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Research article

Dichloroacetate, a pyruvate dehydrogenase kinase inhibitor, ameliorates type 2 diabetes via reduced gluconeogenesis

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ABSTRACT

Aims: Pyruvate dehydrogenase (PDH) catalyzes the decarboxylation of pyruvate to acetyl-CoA, which plays a key role in linking cytosolic glycolysis to mitochondria metabolism. PDH is physiologically inactivated by pyruvate dehydrogenase kinases (PDKs). Thus, activation of PDH via inhibiting PDK may lead to metabolic benefits. In the present study, we investigated the antidiabetic effect of PDK inhibition using dichloroacetate (DCA), a PDK inhibitor.

Main methods: We evaluated the effect of single dose of DCA on plasma metabolic parameters in normal rats. Next, we investigated the antidiabetic effect of DCA in diabetic *ob/ob* mice. In addition, we performed *in vitro* assays to understand the effect and mechanism of action of DCA on gluconeogenesis in mouse myoblast cell line C2C12 and rat hepatoma cell line FaO.

Key findings: In normal rats, a single dose of DCA decreased the plasma level of pyruvate, the product of glycolysis, and the plasma glucose level only in the fasting state. Meanwhile, a single dose of DCA lowered the plasma glucose level, and a three-week treatment decreased the fructosamine level in diabetic *ob/ob* mice. *In vitro* experiments demonstrated concentration-dependent suppression of lactate production in C2C12 myotubes. In addition, DCA suppressed glucose production from pyruvate and lactate in FaO hepatoma cells. Thus, DCA-mediated restricted supply of gluconeogenic substrates from the muscle to liver, and direct suppression of hepatic gluconeogenesis might have contributed to its glucose-lowering effect in the current models.

Significance: PDK inhibitor may be considered as a potential antidiabetic agent harboring inhibitory effect on gluconeogenesis.

1. Introduction

Type 2 diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia. Impaired glucose homeostasis is one of the risk factors associated with type 2 diabetes. Pyruvate dehydrogenase (PDH) plays a pivotal role in glucose homeostasis, and catalyzes the physiologically irreversible conversion of pyruvate to acetyl-CoA [1, 2]. Pyruvate dehydrogenase kinases (PDKs) physiologically regulate the activities of the mammalian PDH [3, 4]. PDK-mediated phosphorylation of PDH leads to decrease in its enzymatic activity and thereby triggers cellular metabolic adaptation in response to nutrient status [3, 4]. In the fed state, PDH is activated to generate energy or to convert glucose into lipids. In the fasted state, PDH is inactivated, then fatty acids are favored as an energy source over glucose, resulting in conservation of gluconeogenic substrates that contribute to maintenance of blood glucose level via gluconeogenesis [5]. There are four isozymes of PDK known in humans, PDK1-4 [6]. The PDKs exhibit unique tissue expression patterns, kinetic properties, and sensitivities to regulatory molecules [7]. The expression levels of PDK2 and PDK4 are uniquely regulated by nutritional factors and hormones [8, 9], and up-regulated in the fasted or diabetic state in rodents and humans [10, 11, 12]. In addition, genetic inactivation of the PDK4 gene in the liver improved hyperglycemia, glucose tolerance, and insulin resistance in diabetic mice [13, 14]. Considering these results, activation of PDH by inhibiting PDK has been proposed as a novel therapy of interest for metabolic diseases including diabetes [15].

Dichloroacetate (DCA) is a non-selective PDK inhibitor, and has already been used clinically for more than 30 years for congenital lactic acidosis [16, 17]. Lately, there has been a rising interest in the anticancer properties of DCA due to the drug's ability to shift metabolism from glycolysis to oxidative phosphorylation [18]. Studies have reported

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anti-hyperglycemic effect of short-term treatment with DCA in animals and patients with diabetes [19, 20]. However, antidiabetic effect of long-term treatment with PDK inhibitors has not been well established. Therefore, in the present study, we examined the antidiabetic effect of DCA using rodent models. We compared the effect of DCA on plasma glucose level in the fed and the fasted state of normal rats. Moreover, we investigated the effect of 3-week treatment with DCA in diabetic *ob/ob* mice. Additionally, we studied the effects of DCA on gluconeogenesis using skeletal myotubes and hepatocytes. Unfortunately, clinical translation of DCA is unlikely because DCA causes a peripheral neuropathy [21]. In this study, we used DCA as a tool to evaluate the antidiabetic effect of PDK inhibition.

2. Materials and methods

2.1. Reagents

All reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA), unless otherwise indicated. Sodium dichloroacetate (DCA) was purchased from Alfa Aesar (Haverhill, MA, USA). It was dissolved in the assay buffer for *in vitro* studies and in 0.5% (w/v) methylcellulose (MC, FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) solution for *in vivo* studies.

2.2. Animals

Seven-week-old male Sprague-Dawley (SD) rats were purchased from CLEA Japan, Inc. (Tokyo, Japan). Seven-week-old Male Lep^{ob}/Lep^{ob} (*ob/ob*; B6.Cg- Lep^{ob}/J), a mouse model of type 2 diabetes, and their non-diabetic untyped littermates as controls (?/+; B6.Cg - Lep^{ob}/J, ?/+ means +/+ or Lep^{ob}/+) were purchased from Charles River Laboratories Japan, Inc. (Yokohama, Japan). All animals were housed in a room with controlled temperature (23 °C), humidity (55%), and lighting for 12 h (7:00 a.m. to 7:00 p.m.). All animals had free access to a standard laboratory chow diet (CE-2, CLEA Japan) and tap water. All animal experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and were approved by the Experimental Animal Care and Use Committee of Takeda Pharmaceutical Company Limited (Osaka, Japan).

2.3. Single dose study in SD rats

DCA (100 mg/kg) or vehicle was administered to eight-week-old male SD rats (n = 5) by gavage in the fed and overnight fasted state. The treatment dosage was determined based on a previous report [22]. After administration of DCA in the fed state, the rats were not allowed to consume food. Blood was collected from the tail vein at pre, 0.5, 1, 2, 4, and 8 h after dosing.

2.4. Single dose study in diabetic ob/ob mice

Sixteen-week-old male *ob/ob* mice were divided in to two groups (n = 5–6) based on their blood glucose levels. DCA (100 mg/kg/day) or vehicle was administered to the mice by gavage. The treatment dosage was determined based on a previous report [23]. Blood was collected from the tail vein at pre, 0.5, 1, 2, 4, and 7 h after dosing.

2.5. Three-week treatment in diabetic ob/ob mice

Nine-week-old male *ob/ob* mice were divided into two groups (n = 7–8) based on their glycosylated hemoglobin and plasma glucose levels, and body weight. Their lean non-diabetic littermates were used as controls (n = 5). DCA (100 mg/kg/day) or vehicle was administered once daily to the mice by gavage for 3 weeks. The day of first dosing was designated as day 0. Blood samples were collected on day 13 and day 20 to estimate blood metabolic parameters. Body weight and food intake were measured after every 3–4 days throughout the study period. Food intake was measured on a per-cage basis.

2.6. Measurement of blood metabolic parameters

Plasma levels of glucose, lactate, total ketone bodies (T-KB), and fructosamine were measured by standard enzymatic procedures using an automatic biochemistry analyzer (7180 Clinical Analyzer, Hitachi Ltd., Tokyo, Japan). Plasma pyruvate level was measured by the Determiner PA kit (Kyowa Medex Co. Ltd., Tokyo, Japan). Rat and mouse plasma insulin levels were determined by a Rat insulin radioimmunoassay kit (catalog No. SRI-13K, EMD Millipore, Burlington, MA, USA) and an Ultra Sensitive Mouse Insulin ELISA Kit (catalog No. M1104, Morinaga Institute of Biological Science, Inc., Yokohama, Japan), respectively. Rat plasma insulin levels below the detection limit (0.0329 ng/mL) were identified as 0.0329 ng/mL. Blood glucose concentration was measured by a blood glucose meter (Accu-Chek Active, Roche Diagnostics K.K., Tokyo, Japan). Glycosylated hemoglobin level was analyzed using an automated glycohemoglobin analyzer (HLC-723 GHb GV, Tosoh Corporation, Tokyo, Japan).

2.7. Cell culture

The mouse myoblast cell line C2C12 was purchased from the American Type Culture Collection (Manassas, VA, USA), and were grown in Dulbecco's modified Eagle growth medium (DMEM, catalog No. 043–30085, FUJIFILM Wako Pure Chemical Corporation) containing 4.5 g/L glucose, 10% (v/v) fetal bovine serum, penicillin (50 units/mL), and streptomycin (50 μg/mL) at 37 °C in a 95% humidified air/5% CO_2 atmosphere. Cells were seeded in collagen I-coated 96-well plates and incubated in the growth medium until confluent. Then the medium was replaced with the differentiation medium, DMEM containing 2% (v/v) horse serum, and incubated up to 8 days to promote fusion into myotubes. The medium was changed every 2–3 days. Myotubes were treated with various concentrations of DCA, 10 mmol/L metformin [24], or 100 nmol/L insulin in Krebs Ringer Henseleit buffer containing 0.2% (v/v) bovine serum albumin (BSA), and 5 mmol/L glucose for 4 h for lactate production. Lactate concentration in the supernatant was determined with the Determiner LA kit (Kyowa Medex). To measure mitochondrial activity, the cells were incubated in the assay buffer containing redox-sensitive reagent, resazurin (alamarBlue, catalog No. DAL1100, Thermo Fisher Scientific) for 2.5 h. The rat hepatoma cell line FaO was purchased from the European Collection of Authenticated Cell Cultures (Salisbury, UK), and were grown in RPMI 1640 (catalog No. 11875093, Thermo Fisher Scientific) containing 10% (v/v) FBS, penicillin (50 units/mL), and streptomycin (50 μg/mL) at 37 °C in a 95% humidified air/5% CO_2 atmosphere. Then, 5×10^4 cells/well were seeded in collagen I-coated 96-well plate and incubated for 24 h. The medium was replaced with serum-free RPMI 1640 medium, and then incubated for 18 h. Cells were treated with various concentrations of DCA or 100 nmol/L insulin in glucose-free DMEM (catalog No. D5030-1L, Sigma-Aldrich) containing 0.2% (v/v) BSA, 2.5 mmol/L sodium lactate, and 0.25 mmol/L sodium pyruvate for 4 h. Glucose concentration in the supernatant was determined with the Amplex Red Glucose/Glucose Oxidase Assay Kit (catalog No. A22189, Thermo Fisher Scientific). T-KB concentration in the supernatant was measured by standard enzymatic procedure using 7180 Clinical Analyzer.

2.8. Statistical analysis

Statistical significance was first determined using the Bartlett's test of homogeneity of variances, followed by the Williams' test ($p \geq 0.05$) or Shirley-Williams test ($p < 0.05$) for dose-dependent studies. Alternatively, statistical significance between two groups was analyzed using the F-test for homogeneity of variances, followed by the Student's *t*-test ($p \geq 0.2$) or Aspin-Welch test ($p < 0.2$). All tests were conducted using a two-

tailed significance level of 5% (0.05). All data are presented as mean ± standard deviation (S.D.).

3. Results

3.1. Single dose of DCA decreased plasma glucose level in the fasted state of normal rats

PDK inhibition promotes PDH activation, which catalyzes an irreversible decarboxylation of pyruvate to acetyl-CoA. To investigate the effect of PDK inhibition, DCA, a PDK inhibitor, was administered to normal rats by gavage, and plasma parameters were measured. In the fed state, single dose of DCA did not affect plasma glucose, pyruvate and lactate levels compared with the vehicle (Figure 1A, B, C). DCA increased plasma T-KB level, which is synthesized from acetyl-CoA, compared with the vehicle (Figure 1D). However, DCA did not affect plasma insulin level (Figure 1E). In contrast to the fed state, single dose of DCA decreased plasma glucose level in the fasted state (Figure 2A). As expected, DCA decreased plasma level of pyruvate, which is a substrate of PDH (Figure 2B). Similar to the fed state, DCA increased T-KB level in the fasted state (Figure 2D). Moreover, DCA administration did not result in change in fasted plasma insulin level (Figure 2E).

3.2. Antidiabetic effect of DCA in diabetic ob/ob mice

To investigate the antidiabetic effect of PDK inhibitor, we evaluated the effect of single dose of DCA on plasma glucose level in *ob/ob* mice, a type 2 diabetes mouse model. The average blood glucose levels of mice in the morning at the time of their allocation to separate groups were 251 ± 43 mg/dL for DCA group and 253 ± 125 mg/dL for vehicle group. DCA significantly decreased plasma glucose level in diabetic *ob/ob* mice under the fed state (Figure 3). Next, we examined the antidiabetic effect of 3-week treatment with DCA in *ob/ob* mice and observed significant reduction in plasma glucose level in DCA-treated mice compared with vehicle-treated *ob/ob* mice (Figure 4A). Plasma level of fructosamine, an indicator of long-term glycemic control [25], was also decreased after DCA treatment (Figure 4B), while plasma insulin level was not affected during the treatment (Figure 4C). Body weight and food intake were not changed after DCA administration during the study period (Figure 4D, E).

3.3. DCA suppressed lactate production in myotubes and decreased glucose production in hepatocytes

Based on the results of animal studies, we believed that the antidiabetic effect of PDK inhibitor might be due to suppression of hepatic gluconeogenesis. Therefore, we examined the effect of PDK inhibition in C2C12 myotubes and FaO hepatoma cells. C2C12 myotubes were incubated in a medium containing glucose for 4 h. We observed that DCA suppressed, whereas metformin and insulin increased the production of lactate, one of the main gluconeogenic substrates, in C2C12 myotubes compared with the control (Figure 5A). Concurrently, DCA increased, whereas metformin and insulin did not affect mitochondrial activity in C2C12 myotubes (Figure 5B). Suppression of lactate production and increase in mitochondrial activity are attributable to PDH activation. In another experiment, FaO hepatoma cells were incubated in glucose-free DMEM supplemented with gluconeogenic substrates such as lactate and pyruvate for 4 h. DCA suppressed glucose production and increased T-KB production, which is synthesized from acetyl-CoA (Figure 6A, B). However, insulin suppressed glucose production without leading increase in T-KB production (Figure 6A, B).

4. Discussion

PDH plays a pivotal role in glucose homeostasis via catalyzing the physiologically irreversible conversion of pyruvate to acetyl-CoA. PDK inactivates PDH through phosphorylation, thereby downregulating this pathway. In the present study, we evaluated antidiabetic effect of PDK inhibition using DCA, a PDK inhibitor, in rodents. Single dose of DCA decreased plasma glucose level in the diabetic as well as the fasted state. Moreover, three-week treatment of DCA reduced the plasma level of fructosamine, an indicator of long-term glycemic control, in diabetic *ob/ob* mice. Additionally, DCA suppressed the production of lactate, one of the major gluconeogenesis substrates, in C2C12 myotubes and suppressed glucose production in FaO hepatoma cells.

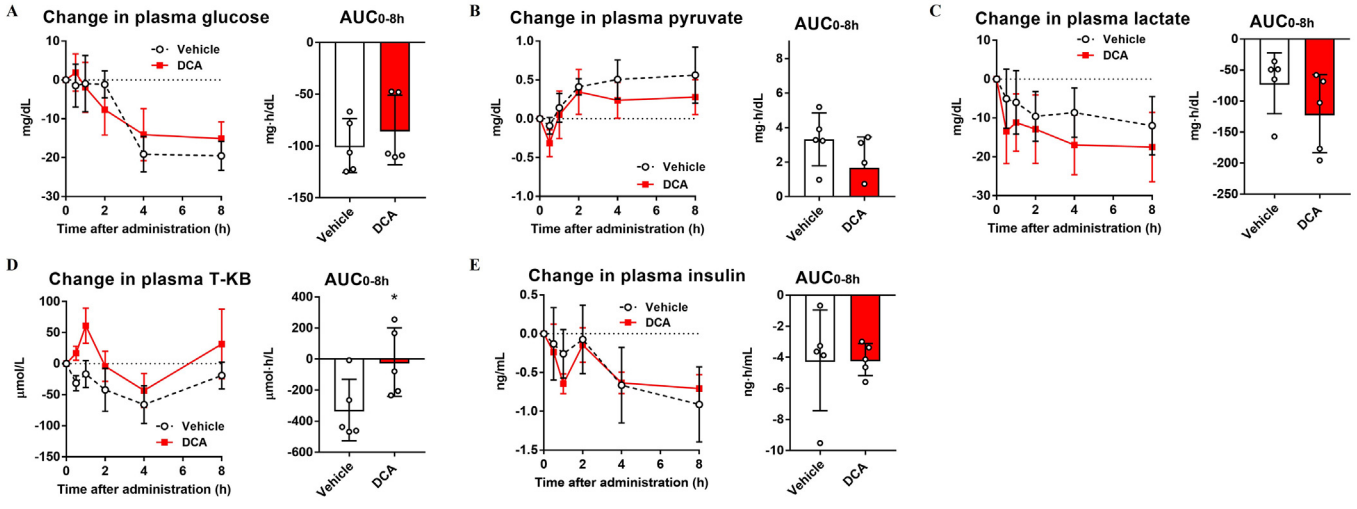

Figure 1. Effect of single dose of DCA on plasma metabolic parameters in the fed state of normal rats. Single dose of DCA (100 mg/kg) was administered to normal rats by gavage in the fed state. The values represent changes from baseline. (A) Glucose (Baseline values were 147 ± 5 mg/dL in DCA group and 149 ± 6 mg/dL in vehicle group), (B) pyruvate (0.577 ± 0.156 mg/dL in DCA group and 0.523 ± 0.138 mg/dL in vehicle group), (C) lactate (30.1 ± 7.6 mg/dL in DCA group and 28.1 ± 4.9 mg/dL in vehicle group), (D) T-KB (194 ± 15 μmol/L in DCA group and 199 ± 28 μmol/L in vehicle group), and (E) insulin (1.46 ± 0.19 ng/mL in DCA group and 1.68 ± 0.42 ng/mL in vehicle group) levels in plasma were measured for 8 h after administration. DCA, dichloroacetate; $AUC_{0-8\ h}$, area under the concentration time curve from 0 to 8 h; T-KB, total ketone bodies. The values are expressed as mean ± standard deviation (S.D.) (n = 5). *p < 0.05 vs vehicle-treated group by Student's *t*-test.

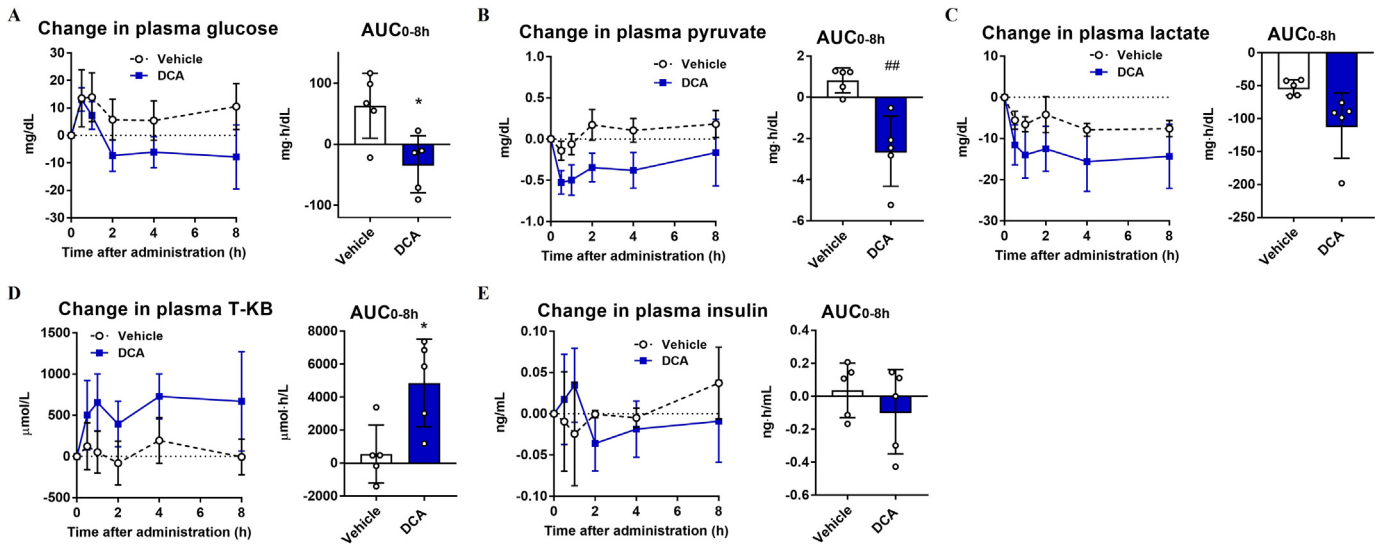

Figure 2. Effect of single dose of DCA on plasma metabolic parameters in the fasted state of normal rats. Single dose of DCA (100 mg/kg) was administered to normal rats by gavage in the fasted state. The values represent changes from baseline. (A) Glucose (Baseline values were 85 ± 7 mg/dL in DCA group and 82 ± 4 mg/dL in vehicle group), (B) pyruvate (0.775 ± 0.178 mg/dL in DCA group and 0.571 ± 0.130 mg/dL in vehicle group), (C) lactate (27.2 ± 6.1 mg/dL in DCA group and 23.1 ± 2.0 mg/dL in vehicle group), (D) T-KB (2200 ± 379 μmol/L in DCA group and 2197 ± 420 μmol/L in vehicle group), and (E) insulin (0.0688 ± 0.0334 ng/mL in DCA group and 0.0859 ± 0.0726 ng/mL in vehicle group) levels in plasma were measured for 8 h after administration. DCA, dichloroacetate; $AUC_{0-8\ h}$, area under the concentration time curve from 0 to 8 h; T-KB, total ketone bodies. The values are expressed as mean ± S.D. (n = 5). *p < 0.05, **p < 0.01 vs vehicle-treated group by Student's *t*-test.

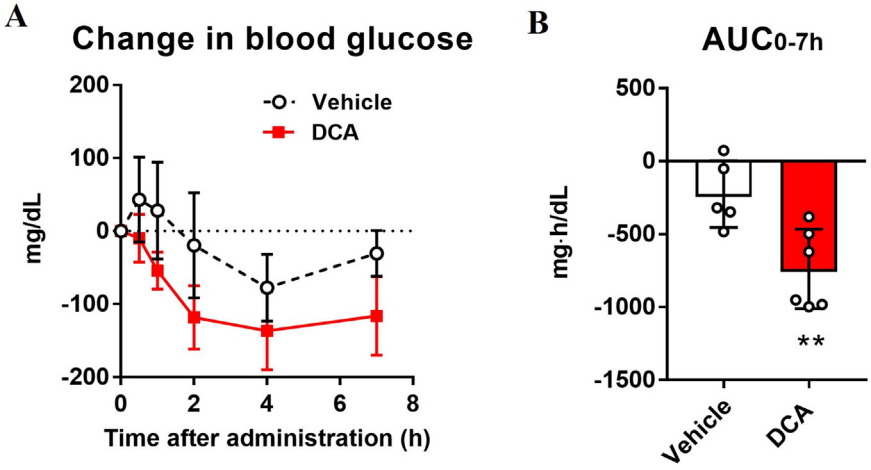

Figure 3. Effect of DCA on blood glucose level in the fed state of diabetic *ob/ob* mice. Single dose of DCA (100 mg/kg) was administered to the diabetic *ob/ob* mice by gavage in the fed state. The values represent changes from baseline. (A) Blood glucose level (Baseline values were 251 ± 43 mg/dL in DCA group and 253 ± 125 mg/dL in vehicle group) was monitored for 7 h after administration. (B) Area under the curve of blood glucose level was calculated. DCA, dichloroacetate; $AUC_{0-7\ h}$, area under the concentration time curve from 0 to 7 h. Values are expressed as mean ± S.D. (n = 5–6). **p < 0.01 vs vehicle-treated group by Student's *t*-test.

Three-week treatment of DCA lowered plasma glucose level and plasma fructosamine level in diabetic *ob/ob* mice. It has been reported that the expression levels of PDK2 and PDK4 were increased in the skeletal muscle and liver in diabetic rats [10]. Moreover, the expression level of PDK4 is increased in skeletal muscle in the patients with type 2 diabetes [11, 26]. Based on these findings, it can be suggested that a significant correlation exists between increased PDK expression and hyperglycemia, and inhibition of this enzyme by DCA might have contributed to a glucose-lowering effect in the current models. Moreover, DCA lowered plasma fructosamine level without increasing insulin level in *ob/ob* mice. Additionally, single dose of DCA did not increase plasma insulin level in normal rats. Hence, the antidiabetic effect of PDK inhibition is likely to be independent of insulin secretion. It has been reported that DCA lowered plasma glucose level in type 1 diabetic animal models, such as alloxan-diabetic dogs and STZ-diabetic rats [19, 27]. Thus, PDK inhibition may be a potential therapeutic strategy for not only type 2 diabetes but also insulin-deficient type 1 diabetes.

In normal rats, single dose of DCA decreased plasma level of pyruvate, the substrate of PDH, and simultaneously increased plasma T-KB level in the fasted state. These findings are suggestive of activation of PDH by DCA, thereby upregulating the pathway of conversion of pyruvate to acetyl-CoA in the current model. Furthermore, DCA lowered plasma glucose level only in the fasted state in normal rats. Induction of PDK2 and PDK4 have been reported in rat liver under starvation [12]. Our observation was consistent with a previous study that showed that PDK4 knockout mice showed lower plasma glucose level only in the fasted state but not in the fed state [28].

The results of *in vivo* studies indicate that antidiabetic effect of DCA might be attributable to suppression of hepatic gluconeogenesis. The liver and skeletal muscle are likely to play important roles in gluconeogenesis by PDK inhibition as the expression levels of PDKs in these tissues are regulated by diabetes or starvation [10, 12]. Therefore, we examined the effect of PDK inhibition in FaO hepatoma cells and C2C12 myotubes. DCA decreased glucose production from pyruvate and lactate in FaO hepatoma cells in a concentration-dependent manner, indicating direct role of DCA in the suppression of hepatic gluconeogenesis.

Pyruvate is the end-product of glycolysis, and its ultimate metabolic fate is determined by the balance between PDH and pyruvate carboxylase

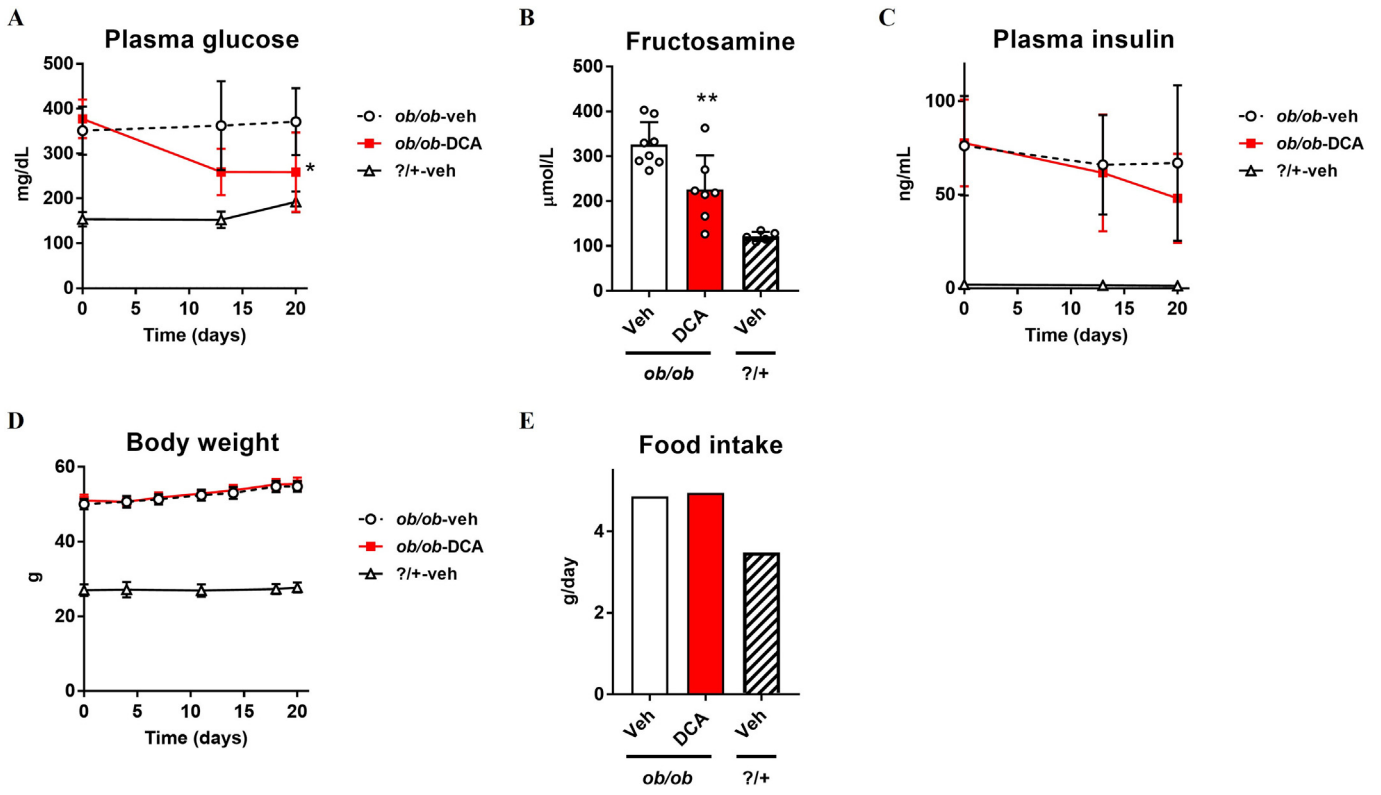

Figure 4. Antidiabetic effect of 3-week treatment with DCA in *ob/ob* mice. DCA (100 mg/kg/day) was administered for 3 weeks in diabetic *ob/ob* mice by gavage. (A) Glucose and (C) insulin levels in the plasma were measured before the study and on days 13 and 20. (B) Plasma fructosamine level was measured on day 20. (D) Body weight and (E) food intake were monitored during the 3-week treatment. Veh, vehicle; DCA, dichloroacetate; ?/+, lean non-diabetic littermates of ob/ob mice. The values are expressed as mean ± S.D. (n = 5–8). *p < 0.05, **p < 0.01 vs vehicle-treated *ob/ob* mice by Student's *t*-test.

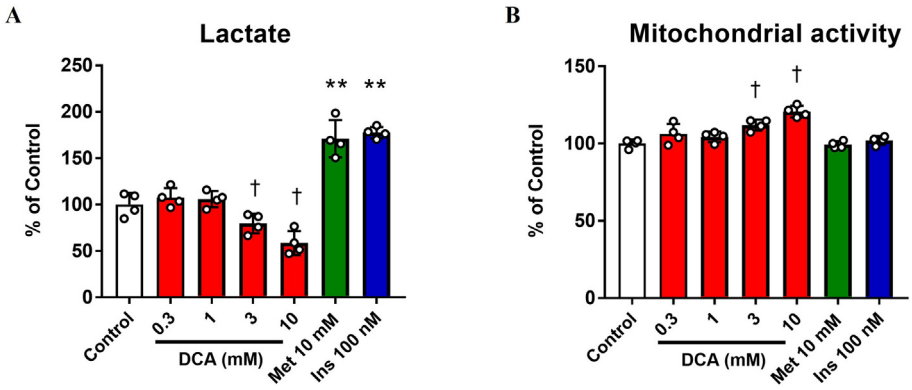

Figure 5. Effect of DCA on lactate production and mitochondrial activity in C2C12 myotubes. C2C12 myotubes were incubated with indicated concentrations of dichloroacetate (DCA), 10 mmol/L metformin (Met), or 100 nmol/L insulin (Ins) for 4 h. (A) Lactate concentration in the supernatant was measured at 4 h. (B) Mitochondrial activity was measured by resazurin at 2.5 h. The values are expressed as mean ± S.D. (n = 4). †p < 0.05 vs control by Williams' test, **p < 0.01 vs control by Student's *t*-test.

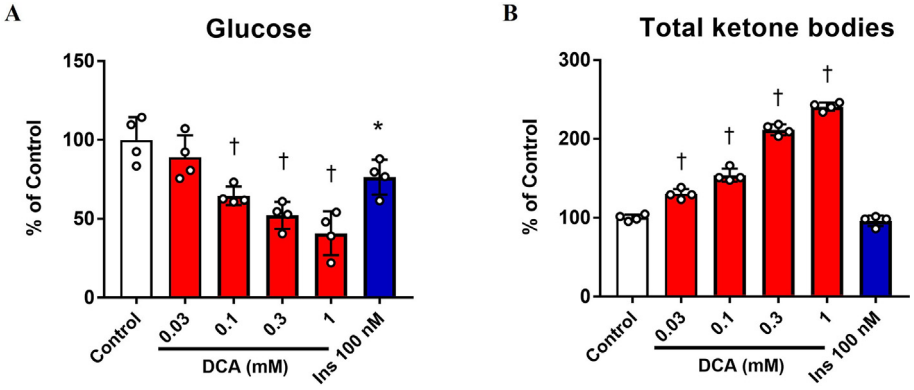

Figure 6. Effect of DCA on production of glucose and total ketone bodies in FaO hepatocytes. FaO hepatoma cells were incubated with indicated concentrations of DCA (dichloroacetate) or 100 nmol/L insulin (Ins) for 4 h. (B) Glucose and (C) total ketone bodies (T-KB) concentrations in the supernatant were measured. The values are expressed as mean ± S.D. (n = 4). †p < 0.05 vs control by Williams' test, *p < 0.05 vs control by Student's *t*-test.

(PC) activity in the liver [1]. Activation of PDH increases pyruvate flux to acetyl-CoA, which is further metabolized in the mitochondria. Stimulation of PC increases pyruvate flux to oxaloacetate, which is critical to augment gluconeogenesis. Thus, activation of PDH via PDK inhibition is thought to reduce pyruvate flux to oxaloacetate, thereby directly suppress hepatic gluconeogenesis. Moreover, DCA increased T-KB production in FaO hepatoma cells, which is synthesized from acetyl-CoA in the mitochondria, and in turn reflects the increased conversion of pyruvate to acetyl-CoA via PDH activation. In addition, single dose of DCA increased plasma T-KB level in normal rats. T-KB is produced as an alternative metabolic source of energy to glucose during starvation or prolonged physical effort. Low concentrations (<7 mmol/L) of circulating ketone bodies may exert beneficial effects on endothelium and the cardiovascular system [29]. It has been reported that a sodium-glucose co-transporter-2 inhibitor ameliorates adverse cardiac remodeling and heart failure in a nondiabetic porcine model by switching fuel utilization from glucose toward T-KB [30]. On the contrary, diabetic ketoacidosis, in which concentration of ketone bodies can reach 25 mmol/L [31], is a serious complication of diabetes mellitus, and further studies are warranted to reveal the safety profile of PDK inhibition.

Both DCA and insulin suppressed glucose production in hepatocytes. DCA suppressed lactate release from C2C12 myotubes in a concentration-dependent manner, which is required for hepatic gluconeogenesis, whereas insulin increased lactate production. Moreover, DCA increased T-KB production in hepatocytes while insulin did not. The findings imply that insulin and PDK inhibitor suppress glucose production via different mechanisms.

Although gluconeogenesis is one of the important contributing factors to hyperglycemia in diabetes, only biguanides are the drugs of choice. Metformin, a biguanide, is known to harbor a concern of lactic acidosis [32]. In the current study, we observed increased lactate production by metformin in myotubes, while DCA decreased it. Some studies reported that a combination of DCA and biguanides leads to suppression of plasma lactate level [33]. Considering that metformin is the preferred treatment option for diabetes, combining PDK inhibitors with metformin may have a potential to achieve better glycemic control and reduced risk of lactic acidosis.

The concentration of DCA observed in the present *in vitro* study is largely in agreement with the concentrations reported in previous studies [34, 35]. Additionally, DCA content in liver and muscle tissues 4 h after intragastrical administration of 100 mg/kg DCA was reported to be 87.9 and 62.2 μg/g tissue weight, which are 0.58 and 0.41 mmol/kg tissue weight, respectively [36]. The tissue concentration of DCA is similar to that observed in *in vitro* assays in this study. There are some limitations to the use of cell lines as a model to "mimic" *in vivo* tissues as metabolic demands in Fao hepatoma cells and C2C12 myotubes *in vitro* might differ from the liver and skeletal muscles *in vivo*.

5. Conclusion

These findings suggest that PDK inhibitors may exert antidiabetic effect by decreasing liver gluconeogenesis via direct suppression of hepatic gluconeogenesis and via reduction in its substrate supply from the skeletal muscle to liver. PDK inhibitors exhibit different characteristics from the conventional diabetic drugs, and therefore, may be considered as a novel attractive therapeutic agent for diabetes.

Declarations

Author contribution statement

Yuko Katayama: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Yayoi Kawata: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Yusuke Moritoh, Masanori Watanabe: Conceived and designed the experiments; Wrote the paper.

Funding statement

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare the following conflict of interests: Yuko Katayama, Yayoi Kawata, Yusuke Moritoh and Masanori Watanabe are/were employees of Takeda Pharmaceutical Company Limited when we conducted these experiments.

Additional information

No additional information is available for this paper.

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References

[1] L.R. Gray, S.C. Tompkins, E.B. Taylor, Regulation of pyruvate metabolism and human disease, Cell. Mol. Life Sci. 71 (2014) 2577–2604.
[2] J. Škerlová, J. Berndtsson, H. Nolte, et al., Structure of the native pyruvate dehydrogenase complex reveals the mechanism of substrate insertion, Nat. Commun. 12 (2021) 5277.
[3] R.A. Harris, K.M. Popov, Y. Zhao, et al., A new family of protein kinases–the mitochondrial protein kinases, Adv. Enzym. Regul. 35 (1995) 147–162.
[4] M.S. Patel, L.G. Korotchkina, Regulation of the pyruvate dehydrogenase complex, Biochem. Soc. Trans. 34 (2006) 217–222.
[5] S. Zhang, M.W. Hulver, R.P. McMillan, et al., The pivotal role of pyruvate dehydrogenase kinases in metabolic flexibility, Nutr. Metab. 11 (2014) 10.
[6] T.E. Roche, J.C. Baker, X. Yan, et al., Distinct regulatory properties of pyruvate dehydrogenase kinase and phosphatase isoforms, Prog. Nucleic Acid Res. Mol. Biol. 70 (2001) 33–75.
[7] M.M. Bowker-Kinley, W.I. Davis, P. Wu, et al., Evidence for existence of tissue-specific regulation of the mammalian pyruvate dehydrogenase complex, Biochem. J. 329 (1998) 191–196.
[8] M.J. Holness, K. Bulmer, N.D. Smith, M.C. Sugden, Investigation of potential mechanisms regulating protein expression of hepatic pyruvate dehydrogenase kinase isoforms 2 and 4 by fatty acids and thyroid hormone, Biochem. J. 369 (2003) 687–695.
[9] B. Huang, P. Wu, M.M. Bowker-Kinley, R.A. Harris, Regulation of pyruvate dehydrogenase kinase expression by peroxisome proliferator-activated receptor-alpha ligands, glucocorticoids, and insulin, Diabetes 51 (2002) 276–283.
[10] G. Bajotto, T. Murakami, M. Nagasaki, et al., Increased expression of hepatic pyruvate dehydrogenase kinases 2 and 4 in young and middle-aged Otsuka Long-Evans Tokushima Fatty rats: induction by elevated levels of free fatty acids, Metabolism 55 (2006) 317–323.
[11] S.S. Kulkarni, F. Salehzadeh, T. Fritz, et al., Mitochondrial regulators of fatty acid metabolism reflect metabolic dysfunction in type 2 diabetes mellitus, Metabolism 61 (2012) 175–185.
[12] P. Wu, P.V. Blair, J. Sato, et al., Starvation increases the amount of pyruvate dehydrogenase kinase in several mammalian tissues, Arch. Biochem. Biophys. 381 (2000) 1–7.
[13] R. Tao, X. Xiong, R.A. Harris, et al., Genetic inactivation of pyruvate dehydrogenase kinases improves hepatic insulin resistance induced diabetes, PLoS One 8 (2013), e71997.
[14] N.H. Jeoung, R.A. Harris, Pyruvate dehydrogenase kinase-4 deficiency lowers blood glucose and improves glucose tolerance in diet-induced obese mice, Am. J. Physiol. Endocrinol. Metab. 295 (2008) E46–E54.
[15] J.H. Jeon, T. Thoudam, E.J. Choi, et al., Loss of metabolic flexibility as a result of overexpression of pyruvate dehydrogenase kinases in muscle, liver and the immune system: therapeutic targets in metabolic diseases, J. Diab. Investig. 12 (2021) 21–31.
[16] P.W. Stacpoole, E.M. Harman, S.H. Curry, et al., Treatment of lactic acidosis with dichloroacetate, N. Engl. J. Med. 309 (1983) 390–396.
[17] P.W. Stacpoole, E.C. Wright, T.G. Baumgartner, et al., A controlled clinical trial of dichloroacetate for treatment of lactic acidosis in adults. The dichloroacetate-lactic Acidosis Study Group, N. Engl. J. Med. 327 (1992) 1564–1569.

[18] P.W. Stacpoole, Therapeutic targeting of the pyruvate dehydrogenase complex/pyruvate dehydrogenase kinase (PDC/PDK) axis in cancer, J. Natl. Cancer Inst. 109 (2017).
[19] G. Ribes, G. Valette, M.M. Loubatieres-Mariani, Metabolic effects of sodium dichloroacetate in normal and diabetic dogs, Diabetes 28 (1979) 852–857.
[20] P.W. Stacpoole, G.W. Moore, D.M. Kornhauser, Metabolic effects of dichloroacetate in patients with diabetes mellitus and hyperlipoproteinemia, N. Engl. J. Med. 298 (1978) 526–530.
[21] P.W. Stacpoole, C.J. Martyniuk, M.O. James, N.A. Calcutt, Dichloroacetate-induced peripheral neuropathy, Int. Rev. Neurobiol. 145 (2019) 211–238.
[22] P.W. Stacpoole, M.G. Gonzalez, J. Vlasak, et al., Dichloroacetate derivatives. Metabolic effects and pharmacodynamics in normal rats, Life Sci. 41 (1987) 2167–2176.
[23] B.K. Min, C.J. Oh, S. Park, et al., Therapeutic effect of dichloroacetate against atherosclerosis via hepatic FGF21 induction mediated by acute AMPK activation, Exp. Mol. Med. 51 (2019) 1–12.
[24] I. Pernicova, M. Korbonits, Metformin-mode of action and clinical implications for diabetes and cancer, Nat. Rev. Endocrinol. 10 (2014) 143–156.
[25] E. Danese, M. Montagnana, A. Nouvenne, G. Lippi, Advantages and pitfalls of fructosamine and glycated albumin in the diagnosis and treatment of diabetes, J. Diabetes Sci. Technol. 9 (2015) 169–176.
[26] A.J. McAinch, L.M. Cornall, R. Watts, et al., Increased pyruvate dehydrogenase kinase expression in cultured myotubes from obese and diabetic individuals, Eur. J. Nutr. 54 (2015) 1033–1043.
[27] H.L. Eichner, P.W. Stacpoole, P.H. Forsham, Treatment of streptozotocin diabetes with di-isopropylammonium dichloroacetate (DIPA), Diabetes 23 (1974) 179–182.
[28] N.H. Jeoung, P. Wu, M.A. Joshi, et al., Role of pyruvate dehydrogenase kinase isoenzyme 4 (PDHK4) in glucose homoeostasis during starvation, Biochem. J. 397 (2006) 417–425.
[29] S. Nasser, V. Vialichka, M. Biesiekierska, et al., Effects of ketogenic diet and ketone bodies on the cardiovascular system: concentration matters, World J. Diabetes 11 (2020) 584–595.
[30] C.G. Santos-Gallego, J.A. Requena-Ibanez, R. San Antonio, et al., Empagliflozin ameliorates adverse left ventricular remodeling in nondiabetic heart failure by enhancing myocardial energetics, J. Am. Coll. Cardiol. 73 (2019) 1931–1944.
[31] L. Laffel, Ketone bodies: a review of physiology, pathophysiology and application of monitoring to diabetes, Diabetes Metab. Res. Rev. 15 (1999) 412–426.
[32] R. DeFronzo, G.A. Fleming, K. Chen, T.A. Bicsak, Metformin-associated lactic acidosis: current perspectives on causes and risk, Metabolism 65 (2016) 20–29.
[33] R. Park, A.I. Arieff, Treatment of lactic acidosis with dichloroacetate in dogs, J. Clin. Invest. 70 (1982) 853–862.
[34] W.R. Mann, C.J. Dragland, C.C. Vinluan, et al., Diverse mechanisms of inhibition of pyruvate dehydrogenase kinase by structurally distinct inhibitors, Biochim. Biophys. Acta 1480 (2000) 283–292.
[35] B.Y. Park, J.H. Jeon, Y. Go, et al., PDK4 deficiency suppresses hepatic glucagon signaling by decreasing cAMP levels, Diabetes 67 (2018) 2054–2068.
[36] O.B. Evans, Dichloroacetate tissue concentrations and its relationship to hypolactatemia and pyruvate dehydrogenase activation, Biochem. Pharmacol. 31 (1982) 3124–3126.