JPP Journal of Pharmacy and Pharmacology

JPP 2008, 60: 1161–1165

Received February 7, 2008
Accepted April 21, 2008
DOI 10.1211/jpp.60.9.0007
ISSN 0022-3573

# Protective effect of caffeine on streptozotocin-induced beta-cell damage in rats

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## Abstract

Many epidemiological studies have shown that coffee consumption reduces the risk of type 2 diabetes mellitus (T2D), although the reasons as to why remain unclear. In this study we investigated the effect of caffeine on pancreatic beta-cell damage in rats using the diabetogenic agent, streptozotocin (STZ). Wistar rats were given intraperitoneal injections of saline or caffeine (10, 50 or 100 mg kg$^{-1}$). After 15 min, the rats were injected with a citrate buffer or 65 mg kg$^{-1}$ STZ. Three days after injection, an oral glucose tolerance test (OGTT) was performed on the rats. Furthermore, three days after the OGTT, the pancreas was isolated and homogenized, followed by determination of insulin content. STZ treatment significantly increased the plasma glucose level compared with the control at all times during the OGTT, which was significantly diminished by caffeine pretreatment at all doses. STZ treatment significantly decreased the plasma insulin level, however, which was not recovered by caffeine pretreatment. Pancreatic insulin content was significantly reduced by STZ treatment compared with the control, which was significantly recovered by caffeine pretreatment at a dose of 100 mg kg$^{-1}$ ($P < 0.01$). We showed that caffeine protects pancreatic beta-cells against STZ toxicity. Further investigation will be required to understand the protective effect of caffeine against beta-cell destruction in T2D.

## Introduction

Recently, many epidemiological studies have shown that coffee consumption reduces the risk of type 2 diabetes mellitus (T2D) (van Dam & Hu 2005), although the reasons remain unclear. Although chlorogenic acid has been suggested as a potential candidate in the mediation of this risk reduction (van Dam 2006), this compound decomposes during roasting, thus the resulting content in roasted coffee is very low (Oka 2007). Caffeine, one of the methylxanthines, is naturally found in beverages such as coffee (Minamisawa et al 2004). Unlike chlorogenic acid, caffeine is stable during roasting, and both lightly and heavily roasted coffee contains very high amounts of caffeine (Minamisawa et al 2004). It is unclear whether caffeine is an effective ingredient in the risk reduction of T2D. However, considering the fact that consumption of green tea containing high amounts of caffeine has also been found to reduce the risk of T2D (Iso et al 2006), caffeine may play a role in preventing T2D.

For many years, caffeine has been studied for its biochemical and physiological effects, including those related to T2D (Spriet et al 1992; Keijzers et al 2002; Park et al 2007). In recent years, it has been shown that caffeine has an effect on immunomodulatory actions (Horrigan et al 2006), which has been suggested to be mediated by the inhibition of cyclic adenosine monophosphate-phosphodiesterase (cAMP-PDE), followed by increasing intracellular cAMP concentrations (Horrigan et al 2006). This indicates that caffeine may have suppressive effects on tissue-degenerating diseases such as T2D.

Many studies have shown that pancreatic beta-cell death is associated with the development of T2D (Robertson et al 2004; Cnop et al 2005). Beta-cell dysfunction and death are induced by various factors such as glucotoxicity and lipotoxicity (Robertson et al 2004; Cnop et al 2005). Recent studies have suggested that pro-inflammatory cytokines, as well as a reactive oxygen species, are involved in glucotoxicity (Donath et al 2005). It has been shown that exposure of human islets to glucose induces beta-cell production of IL-1$\beta$, followed by NF-κB activation (Maedler et al 2002). Furthermore, recently, it has been

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suggested that secretory products of the adipocytes, such as TNF-α, IL-6 and leptin, may be mediators of the ongoing process of beta-cell destruction occurring in T2D (Donath et al 2003). Consequently, in this study, we investigated the effect of caffeine on beta-cell damage in-vivo, using streptozotocin (STZ), which has been found to induce the destruction of pancreatic beta-cells (Szkudelski 2001).

## Materials and Methods

### Animals and treatment

Seven-week-old male Wistar rats were purchased from Japan Laboratory Animals, Inc. (Tokyo, Japan). The rats were housed in a temperature-controlled (23 ± 1°C) room under a 12-h light–dark cycle (light: 0700–1900 h) for one week. The rats had free access to standard rat chow (CE-2; CLEA Japan Inc., Japan) and tap water while housed. The experiments using rats were conducted in accordance with the guidelines and with the approval of the Animal Research Ethics Committee at Tokyo University of Pharmacy and Life Sciences.

The rats, 200–230 g, were assigned to one of five groups of treatment: citrate buffer + saline (Control group); STZ + saline (STZ group); STZ + caffeine (10, 50 or 100 mg kg$^{-1}$) (STZ + Caffeine groups), after being fasted for 18 h. The rats were first intraperitoneally injected with saline or each dosage of caffeine (Wako Pure Chemical Industries Co., Japan) dissolved in saline. Next, after 15 min, the rats were intraperitoneally injected with 0.01 M sodium citrate buffer (pH 4.5) or 65 mg kg$^{-1}$ STZ (Sigma-Aldrich, Japan) dissolved in a citrate buffer. These compounds were filtered through a disposable 0.45-μm sterile filter unit (IWAKI glass, Japan) immediately before injection.

### Oral glucose tolerance test

Three days after injection, an oral glucose tolerance test (OGTT) was performed on the fasting rats. The rats were given an oral administration of glucose (2 g kg$^{-1}$) as a 30% solution, followed by blood collection from a tail vein before, and at 30 and 60 min after administration using a 50-μL heparinized micropipette (Vitrex Medical, Denmark). Blood samples were centrifuged at 3000 rev min$^{-1}$ for 10 min at 4°C, and the plasma samples were stoored at −80°C. Glucose and insulin concentrations were analysed within one week.

### Extraction of pancreatic insulin

Three days after the OGTT, the rats were decapitated. The pancreases were immediately isolated, weighed and homogenized in a cold mixture of 0.7 M HCl–ethanol (1:3 v/v), followed by storage for 24 h at 4°C. The homogenates were centrifuged at 2030 rev min$^{-1}$ for 15 min at 4°C, and supernatants were stored at −80°C until analysis of insulin concentrations within one week.

### Biochemical assay

Plasma glucose concentrations were determined using an enzymatic test kit (Glucose CII-test; Wako Pure Chemical Industries Co.). Insulin concentrations in plasma and the pancreas were determined by commercially available enzyme-linked immunosorbant assays using an ELISA kit (Mercodia, Switzerland). Pancreatic extracts were diluted with 0.1% acid–ethanol before assay. To determine the UV absorption spectrum, a microplate reader (LS-PLATE manager 2001 Win. Ver.2.10; Wako Pure Chemical Industries Co.) was used.

### Statistical analysis

The results are presented as means ± s.e.m. Data comparisons were carried out using one-way analysis of variance and Bonferroni–Dunn test, and $P < 0.05$ was considered as significant.

## Results

### Characteristics of rats

Table 1 shows the main metabolic features of the rats three days after the administration of STZ and various dosages of caffeine. There were no significant differences in body weight baseline (before administration of these compounds, data is not shown). Three days after administration, body weight was significantly reduced by STZ treatment compared with the control, although caffeine pretreatment did not recover STZ-induced reduction of body weight at all doses.

Fasting plasma glucose concentration was significantly increased by STZ treatment compared with the control. Furthermore, caffeine pretreatment, at doses of 50 and 100 mg kg$^{-1}$, significantly protected STZ-induced elevations of plasma glucose levels, which reached the same level of the control ($P < 0.01$, respectively).

Fasting plasma insulin concentration was significantly decreased by STZ treatment compared with the control.

**Table 1** Body weight and fasting plasma glucose and insulin concentrations in rats three days after treatment with citrate buffer + saline (Control group), STZ + saline (STZ group), or STZ + caffeine (10, 50 or 100 mg kg$^{-1}$) (STZ + caffeine groups)

| Group | Body weight (g) | Plasma glucose (mg dL$^{-1}$) | Plasma insulin (ng mL$^{-1}$) |
|---|---|---|---|
| Control | 224 ± 3 | 87 ± 4 | 0.26 ± 0.01 |
| STZ | 209 ± 3** | 206 ± 36** | 0.19 ± 0.028 |
| STZ + caffeine (10 mg kg$^{-1}$) | 204 ± 3*** | 159 ± 61* | 0.15 ± 0.02*** |
| STZ + caffeine (50 mg kg$^{-1}$) | 205 ± 4*** | 105 ± 6## | 0.20 ± 0.02* |
| STZ + caffeine (100 mg kg$^{-1}$) | 207 ± 2*** | 94 ± 3## | 0.17 ± 0.02** |

*$P < 0.05$,**$P < 0.01$ and ***$P < 0.001$, vs control; ##$P < 0.01$ vs STZ group. Bars show means ± s.e.m.

However, in contrast to plasma glucose levels, pretreatment with caffeine, at any dose, did not show protection against decreasing plasma insulin level induced by STZ.

### OGTT

Plasma glucose and insulin concentrations during the OGTT are shown in Figures 1 and 2. STZ treatment significantly increased the plasma glucose level compared with the control at all times during the OGTT; this was significantly diminished by caffeine pretreatment at 30 min at doses of 50 and 100 mg kg$^{-1}$ ($P<0.01$ and $P<0.001$, respectively), and at 60 min at doses of 10, 50 and 100 mg kg$^{-1}$ ($P<0.05$, $P<0.001$ and $P<0.001$, respectively).

STZ treatment significantly decreased plasma insulin levels at all times during the OGTT; however, these levels were not recovered by caffeine pretreatment, unlike glucose.

### Pancreatic insulin content

Figure 3 shows pancreatic insulin contents in the rats three days after the OGTT. Pancreatic insulin content was significantly reduced by STZ treatment compared with the control; this was significantly recovered by caffeine pretreatment at a dose of 100 mg kg$^{-1}$ to almost the same level as in the control ($P<0.01$). Although recovery was also observed at 10 and 50 mg kg$^{-1}$ caffeine, this did not attain statistical significance.

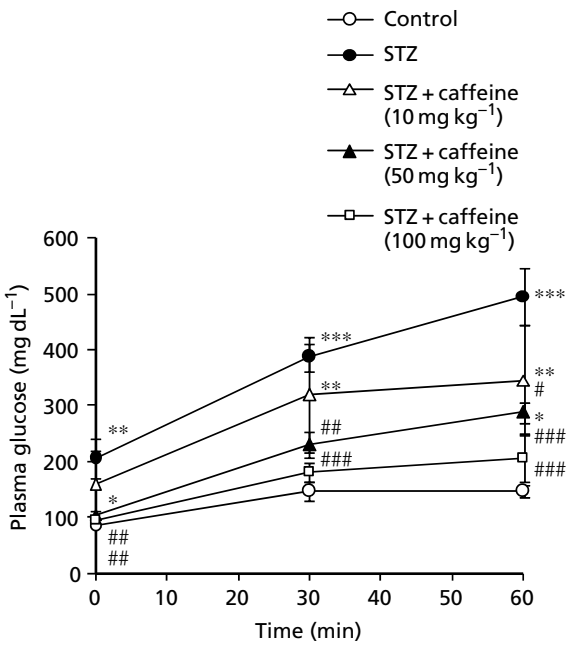


**Figure 1** Plasma glucose concentrations during OGTT. Rats were orally administered 2 g kg$^{-1}$ glucose three days after treatment with citrate buffer + saline (Control group), STZ + saline (STZ group), or STZ + caffeine (10, 50 or 100 mg kg$^{-1}$) (STZ + caffeine groups). Statistical significance from the control is shown by $*P<0.05$,$**P<0.01$ and $***P<0.001$, vs control; $\#\#P<0.01$, $\#\#\#P<0.001$ vs STZ group. Bars show means ± s.e.m.

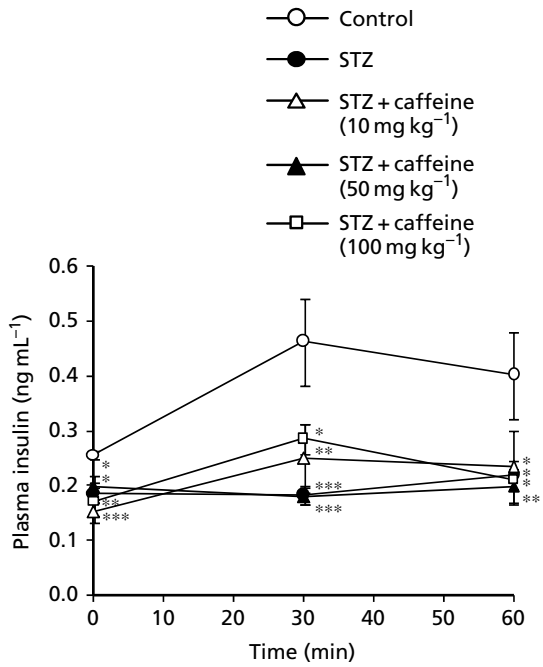


**Figure 2** Plasma insulin concentrations during OGTT. Rats were orally administered 2 g kg$^{-1}$ glucose three days after treatment with citrate buffer + saline (Control group), STZ + saline (STZ group), or STZ + caffeine (10, 50 or 100 mg kg$^{-1}$) (STZ + caffeine groups). Statistical significance from the control is shown by $*P<0.05$,$**P<0.01$ and $***P<0.001$, vs control. Bars show means ± s.e.m.

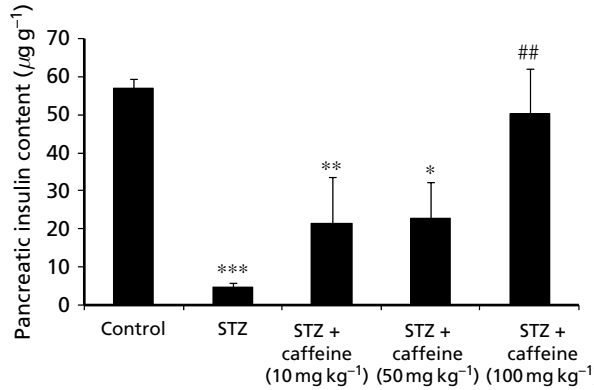


**Figure 3** Pancreatic insulin contents. Rats were decapitated six days after treatment with citrate buffer + saline (Control group), STZ + saline (STZ group), or STZ + caffeine (10, 50 or 100 mg kg$^{-1}$) (STZ + caffeine groups). Then, the pancreas was immediately isolated and homogenized. $*P<0.05$,$**P<0.01$ and $***P<0.001$, vs control; $\#\#P<0.01$, vs STZ group. Bars show means ± s.e.m.

## Discussion

Recent meta-analysis of many epidemiological studies has shown that coffee consumption reduces the risk of T2D, although there have yet to be any studies identifying these effective compounds (van Dam & Hu 2005; van Dam 2006). In this study, we focused on the anti-inflammatory effect of

caffeine. We investigated the effect of this compound on beta-cell damage induced by STZ in-vivo.

First, we examined body weight and fasting plasma glucose and insulin concentrations three days after injection of caffeine and STZ to rats (Table 1). Masiello et al (1998) have reported that pre-administration of nicotinamide can suppress beta-cell damage induced by STZ using rats. In their study, nicotinamide protected against STZ-induced decreases in body weight. However, in our study, caffeine did not show the protective effect against STZ with regard to body weight. Although the reason remains unknown from this study, it has been shown that long-term consumption of caffeine decreases body weight (Greenberg et al 2006). Accordingly, there is a hypothesis that the weight-lowering effect of caffeine might lead to the ineffectiveness of caffeine pretreatment on the recovery of body weight, although caffeine was administered only once in our study. Caffeine recovered STZ-induced elevation of fasting plasma glucose concentrations three days after administration of these compounds. In addition, caffeine administration also recovered pancreatic insulin content. Taking these observations into consideration, it can be postulated that caffeine protected beta-cells against STZ toxicity.

Next, for further investigation of the protective effect of caffeine, we examined blood glucose and insulin concentrations during OGTT three days after injection of caffeine and STZ. In Masiello's study (Masiello et al 1998), they gave nicotinamide to rats 15 min before STZ administration. Although the pharmacokinetics of caffeine differ from those of nicotinamide, we set the dosing intervals between caffeine and STZ at 15 min based on their methodology. In general, an OGTT is carried out for at least 120 min. However, it has been shown that there was a most significant difference in plasma glucose levels between non-treated and STZ-treated rats at 30 min by OGTT (Lo et al 2004). Based on these observations, we performed the test for 60 min in our study. In this study, at all doses administered, caffeine significantly suppressed the STZ-induced elevation of plasma glucose levels (Figure 1). This suppressive effect was dose-dependent both at 30 and 60 min after glucose load. Although caffeine pretreatment recovered STZ-induced reductions in plasma insulin concentrations at 30 min at doses of 10 and 100 mg kg$^{-1}$ (25.0 and 35.7%, respectively), this did not attain statistical significance (Figure 2). In OGTT, it has been shown that elevated plasma insulin concentration, induced by glucose load, reaches a maximum concentration at around 10 min (Lo et al 2004). However, in our study, we examined plasma insulin levels only at 30 and 60 min after glucose load, which may be one reason for the differences not being significant. For investigation of beta-cell damage, we consider that pancreatic insulin levels are more important than plasma insulin levels. In this study, there was a significant difference between the STZ-treatment group and the STZ + caffeine (100 mg kg$^{-1}$)-treatment group with regard to pancreatic insulin levels. Therefore, we did not conduct a further experiment for investigation of plasma insulin levels.

Finally, to confirm the suppressive effect of caffeine on beta-cell damage, we isolated the pancreas from rats three days after OGTT, followed by determination of insulin content. As shown in Figure 3, pretreatment with caffeine almost fully recovered the STZ-induced reduction in pancreatic insulin content at the dose of 100 mg kg$^{-1}$, which was also weakly recovered at 10 and 50 mg kg$^{-1}$. In Masiello's study (Masiello et al 1998), the protective effect on insulin content against STZ was not observed with 100 mg kg$^{-1}$ of nicotinamide. When 350 mg kg$^{-1}$ of nicotinamide was administered, absolute protection was observed, which indicates that the protective effect of caffeine against STZ toxicity was much stronger than that of nicotinamide.

In this study, to investigate the effect of caffeine on STZ-induced beta-cell damage, we used caffeine at doses of 10–100 mg kg$^{-1}$. With regard to plasma glucose concentrations, a low dose of caffeine, 10 mg kg$^{-1}$, showed a significant protective effect against STZ toxicity. Considering that the amount of caffeine in roasted coffee is around 100 mg per cup of coffee (Oka 2007), a dose of 10 mg kg$^{-1}$ is equivalent to six cups of coffee. This dose of caffeine can be considered to be an amount that we can consume in daily life. However, the metabolic body size of rats and man are not similar and, therefore, the dose for rats cannot be similar to the dose for man. Furthermore, 10 mg kg$^{-1}$ of caffeine would be above the typical volume of consumption for many people. In addition, much higher doses were required for a full reversal of plasma glucose levels in this study. Therefore, further investigations at low doses of caffeine are necessary.

Since early times, many researchers have investigated caffeine with regard to its pharmacological actions. In recent years, studies of caffeine have been focused on anti-inflammatory effects (Horrigan et al 2006). As discussed above, caffeine protected beta-cells from STZ-induced damage in-vivo. However the reason remains unknown. STZ has been long used as a diabetogenic agent due to its beta-cell toxicity (Szkudelski 2001). Although this toxic mechanism has yet to be clearly elucidated, it has been suggested that STZ increases reactive oxygen species and subsequent DNA damage. This induces activation of the DNA repair enzyme poly (ADP-ribose) polymerase-1 (PARP-1), which uses nicotinamide adenine dinucleotide (NAD) as a substrate. This results in the depletion of intracellular NAD, followed by ATP depletion, which leads to pancreatic beta-cell death (Szkudelski 2001). A recent study showed that caffeine metabolites exhibit significant PARP-1 inhibiting activity, which was weak in the case of caffeine itself (Geraets et al 2006). According to these observations, caffeine metabolites may protect beta-cells against STZ toxicity by inhibiting PARP-1. In fact, nicotinamide, a PARP-1 inhibitor, has been shown to suppress STZ-induced beta-cell death (Masiello et al 1998).

In addition to the STZ mechanism described above, STZ has been shown to induce activation of NF-κB (Ho et al 2001). Furthermore, it has been shown that the NF-κB inhibitor suppresses STZ-induced beta-cell damage (Eldor et al 2006). Considering the fact that PARP-1 plays a role in NF-κB- and AP-1-mediated production of pro-inflammatory cytokines, such as TNF-$\alpha$, IL-6 and IFN-$\gamma$ (Hassa & Hottiger 2002; Geraets et al 2006), various pro-inflammatory cytokines may be involved in STZ toxic actions. Recently, caffeine has been reported to suppress the production of pro-inflammatory cytokines, such as TNF-$\alpha$, IFN-$\gamma$, IL-2, IL-4 and IL-5, although this compound has been also shown to suppress

production of IL-10, known as an anti-inflammatory cytokine (Horrigan et al 2006). It has been suggested that the immunomodulatory effects of caffeine are mediated via cAMP-PDE, followed by increasing intracellular cAMP concentrations (Horrigan et al 2006). Consequently, caffeine may play a role in the protection of beta-cells against STZ toxicity through the anti-inflammatory effects described above.

### Conclusion

Our data showed that caffeine protects pancreatic beta-cells against STZ toxicity. However, the protective mechanism remains unknown. There are some differences in the processes between beta-cell destruction induced by STZ and those occurring in T2D. Further investigation is required to study the protective effects of caffeine against beta-cell destruction in T2D.

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