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The α-glucosidase inhibitor miglitol suppresses postprandial hyperglycaemia and interleukin-1β and tumour necrosis factor-α gene expression in rat peripheral leucocytes induced by intermittent sucrose loading

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Postprandial hyperglycaemia is thought to increase inflammation in leucocytes. In the present study, we examined whether sucrose loading in rats with moderate postprandial hyperglycaemia induces the expression of cytokines in peripheral leucocytes and whether these inductions are suppressed by inhibiting postprandial hyperglycaemia with the α-glucosidase inhibitor miglitol. One group of streptozotocin-treated rats and age-matched saline-treated rats were orally administered sucrose only, and another group of streptozotocin-treated rats was administered sucrose with miglitol, at a single daily dose for 4 d, under 4 h fasting conditions. Blood glucose levels at 0, 0·25, 0·5, 1, 2 and 3 h and cytokine mRNA in peripheral leucocytes at 0 and 3 h after sucrose loading on days 1 and 4 from the start of sucrose loading were determined. Streptozotocin-treated rats showed moderate postprandial hyperglycaemia (>2000 mg/l) at 0·25–1 h after sucrose loading on days 1 and 4. Postprandial hyperglycaemia was not observed in the miglitol-treated rats loaded with sucrose. Gene expression levels of IL-1β and TNF-α were higher in the streptozotocin-treated rats at fasting on day 1 than in saline-treated rats. Fasting IL-1β and TNF-α gene expression on day 1 were not only increased at 3 h on the same day of sucrose loading, but was also increased at the fasting period on day 4. These inductions on day 4 by intermittent sucrose administration were inhibited by miglitol. The present results suggest that miglitol decreases postprandial hyperglycaemia and intermittent sucrose-induced expression of the IL-1β and TNF-α genes in rat peripheral leucocytes.

Postprandial hyperglycaemia: Miglitol: Interleukin-1β: Tumour necrosis factor-α: Leucocytes

Recent studies have shown that hyperglycaemia directly induces inflammation by enhancing inflammatory cytokines such as IL-1β, IL-6, IL-12, IL-18 and TNF-α, which are mainly expressed in leucocytes including macrophages, monocytes and granulocytes, as well as in many peripheral tissues[1–3]. These cytokines are known to activate macrophages/monocytes and induce the infiltration of macrophages into many tissues[1]. Additionally, several studies indicate that cytokines derived from hyperglycaemia directly induce inflammation without the infiltration of macrophages in many tissues; for example, several cytokines are known to induce apoptosis in many tissues by activating the caspase cascade[4]. Indeed, it has been established that apoptosis of islet β-cells is induced by cytokines such as IL-1β and interferon-γ resulting in an increased risk of developing diabetes by reducing the insulin secretory capacity[5,6]. Also, these cytokines induce macrophage infiltration into the vascular endothelium and increases the risk of developing atherosclerosis[7,8]. Additionally, one of the major causes of insulin resistance is thought to be the production of cytokines, particularly IL-1β, IL-6, IL-12, IL-18 and TNF-α[1]. It has already been reported that hyperglycaemia in individuals with diabetes is associated with the elevated plasma protein levels of circulating inflammatory cytokines such as IL-1β, IL-6, IL-12, IL-18 and TNF-α[9–11]. However, these observations were found after long-term or continuous exposure to hyperglycaemia in animal or cell models and found in fasting levels in human subjects. In human subjects, postprandial hyperglycaemia, which is hyperglycaemia that occurs as a result of glucose uptake into the blood after a meal, normally occurs three times per d, because human subjects typically have three meals per d (i.e. breakfast, lunch and dinner). Recently, epidemiological studies have shown that postprandial hyperglycaemia is associated with increased mortality and the risk of developing CVD in individuals with diabetes[12]. These observations indicate that the accumulation of postprandial hyperglycaemia may enhance inflammation in various tissues, as well as increase the risk of developing diabetes and related complications. Indeed, several epidemiological studies found that the protein expression of some circulating cytokines such as IL-6 and TNF-α is increased in individuals with the metabolic syndrome during the postprandial period[13–15]. These findings indicate that postprandial hyperglycaemia caused by insulin resistance or insulin secretory dysfunction may increase the risk of inflammation by inducing the expression of cytokine genes. However, no studies have shown direct evidence that the gene expression is enhanced by intermittent postprandial hyperglycaemia and that the

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postprandial induction of the genes is decreased by inhibition of postprandial hyperglycaemia.

In the present study, we examined whether postprandial hyperglycaemia after once-daily sucrose loading for 4 d enhances the expression of inflammatory cytokine genes in peripheral leucocytes. Furthermore, we examined whether the induction of these cytokine genes was decreased by inhibiting postprandial hyperglycaemia using the α-glucosidase inhibitor miglitol, which is a strong inhibitor of disaccharides in the brush-border membranes of the small intestine and has a greater effect on reducing glucose fluctuations than other α-glucosidase inhibitors[16,17].

Materials and methods

Animals

Age-matched, male, 7-week-old Sprague–Dawley rats were divided into two groups with similar fasting glucose levels and body weight in both groups. The day after allocation, rats in the first group (*n* 10) received a single injection of streptozotocin (25 mg/kg body weight; Sigma, Tokyo, Japan) dissolved in 0·9 % (w/v) sodium chloride solution into the tail vein to induce a mild glucose-intolerant state. The second group of age-matched control rats was injected with the vehicle alone (saline) (*n* 5). Rats in both groups were given a laboratory chow diet after the streptozotocin or saline treatment. At 5 d after treatment, the streptozotocin-treated rats were divided into two groups of five rats each, with similar fasting glucose level and body weight.

All of the streptozotocin- and saline-treated rats were fasted for 4 h from 09.00 to 13.00 hours. Half of the streptozotocin-treated rats (*n* 5) and saline-treated rats (*n* 5) underwent sucrose loading (2 g/kg body weight) (*n* 5) at a single daily dose for 4 d at 13.30–14.00 hours from day 7 to day 10 after streptozotocin or saline treatment. The other streptozotocin-treated rats (*n* 5) underwent sucrose loading with miglitol (10 mg/kg body weight; Sanwa Kagaku Kenkyusho Co., Ltd, Nagoya, Japan), at a single daily dose for 4 d at 13.30–14.00 hours from day 7 to day 10 after streptozotocin or saline treatment (Fig. 1).

Blood was collected from the tail vein of all rats at 0, 0·25, 0·5, 1, 2 and 3 h after sucrose loading on days 1 and 4. Rats were individually housed in cages and allowed free access to food and tap water, except for times of sucrose loading (13.00–14.00 hours) on each day and while collecting blood (14.00–17.30 hours) on days 1 and 4. The rats were maintained at a stable temperature (22 ± 2°C), humidity (55 ± 5 %) and a 12 h light–dark cycle (light: 07.00–19.00 hours). The experimental procedures used in the present study conformed to the guidelines of the Animal Usage Committee of the University of Shizuoka.

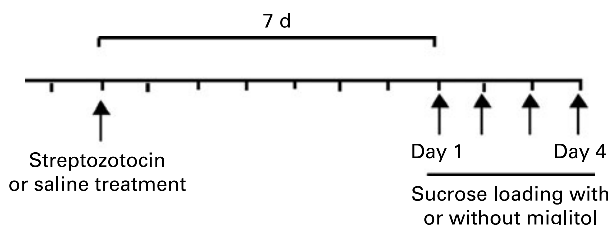

Fig. 1. Experimental design. Rats were treated with low-dose streptozotocin (25 mg/kg body weight) or saline. Once-daily sucrose loading with or without miglitol to the streptozotocin- or saline-treated rats was performed after 4 h fasting for 4 d from 7 to 10 d after streptozotocin or saline treatment.

Parameters

Plasma glucose levels were measured by commercial kits (Wako Pure Chemical Industries, Osaka, Japan).

Real-time reverse transcriptase-polymerase chain reaction analysis

Total RNA was extracted from blood using the PAXgene kit purchased from PreAnalytix (Qiagen/BD Company, Tokyo, Japan). Total RNA samples (50 ng) were converted to cDNA by reverse transcription, using SuperScript™ III RT (Invitrogen, Tokyo, Japan) according to the manufacturer's instructions. To quantitatively estimate the mRNA levels of inflammatory cytokine genes, PCR amplification using universal TaqMan probes (Roche Molecular Biochemicals, Tokyo, Japan) was performed on a Light-Cycler instrument (Roche Molecular Biochemicals) according to the manufacturer's instructions. Sequences of primer sets for amplifying each gene and universal TaqMan probes for detecting amplified signals suggested by the software in the company website (http://www.roche-applied-science.com/sis/rtpcr/upl/index.jsp?id=UP030000) were used. Primer sets used were as follows: IL-1β, 5′-TGTGATGAAAGACGGCACAC-3′, 5′-CTTCTTCTTTGGGTATTGTTTGG-3′, TaqMan probe no. 78; IL-18, 5′-CAACGAATCCCAGACCAGAC-3′, 5′-ACATCCTTCCATCCTTCACAG-3′, TaqMan probe no. 76; TNF-α, 5′-GTCTACTGAACTTCGGGGTGA-3′, 5′-ATGAGAGGGAGCCCATTTG-3′, TaqMan probe no. 119; hypoxanthine guanine phosphoribosyl transferase (HPRT), 5′-AGTCAACGGGGGACATAAAA-3′, 5′-CTTCAACAATCAAGACGTTCTTTC-3′, TaqMan probe no. 22. The cycle threshold (CT) values for each gene and HPRT detected by real-time RT-PCR were converted into signal intensities by the delta–delta method[18], which calculates the difference of one CT value as a two-fold difference between each signal for each gene and the signal for a gene for normalisation (HPRT). The formula used was ($2^{(\text{CT each gene} - \text{CT HPRT})}$).

Statistical analysis

Results are expressed as mean values with their standard errors. The significance of differences between groups was determined by Tukey's multiple-range test based on ANOVA, or paired Student's *t* test, as appropriate. A *P* value of 0·05 or less in the Tukey's multiple-range test or paired Student's *t* test was defined as statistically significant.

Results

Effects of miglitol on postprandial hyperglycaemia in streptozotocin-treated rats

To observe the effects of miglitol on plasma glucose levels after sucrose loading in moderate postprandial hyperglycaemic

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rats, the rats were treated with low-dose streptozotocin (25 mg/kg body weight), which were then assigned to two groups. Fasting glucose levels and body weight in the two streptozotocin-treated groups were confirmed not to be significantly different. On day 7 after streptozotocin or saline treatment, the rats underwent the first sucrose loading with or without miglitol. Once-daily sucrose loading to the streptozotocin-treated rats was performed after 4 h fasting for 4 d. Blood samples were collected on days 1 and 4 of sucrose loading, i.e. 7 d and 10 d, respectively, after streptozotocin or saline treatment. The experimental design is summarised in Fig. 1. The blood glucose levels during the fasting period before sucrose loading on day 1 were not significantly different between the saline-treated rats (1590 (SEM 111) mg/l), the streptozotocin-treated rats subsequently treated with sucrose only (1730 (SEM 144) mg/l) and streptozotocin-treated rats subsequently treated with sucrose and miglitol (1670 (SEM 120) mg/l). Similarly, on day 4, the blood glucose concentrations during the fasting period were not significantly different between the saline-treated rats (1350 (SEM 76) mg/l), streptozotocin-treated rats subsequently treated with sucrose only (1730 (SEM 84) mg/dl) and streptozotocin-treated rats subsequently treated with sucrose and miglitol (1540 (SEM 130) mg/l). However, of the streptozotocin-treated rats, the blood glucose level tended to be lower in the rats also treated with miglitol *v.* without. The blood glucose levels reached the highest concentration at 0·25 h on day 1 (2280 (SEM 117) mg/l) and at 0·5 h on day 4 (2400 (SEM 196) mg/l) after sucrose loading in streptozotocin-treated rats administered with sucrose only, after which they returned to the pretreatment level at 2 h (Fig. 2 (A) and (B)). The blood glucose concentration was significantly different between streptozotocin-treated rats administered with sucrose only and the saline-treated rats at 0.25 h on day 1 and at 0.5 and 1 h on day 4 after sucrose loading. Administration of 10 mg miglitol/kg in combination with sucrose loading of the streptozotocin-treated rats decreased blood glucose concentrations significantly at 0.25 h on day 1 and 0.5 h on day 4 after sucrose loading.

The effects of miglitol on the expression of inflammatory cytokine genes induced by postprandial hyperglycaemia in peripheral leucocytes obtained from streptozotocin-treated rats

To determine whether moderate postprandial hyperglycaemia induces the expression of inflammatory cytokine genes and whether miglitol treatment inhibits the expression of these genes in peripheral leucocytes of streptozotocin-treated rats, we performed real-time RT-PCR using peripheral total leucocyte RNA obtained at 0 and 3 h after sucrose loading with or without miglitol on days 1 and 4 in the streptozotocin- or saline-treated rats. Gene expression of IL-1β, IL-18 and TNF-α was higher in the streptozotocin-treated rats on day 1 compared with the saline-treated rats (Fig. 3). Moreover, IL-1β and TNF-α mRNA on day 1 was increased at 3 h after sucrose loading compared with 0 h ($P<0·05$). In the streptozotocin-treated rats, the expression of the IL-1β and TNF-α genes was also higher at 0 h on day 4 than at 0 h on day 1 ($P<0·05$).

The mRNA levels of IL-1β and TNF-α at 0 and 3 h on day 4 and IL-18 at 3 h on day 4 were higher in the streptozotocin-treated rats administered with sucrose only compared with those co-administered with sucrose and miglitol. The expression of other cytokines (IL-6 and IL-12) did not differ between the streptozotocin-treated and saline-treated groups (data not shown).

Discussion

Recently, epidemiological studies have shown that postprandial hyperglycaemia is associated with increased mortality and the risk of developing CVD in individuals with diabetes[12]. Considering that complications related to diabetes are associated with elevated plasma cytokine levels such as IL-1β, IL-6, IL-12, IL-18 and TNF-α, even though these cytokines levels are normally determined during fasting conditions, it seems likely that intermittent postprandial hyperglycaemia induces complications related to diabetes by enhancing cytokine expression. However, it is unclear whether the expression of cytokine genes is enhanced by intermittent postprandial hyperglycaemia, and whether the induced gene expression can be reduced by attenuating postprandial hyperglycaemia.

In the present study, we investigated whether single or intermittent postprandial hyperglycaemia enhances mRNA expression of inflammatory cytokines in peripheral leucocytes, and whether inhibition of postprandial hyperglycaemia by the α-glucosidase inhibitor miglitol suppresses the induction of these cytokine genes. As shown in Fig. 2, sucrose loading to the streptozotocin-treated rats induced moderate postprandial hyperglycaemia (>2000 mg/l) at 15–60 min on days 1 and 4.

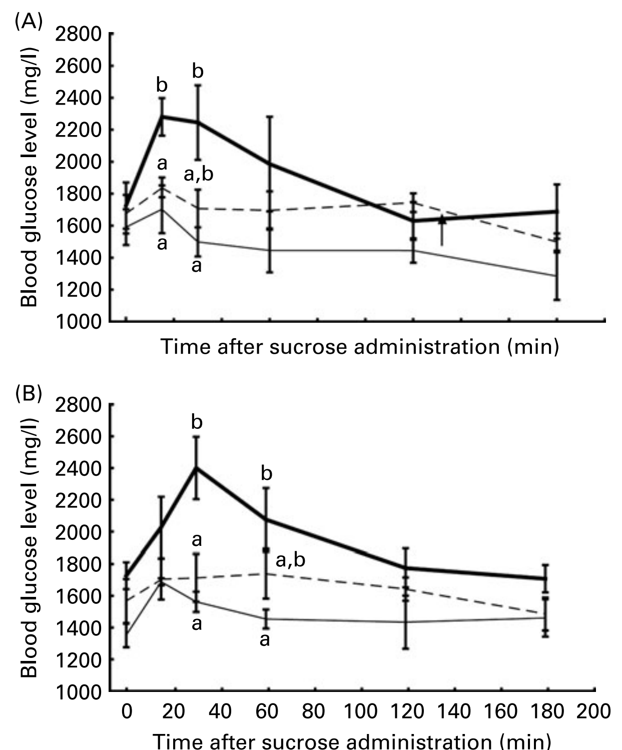

Fig. 2. The effects of miglitol on blood glucose levels after sucrose loading in streptozotocin- and saline-treated rats on days 1 (A) and 4 (B). (——), Saline + sucrose; (▬▬), streptozotocin + sucrose; (– – –), streptozotocin + sucrose + miglitol. Values are means for five rats per group, with standard errors represented by vertical bars. [a,b] Mean values with unlike letters were significantly different ($P<0·05$; Tukey's multiple-range test).

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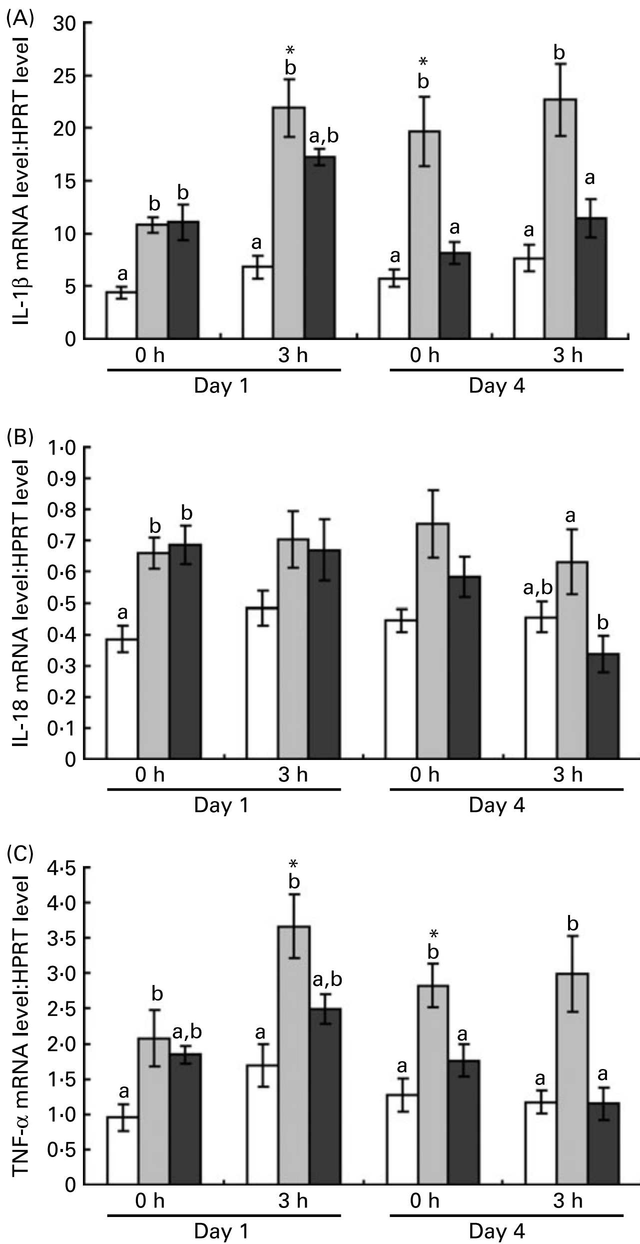

Fig. 3. Quantitative RT-PCR analysis of inflammatory cytokine genes in peripheral leucocytes at 0 and 3 h after sucrose loading in streptozotocin-treated rats treated with (■) or without (■) miglitol. (□), Saline + sucrose. The mRNA levels at 0 and 4 h on days 1 and 4 were analysed by real-time RT-PCR. The results of each sample were normalised for hypoxanthine guanine phosphoribosyl transferase (HPRT) abundance and are expressed as arbitrary units, representing the mean value of the untreated rats as 1. Values are means for five rats per group, with standard errors represented by vertical bars. [a,b] Mean values with unlike letters were significantly different ($P<0·05$; Tukey's multiple-range test). * Mean value was significantly different from that of the sucrose-loaded streptozotocin-treated rats at 0 h on day 1 ($P<0·05$; paired Student's t test).

Miglitol inhibited the postprandial glucose spike in the low-dose streptozotocin-treated rats. The expression of IL-1β, IL-18 and TNF-α genes was increased in the low-dose streptozotocin-treated rats on day 1 but not in the saline-treated rats. Interestingly, the expression of IL-1β and TNF-α genes in streptozotocin-treated rats was higher at 3 h after sucrose loading on day 1 than at 0 h. This result indicates that a single occurrence of postprandial hyperglycaemia enhances the expression of IL-1β and TNF-α in peripheral leucocytes. Furthermore, the expression of IL-1β and TNF-α genes was higher at 0 h on day 4 in streptozotocin-treated rats than at 0 h on day 1. These results indicate that the accumulation of postprandial hyperglycaemia over 4 d by sucrose loading causes further enhancement of IL-1β and TNF-α gene expression in peripheral leucocytes during the fasting period (Fig. 3 (A)). The most important symptom observed in patients with diabetes compared with healthy subjects is that the blood glucose levels at 2–3 h after a glucose load (for example, a mean) have not returned to basal levels (i.e. the fasting state). Thus, it is speculated that human subjects with elevated glucose levels at 2–3 h after a meal may have an increased risk for developing diabetes and associated complications. The present results support this notion because the expression of the IL-1β and TNF-α genes in rat peripheral leucocytes was higher at 3 h than at 0 h after sucrose loading. The response of peripheral leucocytes during the earlier sucrose-loading period with or without miglitol should be investigated, particularly in terms of cytokine gene expression. This is because Kempf *et al.* demonstrated that the oral glucose load and induction of immune mediators such as IL-6 and TNF-α occurs within 30 min of a glucose load in individuals with the metabolic syndrome or type 2 diabetes[15]. Furthermore, miglitol treatment in conjunction with sucrose loading significantly suppressed the induction of IL-1β and TNF-α gene expression by intermittent postprandial hyperglycaemia. It should be noted that the plasma IL-1β level did not change at any time point in any of the groups (data not shown). These results indicate that the induction of IL-1β in peripheral leucocytes is not associated with the plasma concentration. It has been reported that hyperglycaemia in individuals with diabetes is associated with the plasma levels of circulating inflammatory cytokines such as TNF-α, IL-1β, IL-6, IL-12 and IL-18[9–11]. Also, it has been shown that TNF-α and IL-6 are highly expressed in adipose and liver tissues during hyperglycaemia[19,20]. The lack of change in the plasma IL-1β cytokine level in the streptozotocin-treated rats may be because the hyperglycaemia induced in the present study is relatively moderate. Nevertheless, the functions of the increased expression of cytokines in peripheral leucocytes should be investigated.

Recently, many studies have shown that α-glucosidase inhibitors including miglitol prevent the development of insulin resistance[21,22] or insulin secretory dysfunction[16,23], as well as complications such as CVD[24–26]. Indeed, the epidemiological study STOP-NIDDM revealed that inhibition of postprandial hyperglycaemia by α-glucosidase inhibitors in individuals with type 2 diabetes helps prevent the progression of diabetes and complications such as hypertension and CVD[25,27–29]. Although the association between cytokine gene expression in peripheral leucocytes and the development of such complications remains unclear, the suppression of postprandial IL-1β and TNF-α gene induction in peripheral leucocytes by an α-glucosidase inhibitor may reduce the risk of developing such diseases.

In conclusion, the results of the present study suggest that the α-glucosidase inhibitor miglitol suppresses induction of IL-1β and TNF-α gene expression by single or intermittent sucrose loading in rat peripheral leucocytes.

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The authors declare no conflicts of interest.

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