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Research Paper

Dichloroacetate induces regulatory T-cell differentiation and suppresses Th17-cell differentiation by pyruvate dehydrogenase kinase-independent mechanism

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Keywords
dichloroacetate; pyruvate dehydrogenase kinase; reactive oxygen species; Th17; Treg

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Abstract

Objectives Recently, there has been a growing interest in the mechanism of action of dichloroacetate (DCA) for T-cell differentiation; however, this mechanism has not been elucidated in detail. Therefore, this study aimed to investigate the mechanism of action of DCA for Treg and Th17 differentiation with pyruvate dehydrogenase kinase (PDHK) inhibitor (AZD7545) and PDHK knockdown.
Methods Inhibitory activity of DCA and AZD7545 against recombinant PDHK and intracellular PDH phosphorylation was measured. The effects of DCA and AZD7545 on T-cell differentiation were assessed by analysing Foxp3$^+$ T-cell populations for Treg differentiation and IL-17A production for Th17 differentiation. For reactive oxygen species (ROS) production, DCFDA was used as an indicator.
Key findings Dichloroacetate and AZD7545 inhibited PDHK activity of recombinant PDHK and intracellular PDH phosphorylation. DCA was capable of inducing Treg differentiation and suppressing Th17 differentiation. The effects of DCA were independent of PDHK because neither AZD7545 nor knockdown of PDHK1 or PDHK3 affected T-cell differentiation. DCA was determined to be capable of inducing ROS production, and the effects of DCA on T-cell differentiation were shown to be dependent on ROS production.
Conclusions Dichloroacetate possesses Treg induction and Th17 suppression, which is independent of PDHK and dependent on ROS production.

Introduction

T-cell activation initiates a transition from quiescence to rapid cell growth, proliferation and differentiation into functional subsets to drive or suppress the immune response.[1] Th17 and Treg cells represent two CD4$^+$T-cell subsets that share important developmental elements, but each has distinct phenotypes with remarkably opposite activity, Th17 cells being pro-inflammatory and Tregs being anti-inflammatory.[2] The balance between Teffs and Tregs is crucial to providing sufficient immune protection without promoting autoimmunity. Indeed, many autoimmune diseases, including multiple sclerosis and inflammatory bowel disease, involve an imbalance of Teffs to Tregs or decreased Treg function. Emerging evidence has suggested that CD4$^+$T-cell metabolism is highly dynamic and may allow targeting of selection for T-cell populations.[3] Metabolic reprogramming of Th17 cells and Treg cells leads to distinct metabolic programmes. While Th17 cells utilize large amounts of glucose and a high rate of glycolysis to support their energetic needs, Treg cells can oxidize lipids and are able to expand and function even in the absence of glucose.[4,5] Identifying the relationship between the metabolic change and the balance of Th17 cells and Treg cells may provide a way to prevent or suppress autoimmunity.

Dichloroacetate (DCA), a small molecule that inhibits PDHK (thus activating pyruvate dehydrogenase (PDH), the gatekeeper of glucose oxidation), decreases tumour growth in many cancers.[6] Because cancer cells are highly dependent on the glycolytic pathway. To date, four different isoforms of PDK have been identified that have variable expression and sensitivity to inhibition by DCA.[7] The effects of DCA on cancer apoptosis and proliferation are

dependent on PDHK2 inhibition with siRNA completely mimicking DCA effects.[8] These DCA effects can subsequently lead to an increased flow of oxygen in the respiratory chain, which is associated with the enhanced generation of reactive oxygen species (ROS) that may cause apoptosis. T-cell differentiation is related to intracellular metabolism, especially glycolysis. Indeed, DCA has been shown to reduce proinflammatory cytokine production and promote Foxp3 expression within in-vitro and in-vivo inflammatory models.[9,10] However, the molecular mechanisms of the effects of DCA on T-cell differentiation have not been elucidated.

Recent work showed that PDHK1 had effects on T-cell differentiation by shRNA and DCA treatment.[11] But, this study did not show a combination of DCA treatment and knockdown by shRNA. So, whether or not the mechanism of DCA for T-cell differentiation is PDHK dependent or independent has not yet been elucidated. In this study, we investigated the mechanism of action of DCA by employing a combination of DCA, PDHK inhibitors and PDHK siRNA. Although DCA induced Treg cells and suppressed Th17 cells, PDHK inhibitor and PDHK knockdown did not affect the T-cell differentiation. Moreover, DCA showed the same effect for T-cell differentiation in the presence of AZD7545 (PDHK inhibitor) and PDHK siRNA. Instead of PDHK inhibition, ROS production was related to T-cell differentiation by DCA treatment. Although previous studies have not elucidated how DCA affects T-cell differentiation, this study is the first to provide evidence that the mechanism of action of DCA for T-cell differentiation is dependent on ROS production, and not on PDHK activity.

Materials and Methods

Recombinant PDHK activity

To create PDH-PDHK complex, 0.05 U/ml PDH (P7032; Sigma) and 1 μg/ml hPDHK1 (ab110359; Abcam) or 1 μg/ml hPDHK2 (ab110354; Abcam, Cambridge, UK) were mixed in PDH buffer (50 mM MOPS [pH7] (GB61; Dojindo, Kumamoto, Japan)), 20 mM K_2HPO_4 (164-04295; Wako), 60 mM KCl (163-03545; Wako, Tokyo, Japan), 2 mM $MgCl_2$ (310-90361; Wako), 0.4 mM EDTA (15575-020; Life Technologies, Carlsbad, USA), 0.2% Pluronic F-68 (24040-032; Life Technologies, Carlsbad, USA) and 2 mM Dithiothreitol (040-29223; Wako). Before PDH phosphorylation, 16 μl PDH buffer and 2 μl of compounds (30-fold concentration) were added into 40 μl PDH-PDHK complex. PDH phosphorylation was initiated by adding 2 μl of 30 μM ATP (final 1 μM). The reaction was carried out at room temperature for 45 min. To measure the PDH activity, 20 μl substrate mix (5 mM sodium pyruvate (11360070; Life Technologies), 5 mM coenzyme A trisodium salt (035-14061; Wako), 12 mM β –NAD^+ (440500000; Oriental Yeast, Tokyo, Japan) and 5 mM thiamine pyrophosphate (C8754; Sigma, St. Louis, USA) were added into the complex. The 340-nm absorbance, which indicates the amount of NADH, was measured with a plate reader (Molecular Devices, Spectramax 190, Sunnyvale, USA) before adding substrate mix and after incubation for 1 h.

Intracellular PDHK activity

Mouse T-cell line EL4 cells were subcultured at 10^6 cells per well. The cells were cultured at 37 °C for 2 h after adding DCA (326-87772; Wako) or AZD7545 (S7517; SELLECK CHEM, Houston, USA). PDH phosphorylation was measured with a Phosphorylated PDH ELISA Kit (ab115343; Abcam) according to the manufacturer's protocol.

Mice

Six- to twelve-week-old male C57BL/6J mice (Charles River, Wilmington, USA) were used for all experiments. All experiments were approved by the Ethics Committee for Animal Experiments of Daiichi Sankyo Co., Ltd. (certification No. A1501497).

In-vitro Treg and Th17 differentiation

Naive $CD4^+$T cells were isolated with a naïve $CD4^+$T-cell isolation kit (130-104-453; Miltenyi, Bergisch Gladbach, Germany) from mouse splenocytes. Naïve $CD4^+$T cells (5×10^4 cells per well) were cultured in RPMI 1640 supplemented 10% FBS, 1% penicillin–streptomycin and 55 μM β-mercaptoethanol with anti-CD3/CD28 dynabeads (11452D; Gibco, Carlsbad, USA). The following cytokines were added to generate each subset: Treg, 10 ng/ml IL-2 and 10 ng/ml TGFβ; Th17, 100 ng/ml IL-6, 2.5 ng/ml TGFβ, 10 ng/ml IL-1β and 10 ng/ml IL-23. On Day 5 after stimulation, the cells in Treg differentiation were analysed for Foxp3 expression. In Th17 differentiation, sups were collected and the cells were restimulated with 100 ng/ml phorbol 12-myristate 13-acetate (PMA) (P1585; Sigma) and 1 μg/ml ionomycin (I0634; Sigma) for 24 h. IL-17A production was measured with a Mouse IL-17A immunoassay kit (AL514C; PerkinElmer, Waltham, USA). Cell toxicity and proliferation were measured with CellTiter Glo (G7571; Promega, Madison, USA). All cytokines were obtained from Peprotech, California, USA. In most experiments, cells were treated with 1 mM to 30 mM DCA, 1 μM AZD7545 and 1 mM N-acetyl-L-cysteine (NAC).

Foxp3 expression and ROS production

Intracellular Foxp3 was stained with Foxp3 transcription factor staining buffer set (00-5523-00; eBioscience) and anti-Foxp3-APC antibody (17-5773-82; eBioscience). T cells were labelled with DCFDA for 30 min at 37 °C by Cellular Reactive Oxygen Species Detection Assay Kit (Ab113851; Abcam). Labelled cells were incubated for 2 h with vehicle, 10 mM DCA, 1 μM AZD7545, or both 10 mM DCA and 1 μM AZD7545 together. Data were acquired on a FACScant (Beckton Dickinson, Franklin Lakes, USA) and analysed using FlowJo (TreeStar Software, Ashland, USA).

Quantitative PCR

Cells were lysed and total RNA was purified using the RNeasy mini kit (74106; Qiagen, Hilden, Germany) according to the manufacturer's protocol. Total RNA was reverse transcribed using ReverTra Ace qPCR RT Master Mix with gDNA remover (FSQ-301; Toyobo, Osaka, Japan), and quantitative PCR was carried out using Thunderbird probe qPCR mix (QPS-101; Toyobo). Primer and probe sets predeveloped by Applied Biosystems (TaqMan Gene Expression Assays, Appliedbiosystems, Foster City, USA) were used to quantify GAPDH (Mm01246556_m1), PDHK1 (Mm00554300_m1), PDHK2 (Mm00446681_m1), PDHK3 (Mm00455220_m1) and PDHK4 (Mm01166879_m1). GAPDH levels were used as normalization controls and fold induction was calculated using the ΔC_T method.

siRNA Knockdown

Knockdown of PDHK1 and PDHK3 in Naïve CD4$^+$ T cells was done using ACCELL siRNA designed and validated by ThermoFisher/Dharmacon, Carlsbad, USA. A non-targeting siRNA was used as a negative control. Knockdown of PDHK1 and PDHK2 was done using commercially available and validated siRNA from Dharmacon; PDHK1 siRNA (E-054066-00-0005); PDHK3 siRNA (E-052920-00-0005). PDHK1 and PDHK3 knockdown was verified by assessing PDHK1 and PDHK3 mRNA expression. Naïve CD4$^+$ T cells were incubated with the siRNA and ACCELL medium (Dharmacon) including 3% FBS and 50 μM

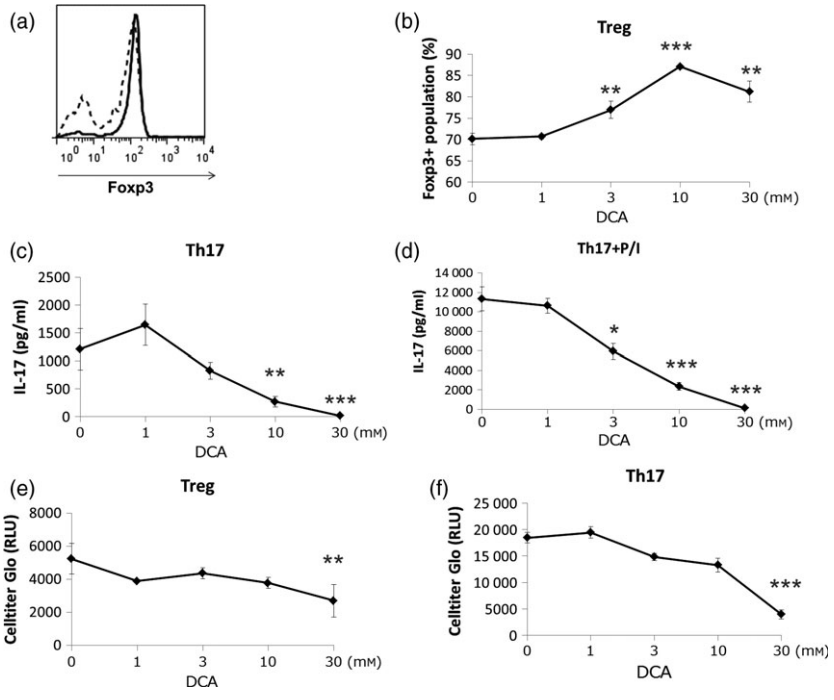

Figure 1 The effect of dichloroacetate on Treg and Th17 differentiation. Naïve CD4$^+$T cells were induced into Treg with Dynabeads Mouse T-Activator CD3/CD28, 10 ng/ml IL-2 and 10 ng/ml TGFβ for 120 h with vehicle, 1, 3, 10 and 30 mM dichloroacetate. The Foxp3 protein expression was determined using flow cytometry (a and b). Naïve CD4$^+$T cells were induced into Th17 with Dynabeads Mouse T-Activator CD3/CD28, 100 ng/ml IL-6, 2.5 ng/ml TGFβ, 10 ng/ml IL-23 and 10 ng/ml IL-1b for 120 h with vehicle, 1, 3, 10 and 30 mM dichloroacetate. The sups were collected, and cells were restimulated with 100 ng/ml PMA and 1 μg/ml ionomcyin. IL-17A in the collected sups and restimulated sups was determined using alphaLISA (c and d). Cell viability and proliferation were determined using CellTiter Glo (e and f). The histogram (a) shows Foxp3 expression in vehicle-treated (dashed line) and 10 mM dichloroacetate-treated (solid line) Treg cells. Data are presented as the mean ± SD (n = 3). Statistically significant differences compared with vehicle: *P < 0.05, **P < 0.01 and ***P < 0.001.

β-mercaptoethanol for 72 h in the conditions of Treg and Th17 differentiation. Cultured cells were collected to measure mRNA expression, Treg, and Th17 differentiation.

Statistical analyses

Data were expressed as means ± standard deviation. Statistical comparisons of the results were made using analysis of variance. Significant differences between the means of the control and test groups were analysed by Dunnett's test. Significant differences between the means of each group were analysed by Tukey's test.

Results

DCA induces Treg differentiation and suppresses Th17 differentiation

We first investigated whether DCA affects T-cell differentiation. In the conditions of Treg and Th17 differentiation, different concentrations (1–30 mM) of DCA were added to the culture media. Compared with the vehicle, DCA showed a significantly induced $Foxp3^+$ population in the Treg differentiation marker (Figure 1a and 1b). The concentration above 3 mM DCA showed Foxp3 induction, and 10 mM DCA showed the strongest effects. DCA also showed significantly suppressed IL-17A production in the Th17 differentiation marker (Figure 1c and 1d). On the other hand, cellular ATP content measured by CellTiter Glo as a cell proliferation and cell toxicity indicator was specifically decreased in only 30 mM DCA applied to Treg and Th17 differentiation (Figure 1e and 1f). DCA at 30 mM may have a toxic effect. Therefore, we investigated the effects of DCA at the concentration of 10 mM in the experiments that followed.

DCA and AZD7545 inhibit recombinant PDHK and intracellular PDHK activity

Dichloroacetate is known as a PDHK inhibitor.[8] AZD7545 has also been reported as a PDHK inhibitor with a higher potency compared to DCA.[12] To confirm the efficacy and potency of DCA and AZD7545 for PDHK inhibition, we investigated recombinant PDHK1/PDHK2 activity. PDHK phosphorylates PDH and inhibits PDH activity, so inhibition of PDHK activity by compounds was

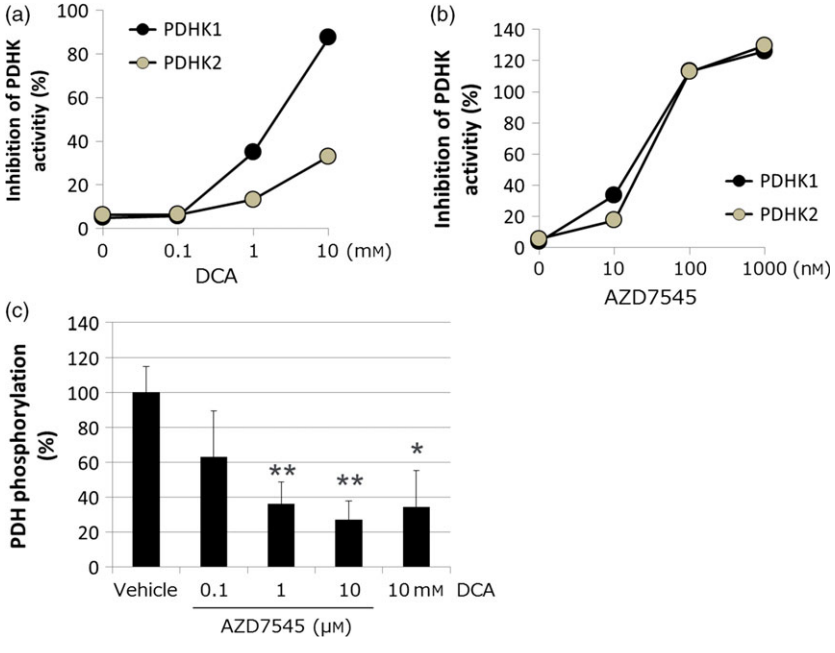

Figure 2 The effects of dichloroacetate and AZD7595 treatment on PDHK1 and PDHK2 inhibition. Dichloroacetate and AZD7595 inhibit recombinant pyruvate dehydrogenase kinase activity and intracellular pyruvate dehydrogenase kinase activity. Inhibitory activity of compounds (0.1, 1 and 10 mM dichloroacetate, and 10, 100 and 1000 nM AZD7595) on recombinant PDHK1 and PDHK2 was evaluated by measuring pyruvate dehydrogenase activity (a and b). The graph shows percent inhibition for 100%, no pyruvate dehydrogenase kinases and 0%, vehicle control. The line graphs show pyruvate dehydrogenase inhibitory activity in the presence of PDHK1 (black circle) and PDHK2 (grey circle). Jurkat cells were treated with vehicle, 0.1, 1 and 10 μM AZD7545 and 10 mM dichloroacetate. Pyruvate dehydrogenase phosphorylation was measured with an assay kit (c). The graph shows pyruvate dehydrogenase phosphorylation for 100%, vehicle control and 0% background in the absence of cell lysis. Data are presented as the mean ± SD ($n = 3$). Statistically significant differences compared with vehicle: $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$. [Colour figure can be viewed at wileyonlinelibrary.com]

measured with PDH activity.[13] DCA and AZD7545 inhibited PDHK activity dose dependently (Figure 2a and 2b). The potency (IC_{50}) was 5.8 mM (PDHK1), and 33% inhibition in 10 mM (PDHK2) in DCA, and 38 nM (PDHK1), and 41 nM (PDHK2) in AZD7545. We next investigated intracellular PDHK inhibitory activity by measuring PDH phosphorylation. DCA and AZD7545 also showed inhibitory effects on intracellular PDH phosphorylation dose dependently (Figure 2c). The potency (IC_{50}) was 70% inhibition in 10 mM in DCA and 29 nM in AZD7545. These results validated AZD7545 as a PDHK inhibitor, which could be used to further investigate PDHK function.

DCA induces Treg differentiation and suppresses Th17 differentiation independent of PDHK mechanism

We next investigated whether DCA affects T-cell differentiation PDHK dependently or independently. In the conditions of Treg and Th17 differentiation, vehicle, 10 mM DCA, 10 μM AZD7545, or both 10 mM DCA and 10 μM AZD7545 together were added to the culture media. Compared with the vehicle, DCA induced significantly an increase in $Foxp3^+$ population regardless of the absence or presence of AZD7545 (Figure 3a and 3b). AZD7545 did not induce any increase in $Foxp3^+$ population. DCA significantly suppressed IL-17A production in the absence or presence of AZD7545 (Figure 3c and 3d). AZD7545 did not suppress IL-17A production. These effects were independent of cell toxicity and proliferation, because cellular ATP content was not changed (Figure 3e and 3f). We next investigated the effects of DCA on T-cell differentiation under the PDHK knockdown condition. The expression of PDHK1 to PDHK4 in naïve $CD4^+$T cells, Th17 cells and Treg cells was measured (Figure 4a). Because PDHK1 and PDHK3 were highly expressed compared with PDHK2 and PDHK4, we focused on PDHK1 and PDHK3. PDHK1 and PDHK3 were knocked down in the presence of PDHK1 siRNA and PDHK3 siRNA, respectively, and compared with control siRNA (Figure 4b and 4c). In the condition of knockdown, Treg and Th17 differentiation were measured with or without DCA treatment. $Foxp3^+$ population was increased by DCA treatment in the absence and presence of PDHK1 siRNA and PDHK3 siRNA (Figure 4d). IL-17A

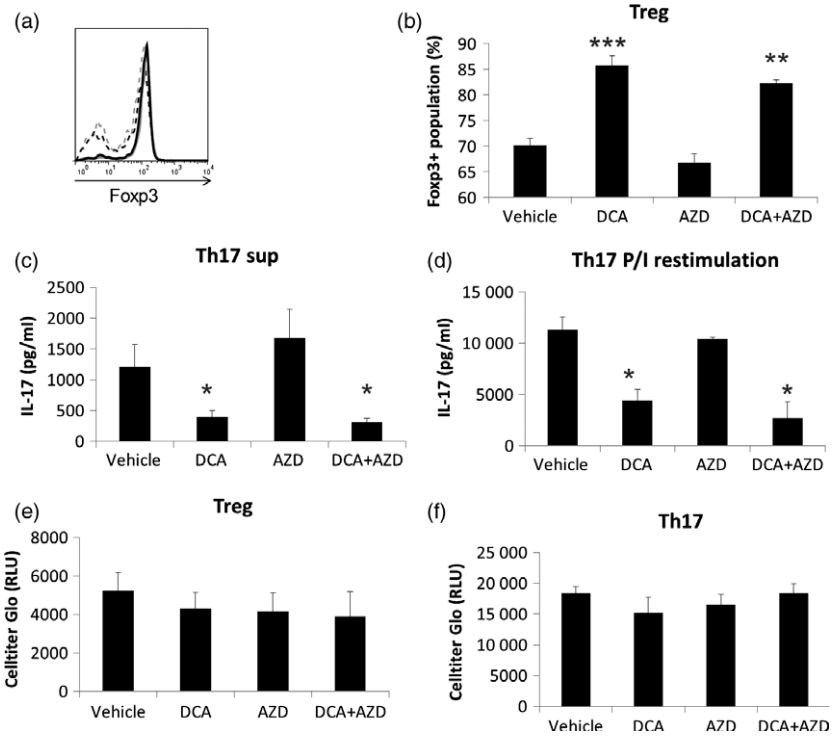

Figure 3 The effects of dichloroacetate and AZD7595 treatment on Treg and Th17 differentiation. Naïve $CD4^+$T cells were induced into Treg with vehicle, 10 mM dichloroacetate, 1 μM AZD7545 or dichloroacetate + AZD. The Foxp3 protein expression was determined using flow cytometry (a and b). Naïve $CD4^+$T cells were induced into Th17 with vehicle, 10 mM dichloroacetate, 1 μM AZD7545 or dichloroacetate + AZD. The sups were collected, and cells were restimulated with 100 ng/ml PMA and 1 μg/ml ionomcyin. IL-17A in the collected sups and restimulated sups were determined using alphaLISA (c and d). Cell viability and proliferation were determined using CellTiter Glo (e and f). Data are presented as the mean ± SD ($n = 3$). Statistically significant differences compared with vehicle: $^*P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

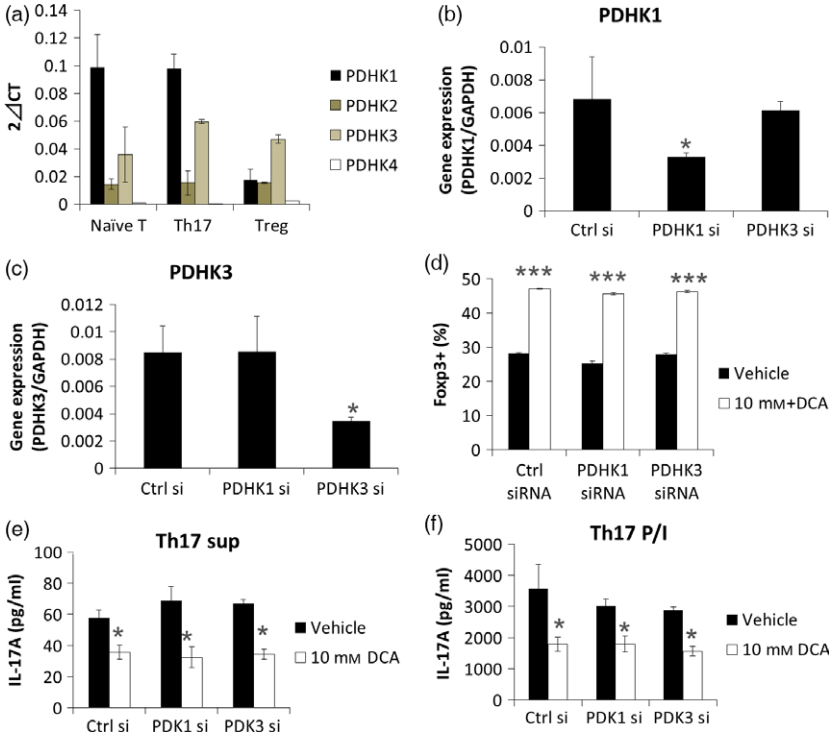

Figure 4 Expression of PDHK1 to PDHK4 on CD4$^+$T cells and the effects of PDHK1 and PDHK3 siRNA on Treg and Th17 differentiation. Gene expressions of PDHK1 to PDHK4 were analysed using real-time PCR on Naïve, Th17 and Treg CD4$^+$T cells (a). Naïve CD4$^+$T cells were induced into Treg and Th17 with vehicle, 10 mM dichloroacetate and Control siRNA, PDHK1 siRNA, PDHK3 siRNA. The gene expression of PDHK1 and PDHK3 was analysed using real-time PCR on siRNA transfected Th17 and Treg CD4$^+$T cells (b and c). The Foxp3 protein expression was determined using flow cytometry (d). IL-17A in the collected sups and restimulated sups was determined using alphaLISA (e and f). Cell viability and proliferation were determined using CellTiter Glo (g and h). Data are presented as the mean ± SD (n = 3). Statistically significant differences compared with vehicle: *P < 0.05, **P < 0.01 and ***P < 0.001. [Colour figure can be viewed at wileyonlinelibrary.com]

production was decreased by DCA treatment in the absence and presence of PDHK1 siRNA and PDHK3 siRNA (Figure 4e and 4f). These results suggest that the effects of DCA on Treg and Th17 differentiation are independent of the PDHKs.

DCA treatment induces ROS production, and Treg differentiation is dependent on ROS

We next investigated the mechanism of Treg differentiation by DCA. To measure ROS production from T cells, DCFDA-labelled Treg and Th17 cells were incubated for 2 h with vehicle, 10 mM DCA, 1 μM AZD7545, or both 10 mM DCA and 1 μM AZD7545 together, and then were analysed using flow cytometry. DCA increased DCFDA fluorescence as an ROS production marker in the presence and absence of AZD7545 (Figure 5a and 5b). AZD7545 did not induce DCFDA fluorescence. To investigate whether the effects of DCA were dependent on ROS production, 1 mM NAC (ROS inhibitor) was treated with DCA in Treg and Th17 differentiation. Treg induction by DCA was inhibited by the presence of NAC (Figure 5d). Moreover, the Th17 suppression effect of DCA disappeared in the presence of NAC (Figure 5e and 5f). These effects were independent of cell toxicity and proliferation, because cellular ATP content was not changed (Figure 5g and 5h). These results suggest that Treg induction by DCA is in fact related to ROS production.

Discussion

Our studies demonstrated several novel findings. We showed that DCA induced Treg and suppressed Th17. On the other hand, PDHK inhibition by PDHK inhibitor or by PDHK knockdown in combination with DCA did not show these effects. These results suggest that the mechanism of DCA for T-cell differentiation is independent of PDHK activity. For the mechanism of DCA, we showed that DCA induced ROS production independent of PDHK and affected T-cell differentiation through ROS production. These results show that the effects of DCA on T-cell differentiation are through not PDHK activity, but ROS production.

A previous study has shown that the effects of DCA are to induce Treg and to suppress Th17 through PDHK

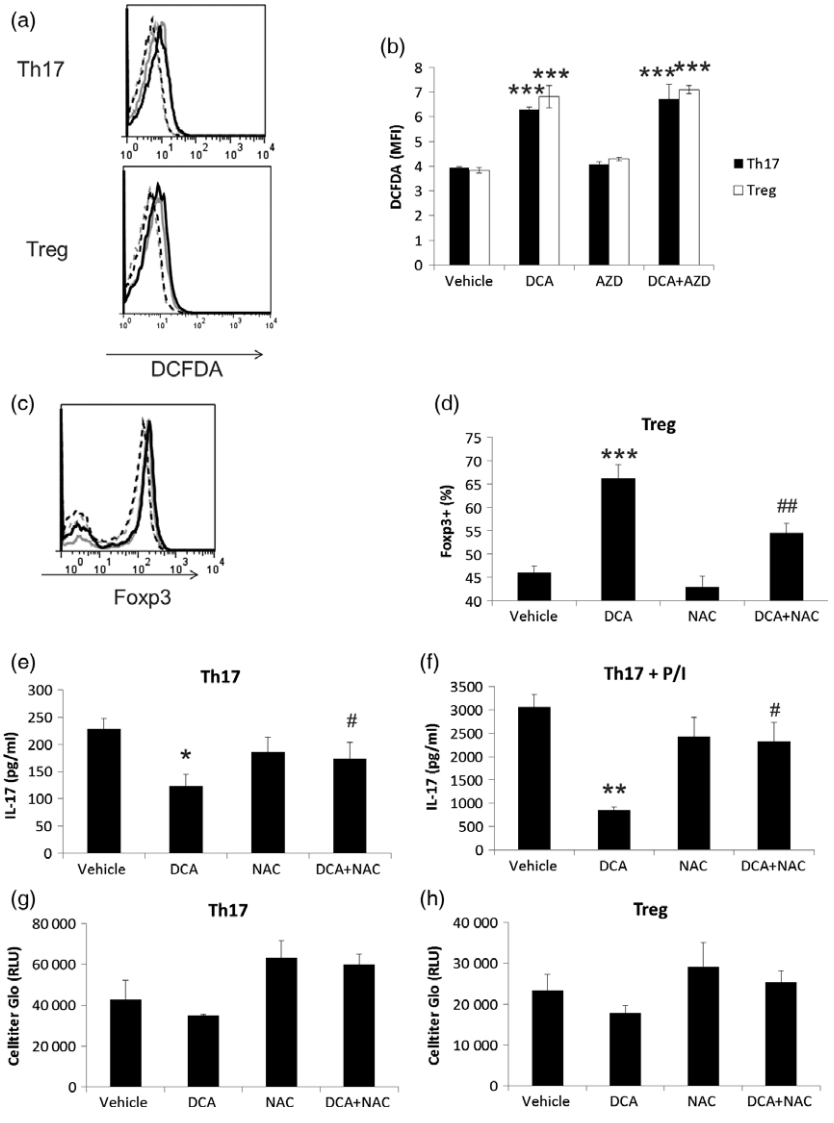

Figure 5 The effects of dichloroacetate and AZD7545 treatment on reactive oxygen species production and the effects of dichloroacetate and N-acetyl-L-cysteine on Treg and Th17 differentiation. Naïve $CD4^+$T cells were induced in Treg and Th17 with vehicle, 10 mM dichloroacetate, 1 mM N-acetyl-L-cysteine or dichloroacetate + N-acetyl-L-cysteine. Differentiated Treg and Th17 were treated with DCFDA. After that, the cells were treated with vehicle, 10 mM dichloroacetate, 1 μM AZD7545 or dichloroacetate + AZD. The fluorescence change of DCFDA as indicator for reactive oxygen species production was determined using flow cytometry (a and b). The Foxp3 protein expression was determined using flow cytometry (c and d). IL-17A in the collected sups and restimulated sups was determined using alphaLISA (e and f). Cell viability and proliferation were determined using CellTiter Glo (g and h). The histogram (a) shows DCFDA fluorescence on vehicle-treated (grey dashed line), dichloroacetate-treated (grey solid line), AZD7545-treated (black dashed line) and dichloroacetate + AZD7545-treated (black solid line) cells. The histogram (c) shows Foxp3 expression on vehicle-treated (grey dashed line), dichloroacetate-treated (grey solid line), N-acetyl-L-cysteine-treated (black dashed line) and dichloroacetate + N-acetyl-L-cysteine-treated (black solid line) Treg cells. Data are presented as the mean ± SD (n = 3). Significant differences between the means of each group were analysed by Tukey test. Statistically significant differences were compared with vehicle: *P < 0.05, **P < 0.01 and ***P < 0.001. Statistically significant differences compared between dichloroacetate treatment and dichloroacetate + N-acetyl-L-cysteine treatment: #P < 0.05 and ##P < 0.01.

inhibition.[11] They showed Treg induction by knockdown of PDHK1 expression in the condition of a co-culture system of T cells and antigen-presenting cells. Results of this study were contradictory to our findings on the mechanism of T-cell differentiation. The point of different point is that they concluded the mechanism of Treg induction by DCA was only dependent on PDHK inhibition. We examined the effects of DCA and PDHK inhibitors for Treg induction in the condition of a co-culture system, which showed similar results in our hands (data not shown). We conducted that

the experiment in the presence of naïve $CD4^+$ T cells only, and not in the condition of a co-culture to clarify the direct effects of DCA on T cells. We concluded DCA induced ROS production independent of PDHKs, which induced Treg induction. Moreover, we investigated the effect by using the highly potent small-molecule AZD7545. AZD7545 has a high potency ($IC_{50} \approx 40$ nM for PDHK protein and $IC_{50} = 29$ nM for intracellular activity) compared with DCA. AZD7545 treatment did not affect T-cell differentiation with or without DCA treatment. Other PDHK inhibitors also did not show any effects (data not shown). Moreover, knockdown of PDHK1 and PDHK3, highly expressed in $CD4^+$T cells compared with PDHK2 and PDHK4, did not affect T-cell differentiation with or without DCA treatment. Our approach using small-molecule inhibitors and knockdown in combination with DCA is thought to show more clear results than the previous study.[11]

A recent study has shown the other effects of DCA that induced T-cell apoptosis in addition to Treg induction.[14] This study also showed the effects of DCA upon glycolysis, such as decreasing HK2 and Glut1 expression. There was no change of ATP contents shown in our results, which result did not estimate the induction of T-cell apoptosis by DCA treatment. This discrepancy may come from the difference of species (mouse or human) and the used cells (T cells only or MLR (T cell and APC)). We assessed DCA's direct effects on T cells. In the other study, they might assess the indirect effects of DCA on T cells mediated by other cells like APC. In cancer cells, DCA increases cell apoptosis and cell death by ROS production.[15] But in this study, DCA did not increase cell death or suppress proliferation on T cells according to the results of the cellular ATP content. This is because cancer cells and T cells may have a different metabolic character. For T-cell differentiation, previous studies have shown that ROS production is related to Treg and Th17 differentiation, as the investigation of NAC (ROS inhibitor) treatment has demonstrated.[11,16,17] Indeed, DCA treatment induced ROS production independent of PDHKs, induced Treg cells and suppressed Th17 cells. Other studies have shown that DCA inhibits mTOR in cancer cells.[18,19] We also investigated whether the mTOR signal was affected by DCA treatment, but DCA did not show any effect on the mTOR signal (data not shown). The mechanism of action of DCA for promoting T-cell differentiation and ROS induction was found to not be dependent on either the PDHKs or mTOR.

Treg cells and Th17 cells are related to several autoimmune diseases.[20,21] Drug development for Th17 suppression has been investigated for targeting Rorγt, IL-17, IL-23, etc, for the treatment of psoriasis in clinical settings.[22] Although there are several approaches for inducing Treg production, including mTOR inhibition, the study did not proceed into clinical trial[23] because mTOR inhibitor also affects T-cell proliferation and causes other effects related to adverse effects.[24] On the other hand, DCA can modulate the balance of Treg and Th17 differentiation without affecting T-cell proliferation. DCA has clinically been developed for lactic acidosis and cancer.[25,26] As we have already mentioned, DCA has the effect of improving the balance of Treg/Th17. DCA itself may have the potential of being a drug applicable against autoimmune diseases. Moreover, identifying the mechanism of action of DCA, including the ROS production, may reveal a promising target for drug discovery that is applicable against autoimmunity.

Conclusion

In summary, we investigated the effects and mechanism of action of DCA for T-cell differentiation. Importantly, this study shows that the effects of DCA on Treg induction and Th17 suppression are not PDHK dependent, but ROS dependent. In the future, if the mechanism of action of DCA can be elucidated in detail, it may bring the discovery of a drug target for Treg induction and Th17 suppression.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflict of interests to disclose.

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