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Effect of dimethyl fumarate on rats with chronic pancreatitis

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ABSTRACT

Objective: To discuss the effect of dimethyl fumarate (DMF) on rats with *L*-arginine induced chronic pancreatitis (CP).**Methods:** Male Wistar rats were given DMF treatment (25 mg/kg) by oral lavage method; then Wistar rats were given the intraperitoneal injection of *L*-arginine for 5 times (250 mg/100 kg, twice per time, each interval of 1 h) for building of CP model. Rats were divided into control group, CP group, DMF group and CP + DMF group. Rats in CP + DMF group were given the oral intragastric administration of DMF (25 mg/kg), while rats in control group and CP group were given the equal volume of normal saline. The weight of rats was evaluated and the intraperitoneal glucose tolerance test was performed (IPGTT, 2 g/kg). The islet of rats was isolated and then flow cytometry was employed to evaluate the quality and activity of islets. Meanwhile, the histology of non-endocrine tissues and levels of myeloperoxidase (MPO) and malondialdehyde (MDA) were detected.**Results:** Compared with control group, the weight of rats in CP group was significantly reduced at week 2, 4 and 6; the blood glucose significantly increased, AUC increased, the histopathological scores of pancreatic atrophy, acinar injury, edema and cellular infiltration increased, levels of MDA and MPO increased, the islet equivalent and islet activity decreased at 0, 30, 60, 120 and 180 min. Compared with CP group, the weight of rats in CP + DMF group significantly increased at week 2, 4 and 6; the blood glucose significantly decreased, AUC decreased, the histopathological scores of pancreatic atrophy, acinar injury, edema and cellular infiltration decreased, levels of MDA and MPO decreased, the islet equivalent and islet activity increased at 0, 30, 60, 120 and 180 min.**Conclusions:** DMF treatment can improve CP induced by *L*-arginine and islet function in rats.

1. Introduction

Chronic pancreatitis (CP) is a kind of progressive inflammatory diseases, which would cause the damage and fibrosis of pancreas parenchyma and eventually lead to endocrine and exocrine function obstacle [1–3]. Statistics indicated that CP incidence was still increasing [4]. In a structured questionnaire, CP patients indicated CP disease had the severe impact on their body, social and psychological health [5]. At present, the

main therapeutic method for CP is supportive care, including nutrition and pain control [6,7]. But the specific pathogenesis has not been clear and thus the clinical effect of main treatments was poor [8,9].

Dimethyl fumarate (DMF) is used in the treatment of patients with multiple sclerosis [10,11]. The mechanism for DMF has not been clear yet. However, its unique antioxidative and anti-inflammatory features have already been proved [12–14]. CP could induce the oxidative stress and inflammation response in islet endocrine and non-endocrine cells. Thus in this study, it's presumed that DMF might inhibit the development of CP through the antioxidant and anti-inflammatory response. By building the rat model of CP and feeding rats with the certain dose of DMF, it is to discuss the inhibition and mechanism of DMF on the inflammation and oxidative stress of rats with CP.

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2. Materials and methods

2.1. Materials

2.1.1. Animals

Male Wistar rats (250–300 g) were purchased from Hebei University of Engineering laboratory, and all rats were raised in a sterile environment at $(22 \pm 2)^\circ\text{C}$, free access to eating and drinking, with fasting for 12 h before experiment.

2.1.2. Reagents

L-arginine and DMF were purchased from Sigma; HE, TMRE and 7-AAD dye from Sigma; malondialdehyde (MDA) kit and myeloperoxidase (MPO) kit from Nanjing Jiansheng Biological Products Research Institute; collagenase V from Sigma; dithizone (DTZ) from Shanghai No.3 Reagent Factory; rabbit-derived polyclonal insulin and glucagon antibody from Santa.

2.2. Methods

2.2.1. Building of CP model

L-arginine was dissolved in NS (20%), with the intraperitoneal injection of *L*-arginine (250 mg/100 kg, twice per time, every interval of 1 h) for 5 times (day 1, 5, 9, 13 and 17); after 7 weeks, rats were put to death. From 24 h before building of CP model, rats were given DMF treatment (25 mg/kg) by oral lavage method. Rats in the control group and CP group were given the equal volume of normal saline. After 7 weeks of administration, rats were put to death.

2.2.2. Intraperitoneal glucose tolerance (GT) test (IPGTT)

At week 7 of modeling, the intragastric administration of 2 g/kg glucose was performed and the blood was collected from the caudal vein. The trace glucose meter was employed to detect the blood glucose of rats at fasting, 30, 60, 90, 120 and 180 min after dining, as well as the area under the curve (AUC), $\text{AUC} = 1/2 \text{ fasting value} + \text{value at 1 h} + \text{value at 2 h} + 1/2 \text{ value at 3 h}$.

2.2.3. Histological analysis

After 7 weeks, rats were put to death. Then the pancreas of rats was taken out for stabilization and the biochemical analysis was performed. Firstly, pancreas was fixed with 10% of formalin, embedded with paraffin, sliced into 5 mm pieces and then HE staining was conducted. Schmidt standard was adopted to evaluate the histological analysis based on the area of lesions, alveolus atrophy, acinar cell damage, fibrosis, interstitial edema and inflammatory cell infiltration.

2.2.4. Pancreas MDA and MPO detection

The partial pancreatic tissues of rats were collected for weighing. After that, the equivalent NS was added and 1% of homogenate (made from low temperature homogenate machine) was used for being centrifuged at room temperature (3000 g/min, 10 min) to obtain the supernatant. Finally, MDA content was detected according to the procedure of MDA kit. The extra partial pancreatic tissues of rats were collected for weighing. After that, the equivalent NS was added and 1% of homogenate (made from low temperature homogenate machine) was used for

being centrifuged at room temperature (3000 g/min, 10 min) to obtain the supernatant. Finally, MDA content was detected using the chemoenzymatic method. Operations should be strictly in accordance with the instruction manual.

2.2.5. Islet isolation and endocrine function evaluation

After taking out of the pancreas and weighing, common bile duct puncture and injection of cold collagen V (10 mL) digestive juices were conducted, which could make the pancreas adequate swelling. Afterwards, pancreas was put into cold collagen V (5 mL) digestive juices and digested in 37°C water bath for 10 min; then Hanks solution (contained 10% of fetal calf serum) was used for digestion termination, mesh screen filtration, centrifugal collecting of the islet. 50 μL of DTZ working solution was mixed with 50 μL of islet for 5 min. The cell mass numbers of scarlet DTZ staining was counted under the microscope, which was equivalent to the (150 μm in diameter) islet equivalent quantity according to formula conversion and then the total obtained islet equivalent quantity was calculated.

2.2.6. Islet activity evaluation

Cultured islet was separated into single-cell suspension liquid. Cells were placed into flow cytometry instrument for TMRE staining after being washed. TMRE was selectively combined with mitochondrial membrane to detect the cell apoptosis. After being washed, cell 7-AAD was stained. 7-AAD combined with cell DNA indicated the cell membrane permeability changes after cell death.

2.2.7. Islet alpha/beta cell ratio

Single-cell suspension liquid was placed in 4% of paraformaldehyde and was fixed for 10 min at room temperature. After continuous PBS washing, cell was incubated by polyclonal insulin in rabbit and anti-Glucagon. Afterwards, the goat-anti-rabbit secondary antibody was used for marking and the flow cytometry (FCM) was employed for the analysis.

2.2.8. Statistical analysis

Data was expressed by mean \pm sd. The *t* test or one-way analysis of variance was employed for the comparison between groups, where $P < 0.05$ indicated the statistical difference.

3. Results

3.1. Effect of DMF on weight of rats with CP

As shown in Table 1, as the time of modeling passed, the weight of rats in control group, CP group and CP + DMF group significantly increased, but the weight of rats in CP group was significantly lower than that in control group at week 2, with the

Table 1

Weight changes in each group (g).

Group	Week 2	Week 4	Week 6
Control group	110.45 \pm 10.78	167.42 \pm 15.63	198.42 \pm 15.63
CP group	105.39 \pm 10.20*	132.37 \pm 13.24**	167.37 \pm 15.38**
CP + DMF group	108.42 \pm 10.17#	158.48 \pm 16.13###	188.52 \pm 18.29###

Compared with control group, * $P < 0.05$, # $P < 0.01$; compared with CP group, ** $P < 0.05$, ### $P < 0.01$.

Table 2

IPGTT results in each group (mmol/L).

Group	0 min	30 min	60 min	120 min	180 min
Control group	4.05 ± 0.40	6.72 ± 0.66	6.34 ± 0.62	5.26 ± 0.53	4.16 ± 0.42
CP group	5.73 ± 0.52**	8.96 ± 0.90**	9.03 ± 0.92**	7.28 ± 0.75**	6.57 ± 0.66**
CP + DMF group	4.89 ± 0.50 ^{##}	7.31 ± 0.73 ^{##}	7.04 ± 0.70 ^{##}	6.11 ± 0.60 ^{##}	4.86 ± 0.49 ^{##}

Compared with the control group, [#]*P* < 0.01; compared with CP group, ^{##}*P* < 0.01.

statistical difference; the weight of rats in CP + DMF group was significantly higher than that in CP group, with the statistical difference (*P* < 0.05). The same situation was found at week 4 and 6. It indicated that, as the time of modeling passes, the weight of CP rats increased slowly, which was lower than that in the normal group. After being given GMF, the weight of CP rats was recovered gradually.

3.2. Improvement of DMF for GT in rats with CP

The results showed that the blood glucose level in CP group was significantly higher than that in the model group; while the blood glucose level in CP + DMF group was significantly lower than that in CP group, with the statistical difference (*P* < 0.01). Meanwhile, the same situation was found at 30 and 60 min. But at 120 and 180 min, the blood glucose level in control group, CP group and CP + DMF group was decreased a bit, but the one in CP group was still significantly higher than that in the model group and the one in CP + DMF group was significantly lower than that in CP group, with the statistical difference (*P* < 0.01) (Table 2).

The results showed that AUC value in CP group (22.46 ± 2.50) mmol/L·h⁻¹ was significantly higher than that in the control group, (15.70 ± 1.45) mmol/L·h⁻¹; while the one in CP + DMF group, (18.02 ± 1.82) mmol/L·h⁻¹ was significantly lower than that in CP group, with the statistical difference (*P* < 0.01).

3.3. Improvement of DMF for histopathology in rats with CP

L-arginine could cause pancreatic histologic changes in rats, including serious pancreatic acinar structure damages, edema and fatty infiltration. Oral administration of DMF treatment

could significantly improve the above symptoms. As shown in Table 3, the histopathological score of the area of lesions, acinar cell damage, edema and inflammatory cell infiltration in CP group was significantly higher than that in the control group; while the histopathological score in CP + DMF group was significantly lower than that in CP group, with the statistical difference (*P* < 0.01).

3.4. Improvement of DMF for biochemical parameter in rats with CP

Compared with the control group, the levels of MPO and MDA in CP group significantly increased. After the treatment of DMF, the levels of MPO and MDA in CP group significantly decreased. The islet equivalent and islet activity in CP group were significantly lower than those in the control group; while the islet equivalent and islet activity in CP + DMF group were significantly higher than those in CP group (Table 4).

4. Discussion

The results indicated the obvious difference of pathology, histology and pancreas biochemical markers between CP + DMF group and CP group. The weight of rats in CP + DMF group and CP group at week 2, 4 and 6 was significantly higher than that in CP group; while the blood glucose level at 0, 30, 60, 120 and 180 min was significantly lower than that in CP group and the histopathological score of the area of lesions, acinar cell damage, edema and inflammatory cell infiltration was significantly lower than that in CP group, which indicated that DMF could significantly improve the pathological changes of pancreas, the blood glucose level and weight of CP rats. The results of comparison in

Table 3

Histopathological score of rats in each group.

Group	Area of lesions	Acinar cell damage	Edema	Fibrosis	Inflammatory cell infiltration
Control	0.00 ± 0.00	0.00 ± 0.00	0.20 ± 0.02	0.00 ± 0.00	0.00 ± 0.00
DMF	2.10 ± 0.20**	2.90 ± 0.30**	1.30 ± 0.20**	0.50 ± 0.30	2.70 ± 0.30**
CP + DMF	0.80 ± 0.10 ^{##}	1.40 ± 0.20 ^{##}	0.90 ± 0.10 ^{##}	0.50 ± 0.40	1.00 ± 0.10 ^{##}

Compared with the control group, [#]*P* < 0.01; compared with CP group, ^{##}*P* < 0.01.**Table 4**

Levels of MPO and MDA and islet equivalent and islet activity of rats in each group.

Group	MPO (U/mg protein)	MDA (mmol/mg protein)	Islet equivalent (IEQ/g)	Islet activity (%)
Control group	0.70 ± 0.05	0.24 ± 0.02	2873.5 ± 290.4	80.30 ± 8.60
CP group	1.32 ± 0.14**	1.08 ± 0.10**	2451.4 ± 243.5**	49.60 ± 5.10**
CP + DMF group	0.89 ± 0.09 ^{##}	0.54 ± 0.05 ^{##}	2732.8 ± 275.3 ^{##}	74.50 ± 7.30 ^{##}

Compared with the control group, [#]*P* < 0.01; compared with CP group, ^{##}*P* < 0.01.

the degree of fibrosis between CP group and control group showed no significant difference, but it showed the certain tendency of damage. Besides, DMF could also improve the fibrosis of pancreas of L-arginine induced CP rats. The flow cytometry was employed to evaluate the quality and activity of islets. The results also indicated that DMF had the obvious protection for the islet of L-arginine induced CP rats.

DMF had unique antioxidative and anti-inflammatory feature. Although mechanism of DMF was not clear, its side-effect was smaller, which made its use for more than 20 years. Fumarate was mainly used in the treatment of psoriasis, which was based on its inhibition of lymphocyte proliferation. Recent reports found that DMF selectively increased Th2 cytokines and inhibited Th1 responses [15]. There were also researches showing that DMF decreased the gene expression of pro-inflammatory cytokines and chemotactic factors, and increased the expression of antioxidant molecules [16–18]. These findings suggested that DMF could be used in the treatment of autoimmune and inflammatory diseases, including multiple sclerosis. Other scholars reported that DMF played its antioxygenation role through Nrf2 activator [14,15]. DMF was likely to have multiple effective anti-oxidation mechanisms.

MPO is the marker of inflammatory stress, while MDA is the by-product of lipid peroxidation, as the marker of cell injury and oxidative stress. The results of this study indicated that the levels of MPO and MDA were significantly increased in CP group. After the treatment of DMF for CP rats, the levels of MDA and MPO significantly decreased, which indicated that DMF could improve the inflammatory and oxidative stress of CP rats. Other researches also found that the antioxidant effect of DMF could protect the islet from the effect of L-arginine induced inflammatory response [19,20]. It also indicated that DMF could reduce the levels of MPO and MDA to inhibit the inflammatory and oxidative stress of CP rats and thus protect the islet.

In conclusion, long-term DMF treatment can effectively improve the histopathological changes, biochemical abnormalities and beta cell function in rats with CP. In the further studies, it is necessary to confirm the efficacy and side effects of DMF in the clinical practice.

Conflict of interest statement

We declare that we have no conflict of interest.

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