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Oral administration of *Dictyostelium* differentiation-inducing factor 1 lowers blood glucose levels in streptozotocin-induced diabetic rats

Ritsuko Kawaharada [a,1], Akio Nakamura [b,1], Katsunori Takahashi [c], Haruhisa Kikuchi [d], Yoshiteru Oshima [d], Yuzuru Kubohara [e,f,*,1]

[a] Department of Health and Nutrition, Faculty of Health and Welfare, Takasaki University of Health and Welfare, Takasaki 370-0033, Japan
[b] Department of Molecular Pharmacology and Oncology, Gunma University School of Medicine, Maebashi 371-8515, Japan
[c] Department of Medical Technology, Faculty of Health Science, Gunma Paz College, Takasaki 370-0006, Japan
[d] Laboratory of Natural Product Chemistry, Graduate School of Pharmaceutical Sciences, Tohoku University, Sendai 980-8578, Japan
[e] Department of Molecular and Cellular Biology, Institute for Molecular and Cellular Regulation, Gunma University, Maebashi 371-8512, Japan
[f] Department of Health Science, Graduate School of Health and Sports Science, Juntendo University, Inzai City 270-1695, Japan

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ABSTRACT

Aims: Differentiation-inducing factor 1 (DIF-1), originally discovered in the cellular slime mold *Dictyostelium discoideum*, and its derivatives possess pharmacological activities, such as the promotion of glucose uptake in non-transformed mammalian cells *in vitro*. Accordingly, DIFs are considered promising lead candidates for novel anti-diabetic drugs. The aim of this study was to assess the anti-diabetic and toxic effects of DIF-1 in mouse 3T3-L1 fibroblast cells *in vitro* and in diabetic rats *in vivo*.

Main methods

We investigated the *in vitro* effects of DIF-1 and DIF-1(3M), a derivative of DIF-1, on glucose metabolism in 3T3-L1 cells by using capillary electrophoresis time-of-flight mass spectrometry (CE-TOF-MS). We also examined the effects of DIF-1 on blood glucose levels in streptozotocin (STZ)-induced rats.

Key findings.

CE-TOF-MS revealed that 20 μM DIF-1 and 20 μM DIF-1(3M) promoted glucose uptake and metabolism in 3T3-L1 cells. Oral administration of DIF-1 (30 mg/kg) significantly lowered basal blood glucose levels in STZ-treated rats and promoted a decrease in blood glucose levels after oral glucose loading (2.5 g/kg) in the rats. In addition, daily oral administration of DIF-1 (30 mg/kg/day) for 1 wk significantly lowered the blood glucose levels in STZ-treated rats but did not affect their body weight and caused only minor alterations in the levels of other blood analytes.

Significance: These results indicate that DIF-1 may be a good lead compound for the development of anti-diabetic drugs.

1. Introduction

Differentiation-inducing factor 1 (DIF-1) (Fig. 1A) is a physiologic signal molecule that induces stalk cell differentiation in the cellular slime mold *Dictyostelium discoideum* [1–3]. In addition, DIF-1 and its derivatives (DIFs) have been shown to exhibit anti-proliferative and anti-metastatic activities and to occasionally induce cell differentiation *in vitro* in mammalian tumor cells [4–11]. Thus, DIFs are expected to have therapeutic potential for the treatment of cancer. Several studies have investigated the mechanism(s) underlying the actions of DIFs in tumor cells [6–9,12–16]; however, the precise mechanism or mechanisms involved have yet to be elucidated.

Previously, we found that DIF-1 stimulates glucose consumption in *in vitro* cultures of mouse 3T3-L1 fibroblasts and 3T3-L1 adipocytes [17]. In these cells, DIF-1 induces the translocation of glucose transporter 1 (GLUT-1) from intracellular vesicles to the plasma membrane, thereby promoting glucose uptake [17] (Fig. 1B); however, the fate of the glucose that is taken up by the cells has not yet been elucidated. It is noteworthy that the mechanism by which DIF-1 stimulates glucose uptake differs from that used to suppress tumor cell growth [17]. Among the derivatives of DIF-1 tested to date, DIF-1 and DIF-1(3M) (Fig. 1A) possess the most potent ability to promote glucose uptake [17,18]. Moreover, intraperitoneal injection of KK-Ay diabetic mice with DIF-1(3 M) was shown to lower blood glucose levels after feeding [18]. These observations suggest that DIF-1 and its derivatives may have

Abbreviations: DIF, differentiation-inducing factor; MC, methylcellulose; OGTT, oral glucose tolerance test; STZ, streptozotocin.

* Corresponding author at: Graduate School of Health and Sports Science, Juntendo University, Inzai City 270-1695, Japan.
E-mail address: ykuboha@juntendo.ac.jp (Y. Kubohara).

[1] These authors contributed equally to this work.

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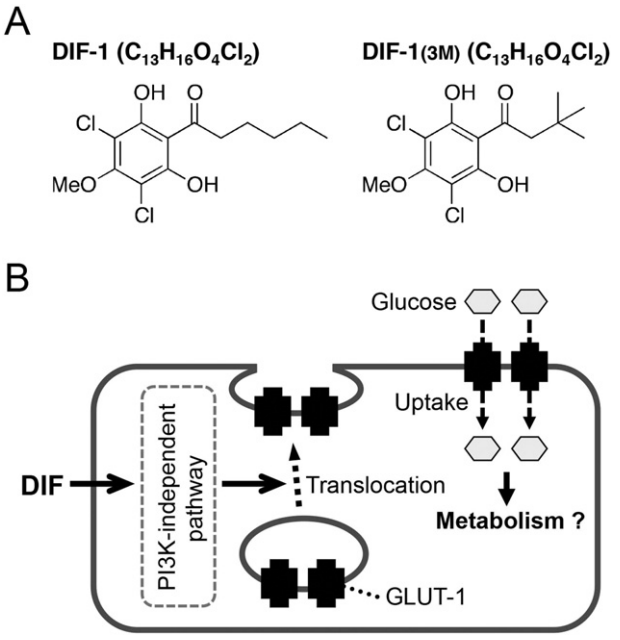

Fig. 1. A. Chemical structures of DIF-1 and DIF-1(3 M). DIF-1, 1-(3,5-dichloro-2,6-dihydroxy-4-methoxyphenyl)hexan-1-one. DIF-1(3M), 1-(3,5-dichloro-2,6-dihydroxy-4-methoxyphenyl)-3,3-dimethylbutan-1-one. The chemical formulae for the compounds are provided in parentheses. B. Proposed scheme for the mechanism underlying the actions of DIFs in mammalian cells expressing GLUT-1. DIF-1 has been shown to induce the translocation of GLUT-1 (from intracellular vesicles to the plasma membrane) via a phosphoinositide 3-kinase (PI3K)-independent pathway, thereby promoting glucose uptake [17]. In addition, DIF derivatives have been suggested to penetrate the plasma membrane [16]. Thus, regardless of the presence of the insulin signaling system that involves PI3K, DIFs would promote glucose uptake in cells expressing GLUT-1 [17]; however, the fate of the glucose taken up by the cells remains to be elucidated.

therapeutic potential for the treatment of obesity and type 2 diabetes. However, it is not currently known whether DIFs are effective in type 1 diabetes.

In this study, we investigated the *in vitro* effects of DIF-1 and DIF-1 (3M) on glucose metabolism in confluent 3T3-L1 fibroblast cells by using capillary electrophoresis time-of-flight mass spectrometry (CE-TOF-MS). We found that both DIF-1 and DIF-1(3M) tend to accelerate glucose metabolism without affecting ATP production. In addition, to further assess the anti-diabetic and toxic effects, if any, of DIF-1 *in vivo*, we orally administered DIF-1 to streptozotocin (STZ)-induced diabetic rats, a model of type 1 diabetes. We found that oral administration of DIF-1 lowered basal blood glucose levels in STZ-treated rats but caused only negligible changes in other blood analytes. Our results suggest that DIF-1 may be a good lead compound for the development of anti-diabetic drugs.

2. Materials and methods

2.1. Cells, reagents, animals, diet, and STZ treatment

Mouse 3T3-L1 fibroblasts were used in this study; the cells were maintained at 37 °C (5% CO_2) in DMEM-HG (Dulbecco's Modified Eagle's Medium [DMEM] containing a high concentration [4500 mg/l] of glucose [SIGMA, D5796] supplemented with 75 μg/ml penicillin, 50 μg/ml streptomycin, and 10% [v/v] fetal calf serum [FCS]). DIF-1 and DIF-1 (3 M) were synthesized as previously described [10] and stored at −20 °C until use. STZ and a methylcellulose (MC) solution were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Four-week-old male Wistar rats were purchased from SLC Japan, Inc. (Shizuoka, Japan). Diabetic rats were induced by intravenous injection of STZ (40 mg/kg) in a 0.05 M citrate buffer (pH 4.5). All rats were fed standard rat chow diet (Nippon Clea Co., Tokyo, Japan).

2.2. Analysis of metabolites by use of CE-TOF-MS (metabolome analysis)

3T3-L1 cells were seeded in six 9-cm tissue culture dishes, each containing 10 ml of DMEM-HG, and incubated for 4 days until they reached

Table 1
Metabolite levels in control and DIF-treated cells.

Metabolite	Concentration (pmol/10^6 cells)				
	Control	DIF-1		3M	
	Mean (SD)	Mean (SD)	*P* value	Mean (SD)	*P* value
Glucose 6-phosphate (G6P)	697 (53)	628 (19)	0.448	483 (4.7)	0.177
Fructose 6-phosphate (F6P)	237 (5.0)	202 (2.5)	0.061	154 (8.4)	0.028
Fructose 1,6-diphosphate (F1,6P)	886 (97)	727 (87)	0.430	754 (67)	0.489
Glyceraldehyde 3-phosphate (G3P)	60 (0.6)	56 (20)	0.982	55 (18)	0.973
3-Phosphoglyceric acid (3PG)	340 (11)	368 (98)	0.960	423 (92)	0.655
2-Phosphoglyceric acid (2PG)	74 (4.8)	92 (6.1)	0.153	102 (34)	0.685
Phosphoenolpyruvic acid (PEP)	109 (1.8)	114 (22)	0.964	158 (24)	0.336
Pyruvic acid (Pyr)	204 (0.8)	238 (128)	0.964	330 (4.5)	0.021
Acetyl CoA_divalent (AcCoA)	N.D. (N.A.)	N.D. (N.A.)	–	N.D. (N.A.)	–
Malonyl CoA_divalent (MalCoA)	N.D. (N.A.)	N.D. (N.A.)	–	N.D. (N.A.)	–
Citric acid (Cit)	910 (109)	2312 (914)	0.511	2941 (427)	0.243
cis-Aconitic acid (cis-Aco)	102 (6.9)	202 (62)	0.427	202 (55)	0.384
Isocitric acid (IsCit)	54 (7.8)	127 (56)	0.246	152 (35)	0.139
2-Oxoglutaric acid (2OG)	519 (31)	438 (46)	0.361	795 (20)	0.030
Succinic acid (Suc)	238 (30)	572 (85)	0.149	*2722 (41)	0.001
Fumaric acid (Fum)	153 (8.4)	230 (77)	0.612	590 (94)	0.157
Malic acid (Mal)	593 (33)	860 (207)	0.506	2042 (93)	0.027
NAD^+	1605 (289)	1397 (543)	0.943	1563 (360)	0.999
ADP	781 (32)	874 (11)	0.211	826 (172)	0.969
ATP	10,060 (994)	8412 (881)	0.426	8645 (2036)	0.783

Confluent 3T3-L1 cells were incubated for 3 h with 0.1% DMSO (Control), 20 μM DIF-1, and 20 μM DIF-1(3M) in duplicate, and intracellular metabolite levels per 10^6 cells were determined by use of CE-TOF-MS as described in the Materials and methods section.
N.D., not detected.
N.A., not available.
The *P* value for each metabolite versus Control is indicated.
* $P < 0.01$ versus DIF-1 group (by ANOVA).