Effects of Long-Term Administration of Hesperidin and Glucosyl Hesperidin to Spontaneously Hypertensive Rats

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Summary Hesperidin (HES) is a flavonoid contained in citrus fruit peel. We investigated the effects of long-term administration of HES and its newly developed water soluble analogue, glucosyl hesperidin (GHES), to spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto rats (WKY). Animals were fed with diets containing HES or GHES (30mg/d/kg body weight) for 25 wk. While the daily food intake and the body weight of administered rats were not different from those of the non-administered control rats in both SHR and WKY through the experimental period, the blood pressure and heart rate of SHR administered HES or GHES for longer than 15 wk decreased as compared to the control group. The blood pressure and heart rate of WKY were not changed by the long-term administration of HES or GHES. These results suggest that HES and GHES have anti-hypertensive effects on hypertensive animals.

Key Words hesperidin, blood pressure, hypertension, long-term administration

Hesperidin (HES), or hesperetin 7-rhamnoglucoside, is a flavanone with a molecular weight of 610.6 daltons and present in the fruit, leaves and bark of orange and other citrus species. Flavanone and other related chemical subspecies (catechines, chalcones, etc.) constitute the chemical series of flavonoids. Flavonoids are known as an antioxidant (1, 2) and have a variety of other biological effects in mammalian cell systems including the inhibition of enzyme activities (3, 4). Therefore, flavonoids seem to be applicable to a wide range of therapies (5).

HES was once called “vitamin P” and is known for its effects on the vascular system, inhibition of increment of blood vessel permeability and enhancement of capillary resistance (6). HES is also reported to have a quick-acting antihypertensive effect (7, 8). However, its mechanism is still unclear and little attention has been given to the effect of long-term administration. Additionally, because of very low water solubility, utilization has been restricted. The aim of the present study is to investigate the effects of long-term administration of HES and glucosyl hesperidin (GHES), which is made water soluble by the addition of one glucose molecule to the HES molecule (9), on spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto rats (WKY).

Materials and Methods

Animals and diets. Eighteen male SHR and WKY rats aged 3 wk were purchased from Charles River Japan (Kanagawa, Japan) and housed in an air-conditioned room (25°C) with a 12 h light/dark cycle (light: 8:00–20:00). They were given free access to a stock diet (MF, Oriental Yeast, Chiba, Japan) and water. Two weeks later, the body weight, daily food intake and blood pressure of each animal were measured. Then, they were divided into three groups, and each group was fed for 25 wk on one of the following diets: diet containing HES (30 mg/d/kg body weight), diet containing GHES (30 mg/d/kg body weight) and control diet containing neither HES nor GHES. HES was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and GHES was obtained from Toyo Sugar Refining Co., Ltd. (Tokyo, Japan).

This experimental design was approved by the Animal Experiment Committee of Okayama University and the rats were managed in line with the Guidelines for Care and Use of Laboratory Animals.

Measurement of blood pressure and heart rate. Systolic or mean blood pressure and heart rate of pre-warmed conscious rats were measured once a week using the indirect tail cuff method (BP-98A, Softron, Tokyo, Japan). The mean of at least three successive measurements was taken as the data for blood pressure and heart rate.

Statistics. Data was obtained as the mean±SE (n=6) and analyzed by Student’s t-test. The difference from the control was considered significant at p<0.05.

Results

Body weights of SHR and WKY increased with age and there was no significant difference between rats administered HES or GHES and control rats throughout the experimental period (Fig. 1). The addition of HES or GHES to diet did not cause a change in the daily food intake of SHR or WKY (data not shown).
Among the three groups, the heart rate of SHR was reduced slightly until 15 wk of age, and after 20 wk, was lower in both the HES and GHES groups as compared to the control group. The effect of HES was especially conspicuous (Fig. 2A). In contrast to this, the heart rate of WKY decreased constantly, which was common among the three groups, and a significant difference from the control group was scarcely observed.
throughout the experimental period (Fig. 2B).

The systolic blood pressure of SHR markedly increased until 10 wk of age. After that, significantly lower blood pressures were often observed for the HES and GHES groups as compared to the control group (Fig. 3A). In contrast to this, the blood pressure of WKY did not increase apart from a few exceptions at early ages (Fig. 3B). Noteworthy is the fact that the administration of HES or GHES exerted almost as much effect on the mean blood pressure as on the systolic blood pressure in both SHR and WKY (data not shown).

**Discussion**

In the present study, the antihypertensive effects from the long-term administration of HES and GHES were observed in the hypertensive rats but not in the normotensive rats. This experiment was first performed under the condition of long-term, low-dose administration of HES. It has been reported that short-term treatment with higher doses of HES exerts a hypertensive effect on SHR and Wistar (7, 8). The hypertensive effect of HES and GHES shown on WKY of early ages in this study may be consistent with the previous data. These short-term effects may be explained by stimulated diuresis (7). Various flavanones serve as an inhibitor of cyclic AMP phosphodiesterase responsible for diuresis (10). Another study has showed that methylhesperidin reduces blood pressure and that this effect arises from the inhibition of voltage-dependent Ca\(^{2+}\) channel and Ca\(^{2+}\) release generated by norepinephrine (11). Flavonoids influence the activity of various enzymes such as phospholipase A\(_2\) (3), lipoxygenase (4) and cyclooxygenase (12), which probably regulate platelet aggregation, erythrocyte adhesion and blood rheology. Recently many reports deal with the reactivity of flavonoids on active oxygen species. HES exerts an antioxidant effect (1, 2) that may play a role in antihypertension such as the preservation of endothelial nitric oxide and/or the prevention of low-density lipoprotein oxidation (13). The former is related to the short-term effect, while the latter may be available for preservation of the intact structure of the vessel wall (14), which needs long-term administration.

The long-term administration of HES and GHES exerted an antihypertensive effect only on SHR. Therefore, investigation as to whether or not the appropriate dose of HES or GHES customarily has a good effect on humans suffering from or liable to hypertension may be invaluable. HES and GHES also reduced the heart rate of SHR but not that of WKY in this study. Galati et al. (7) described that a modification of cardiac rhythm is not observed in the case of short-term HES treatment. In general, however, blood pressure is closely related with heart rate. Further investigation is needed to clarify the mechanism by which HES and GHES decrease the blood pressure and heart rate of SHR.

In view of the findings reported here and elsewhere, it seems likely that the long-term administration of HES or GHES brings about antihypertension in hypertensive rats and that the improvement of water solubility will contribute to the practical expansion of flavonoid utilization.

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