Antihyperglycemic Effects of Methylenebisalicylic Acid

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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.2 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 (IC50 values; 20 ± 1 µM and 19 ± 1 µM, respectively) and suppressed diet-induced weight gain in a mouse model system.4,6 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 αβ kinase-β (IKK-β), a recently identified target for diabetes/obesity drug development.3 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IKK-β and was not an effective inhibitor of either PTP1B (IC50 = 3600 ± 800 µM)4,5 or IKK-β (no inhibition at 10 µM, unpublished result).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

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 + MDSA14 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). 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).

Figure 1. Compounds used or referred in this study.
The results by Radomski et al. did not exhibit an antiobesity effect; this is inconsistent with the findings of Bassett et al. without decreasing the fasting glucose level. Conversely, MDSA was found to improve glucose tolerance in vivo in a study using a 1-way ANOVA, where *p < 0.05 and **p < 0.005. The improvement of glucose tolerance by MDSA is apparently not due to an inhibition of PTP1B or ring a common scaffold. The improvement of glucose tolerance pharmacological effects requires further study.

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. 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.

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References and Notes

12. Mouse experiment: Twenty one mice (C57BL/6J Mls 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 as 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% H2O to make a dough. For the treatment with MDSA, 2.5 g of MDSA was dissolved in H2O (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 seccobarbital 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).