6J Jms Slc, 4-wk old, male, 17 ~ 19 g, Japan SLC, Inc., Haruno Breeding branch, Japan) were individually housed and maintained in a 12 h light/dark cycle at 22 ± 2 °C. Food and water were available *ad libitum*. A high fat diet (HFD, D12451, New Brunswick, NJ, USA) and low fat diet (LFD, D10012G, New Brunswick) containing 45% and 16% of the calories from fat, respectively, were either in pellet or powder form. All mice were acclimatized for 1 wk (LFD), with 14 mice fed a HFD for the first 8 wk of the study for the development of obesity and diabetes; the remaining 7 were fed a LFD. The mice assigned to the LFD group were maintained on this diet throughout the study as a lean control group. At week 8, all the HFD-fed mice were divided into two groups. Each group was then given a HFD or HFD plus MDSA for 4 wk. The concentration of MDSA in the diets was 5.0 g/kg of diet (0.5% w/w). The LFD was provided in pellet form throughout the experiment. Conversely, the obese/diabetic control and MDSA-treated mice groups were fed with a HFD powdered food mixed with 10% H_2O to make a dough. For the treatment with MDSA, 2.5 g of MDSA was dissolved in H_2O (50 mL containing 2.0 equivalents of NaOH solution), mixed in powdered food (500 g), and kneaded to form a dough.

    Body weight and food intake were recorded every 3 d throughout the study. For the glucose tolerance test, mice were fasted for 6 h and glucose (1.0 g/kg of body weight) injected intraperitoneally. Blood glucose levels were measured from tail bleeds with a glucometer (Accu check active, Roche diagnostics, Ireland) at 0 (prior to glucose administration), 20, 40, 60, 90, and 120 min after glucose injection.

    After a 5 d recovery period on their own diet (the test group on HFD plus MDSA), mice were fasted overnight and blood collected by cardiac puncture under secobarbital anesthesia. Liver, lung, kidney, and white adipose tissue were excised and weighed. Plasma was analyzed for glucose, triglyceride, total cholesterol and free fatty acids using diagnostic kits (Glucose C2, TG E, T-Cho E and NEFA C from Wako Pure Chemical Industries, Ltd. Osaka, Japan).
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