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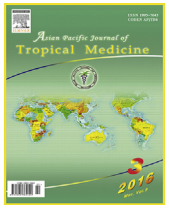

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Effect of dimethyl fumarate on rats with chronic pancreatitis

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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 ± 2) °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; collagenaseV from Sigma; dithizone (DTZ) from Shanghai No.3 Reagent Factory; rabbit-derived polyclonal insulin and glucagonantibody 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. Feom 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), AUC = 1/2 fasting value + value at 1 h + value at 2 h + 1/2 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 ± 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 ± 10.78	167.42 ± 15.63	198.42 ± 15.63
CP group	105.39 ± 10.20*	132.37 ± 13.24**	167.37 ± 15.38**
CP + DMF group	108.42 ± 10.17$^{\#}$	158.48 ± 16.13$^{\#\#}$	188.52 ± 18.29$^{\#\#}$

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