Inhibitory effects of alpha-cyclodextrin and its derivative against sucrose-induced hyperglycemia in an in vivo evaluation system

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Summary
Cyclodextrins (CyDs) are cyclic oligosaccharides consisting of six to eight glucose residues. Administration of α-CyD (six glucose residues) inhibits sucrose-induced hyperglycemia in humans. Here we show that oral administration of α-CyD and dimethyl α-CyD suppresses sucrose-induced hyperglycemia in an in vivo evaluation system using silkworms. On the other hand, β-CyD (seven glucose residues), γ-CyD (eight glucose residues), and their derivatives did not show the suppressive effect. These findings suggest that dimethyl α-CyD is a new inhibitor against sucrose-induced hyperglycemia and the silkworm system is useful for evaluation of suppressive activities of α-CyD derivatives against postprandial hyperglycemia.

Keywords: Cyclodextrin, hyperglycemic activity, silkworm, sucrose-induced hyperglycemia

1. Introduction

Elevated blood glucose levels due to excessive intake of sucrose, a major sweetener that is added to a variety of foods, lead to the development and worsening of lifestyle-related diseases, such as obesity and diabetes (1). Establishment of strategies for suppressing increases in blood glucose levels caused by excess sucrose intake is expected to prevent lifestyle-related diseases (2). Blood glucose levels after intake of sucrose are regulated by various different kinds of steps (3). Therefore, evaluation of active substances that suppress increases in blood glucose levels caused by sucrose intake requires experiments using whole animals.

Silkworm is an invertebrate animal and has several advantages as an experimental animal compared to mammals, including lower breeding costs and fewer ethical problems with regard to animal welfare (4-8). We have established in vivo experimental systems using silkworm for evaluating therapeutic activities of anti-diabetic drugs (8-10). A hyperglycemic silkworm model is useful for evaluating therapeutic activity of human insulin (8,9,11). We also developed a diabetic silkworm model for evaluation of anti-diabetic agents such as pioglitazone and metformin (8,10). Hemolymph glucose levels of silkworms are increased by intake of sucrose, the increase is suppressed by administration of α-glucosidase inhibitors that are used for treatment of diabetes in clinical (12). This means that in vivo evaluation systems using silkworms are useful to search substances for blood glucose control of humans.

Cyclodextrins (CyDs) are cyclic oligosaccharides consisting of six to eight glucose residues linked with α-1,4-glycosidic bonds. α-cyclodextrin (α-CyD) is composed of six glucose residues, β-cyclodextrin (β-CyD) of seven glucose residues, and γ-cyclodextrin (γ-CyD) of eight glucose units. CyD derivatives are clinically used for improving drug solubility and drug delivery (13). Moreover, α-, β-, and γ-CyDs have a capacity to inhibit α-amylase or glucoamylase, which degrade starch to maltose (14). Suppressive effect of α-CyD against increase in blood glucose level caused by intake of white rice has been demonstrated in human clinical trials (15). Administration of α-CyD also inhibits sucrose-induced hyperglycemia in humans (16). However, there is no report regarding comparative study of CyDs and its derivatives on the elevated blood glucose level after ingestion of sucrose.

In this study, we compared the suppressive effects of CyDs and its derivatives against sucrose-induced hyperglycemia in silkworms...
hyperglycemia in silkworms. α-CyD and dimethyl α-CyD have an activity to suppress sucrose-induced hyperglycemia in silkworms. Our findings suggest that silkworm system is useful to search for α-CyD derivatives that inhibit postprandial hyperglycemia by intake of sucrose.

2. Materials and Methods

2.1. Silkworm rearing conditions

Silkworms were reared according to the previously reported method (12). The eggs of the silkworm (Hu Yo × Tukuba Ne) were purchased from Ehime sericulture incorporated company (Ehime, Japan). Larvae hatched from the eggs were fed an artificial diet (Silkmate 2S, Nihon Nosan Corporation, Tokyo, Japan) and reared to the fifth-instar stage at 25-27 °C. A 10% (w/w)-sucrose or glucose diet was prepared by mixing Silkmate 2S and sucrose. A chemical-containing diet was prepared by mixing with 10%-sucrose diet.

2.2. Determination of glucose level in silkworm hemolymph

Glucose levels in the hemolymph were determined by the method described previously (12). Hemolymph was collected from the silkworms through a cut on the first proleg. Glucose levels in the hemolymph were determined using a glucometer (Accu-Check, Roche).

2.3. Chemicals

Cyclodextrins (CyDs) used in this study was listed in Table 1. HP-β-CyD with an average degree of substitution of hydroxypropyl group of 4.4 and HP-β-CyD with an average degree of substitution of hydroxypropyl group of 4.6 were kindly provided by CyDing Co., Ltd. (Kumamoto, Japan). Other CyDs used in this study were purchased from Wako Pure Chemical Industries (Osaka, Japan).

3. Results

3.1. Evaluation of inhibitory effects of α-, β-, and γ-CyDs against sucrose-induced hyperglycemia in silkworms

We first tested whether α-, β-, and γ-CyDs and their derivatives have capacities to suppress sucrose-induced hyperglycemia in silkworms. CyDs used in this study was listed in Table 1. We previously reported that acarbose showed suppressive effect against sucrose-induced hyperglycemia in silkworms (12). Therefore, acarbose was taken as a positive control of the experiment. The glucose levels in hemolymph of silkworms, which ingested diet containing 10% sucrose and α-CyD, was much lower than that of silkworms, which fed 10% sucrose diet (control) (Figure 1). On the other hand, the glucose levels in hemolymph of silkworms, which ingested diet containing 10% sucrose and β-CyD, γ-CyD, HP-β-CyD, HP-γ-CyD, M-β-CyD, or DM-β-CyD, were indistinguishable from the control (Figure 1). These results suggest that α-CyD has higher inhibitory capacity against sucrose-induced hyperglycemia than β-CyD, γ-CyD, and their derivatives.

3.2. Inhibitory activity of an α-CyD derivative against sucrose-induced hyperglycemia in silkworms

We next tested suppressive effects of DM-α-CyD,
an α-CyD derivative, against sucrose-induced hyperglycemia in silkworms. The glucose levels in hemolymph of silkworms feeding diet containing 10% sucrose and DM-α-CyD were much lower than that of silkworms feeding diet with a 10% sucrose (control) (Figure 2). The result suggest that DM-α-CyD also has inhibitory activity against sucrose-induced hyperglycemia in silkworms.

4. Discussion

In this study, we demonstrated that α-CyD suppressed sucrose-induced hyperglycemia using a silkworm system. β- and γ-CyDs did not show the suppressive effect in the system. Furthermore, we showed that dimethyl-α-CyD had an activity to suppress sucrose-induced hyperglycemia. CyDs are used for pharmacological applications, such as increase of solubility of drugs, therefore, their safety against humans are established (13). We propose here that identification of CyD derivatives by using the silkworm system will give fruitful results of finding new substances that suppress postprandial hyperglycemia in humans.

We also revealed that β- and γ-CyDs did not show suppressive capacity against sucrose-induced hyperglycemia in silkworms. The finding suggests that the structure of cyclic oligosaccharides composed six of glucose linked with α-1, 4-glycosidic bond are important for the suppressive functions. Moreover, dimethyl α-CyD, an α-CyD derivative, also showed the suppressive effect against sucrose-induced hyperglycemia in silkworms. This is the first report that dimethyl α-CyD has a capacity to suppress sucrose-induced hyperglycemia. To find a compound having higher capacities among derivatives of α-CyD is a subject in future.

Mammals such as mice and rats have been conventionally used for the evaluation of substances that exhibited suppressive action against increased blood glucose levels after ingestion of sucrose. Use of a large number of mammals for experiments is raising a problem of not only the cost but also animal welfare point of view. Silkworms have advantages as experimental animals being less expensive and having less ethical problems than mammals. Thus, we propose to use silkworms as a pre-step of using mammals for screening of effective substances for suppression of sucrose-induced hyperglycemia. Taken together, the in vivo evaluation system using silkworms may be useful to search α-CyD derivatives that have higher inhibitory activity against sucrose-induced hyperglycemia.

Acknowledgements

We thank Kana Hashimoto, Mari Maeda, and Miki Takahashi (Genome Pharmaceuticals Institute Co., Ltd, Tokyo, Japan) for their technical assistance in rearing the silkworms. This research was funded by JSPS KAKENHI grant number JP15H05783 (Scientific Research (S) to KS), and JSPS KAKENHI grant number JP17K08288 (Scientific Research (C) to YM). The project was also supported by Genome Pharmaceuticals Institute Co., Ltd (Tokyo, Japan) and CyDing Co., Ltd (Kumamoto, Japan).

References


(Received May 18, 2018; Accepted May 28, 2018)