

The Efficacy of Marmot Brown Fat in Treatment of Acute Pancreatitis

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Objectives: The brown fat of marmots is called "human meat" among Mongolian people because they abstain from eating it. Our experimental study is the first academic-based study to investigate the effect of marmot brown fat (MBF) on acute pancreatitis (AP). **Methods:** This study used 82 healthy female Wistar rats (250-280 g). The study rats were divided into 4 groups: (1) relatively healthy (no medication and no AP, n = 10), (2) caerulein-induced AP rats without any treatments (n = 24), (3) AP rats fed by the MBF suspension (n = 24), and (4) AP rats injected with sandostatin (n = 24). The serum α -amylase level (SAAL) and histological examination were observed. **Results:** Significantly increased SAAL (group 1: 1347.10 \pm 10.76 units/L vs group 2: 1804.50 \pm 134.32 units/L) was confirmed in rats with caerulein-induced, mild AP. In both treatment groups 3 and 4, the SAAL was significantly decreased on the fifth day after treatment. Interestingly, advanced damage of pancreatic cells was observed in group 3. Additionally, the health condition of group 3 rats was poor, and spleen and lung tissue damage was detected by histological examination. **Conclusion:** Our study results suggest that the MBF suspension might be stimulating pancreatic juice secretion, therefore we conclude that the MBF suspension is not beneficial in the treatment of AP.

Keywords: Ceruletide; Pancreatitis, Acute Necrotizing; Marmota; Adipose Tissue, Brown

Introduction

Acute pancreatitis (AP) is an acute inflammation of the pancreas characterized by swelling and at times even destruction of pancreatic tissue. The most common causes of AP are

gallstones and excessive alcohol consumption. Other causes include medication, abdominal trauma, infections, and genetic abnormalities of the pancreas [1]. The retroperitoneal space location of the pancreas makes biopsy, detecting

histopathological structure and dysfunction, and controlling action of medication difficult in the clinical practice. Therefore, animal-based experimental study is useful to medical science in this case.

Mongolian statistical data from 2005 to 2009 showed pancreatitis and other related diseases increasing 1.7-fold during these 5 years and 41.4% of pancreatitis patients were treated in the Ulaanbaatar city [2]. Normal treatment of AP uses scientific medications, which follows treatment guidelines [3]. However, for many years Mongolian people have been using marmot brown fat (MBF) for treatment of pancreatitis. The Mongolian people called it "human meat" because they abstain from eating it.

The marmot is a species of the rodent in the family Scuriidae. It is found in China (Inner Mongolia), northern and western Mongolia, and Russia. By our hypothesis the "human meat" of the marmot might have been firstly noted in a small religious sect of the Tibetan medicine. A Russian study based on questionnaires and interviews showed that all organs of marmot are used for treatment of some disease [4]. But, no previous scientific results describing an association between MBF and pancreatitis were found in the literature. Therefore in this animal-based experimental study we purposed to the determine effect of MBF on caerulein-induced AP.

Materials and Methods

The experiment was conducted with 82 female Wistar rats weighing 250-280 g. They were maintained in a room at a controlled temperature of $22 \pm 2^\circ\text{C}$ with 12-hour light/dark cycles and fed an *ad libitum* diet. This study was conducted in accordance with the "Ethics of Biomedical Study Guideline for Animal-Based Experiment" of the Mongolian Ministry of Health. The MBF was taken out from the subclavian of the marmot for the purpose of treating AP of rats under a license of slaughter from the Mongolian Ministry of Nature, Environment, and Green Development.

1. Caerulein -induced AP

AP was induced by tail vein injection of caerulein (5 $\mu\text{g}/\text{kg}$, Sigma Aldrich, USA) four times at one-hour intervals [5]. Mild AP was confirmed 12 hours after the last dose of caerulein by the serum α -amylase level (SAAL) and a histological evaluation

of the pancreas [6]. During the experimental time, the rats were fed by liquid water since standard food might have induced some signs of pancreatitis or influenced biochemical results.

2. Treatment of AP

A suspension of MBF was prepared so that rats could be fed by catheter, so as to avoid digestion in the mouth of the rats. To prepare the MBF suspension, first the extracted brown fat was transported at -20°C in a dedicatory icebox. Next, connecting tissue and capsules from pestled MBF preparation were removed. Then, 20 mL distilled water was added to the remaining parts. This MBF solution was administered one time daily over three days. The daily dose was 2 mL per day.

For rats treated with sandostatin, a 4- $\mu\text{g}/\text{kg}$ dose of sandostatin (0.05 mg/mL, Octreotide, Novartis Pharma, Switzerland) was administered through their tail vein. The tail vein injection method was in accordance with the "Institutional Animal Care and Use Committee, IACUC" protocol [7]. The sandostatin was administered one time daily over three days as 0.3 mL of solution made from 0.1 mL sandostatin and 10 mL of 0.9% saline.

3. Experimental protocols

The caerulein-induced AP model consisted of four groups in this study. Group 1 was 10 control rats. Group 2, 3, and 4 were caerulein-induced AP Groups with 24 rats each. Group 3 was treated with the suspension of MBF by catheter and group 4 was treated with sandostatin injection through their tail vein 12 hours after the last dose of caerulein. Group 2 did not have any treatment. Experimental study was continued for 12 days.

4. Biochemical analysis

Cardiac puncture in accordance with "Guidelines for Collection of Blood Laboratory Animals" was used to collected blood from the rats [6]. Cardiac puncture is the preferred technique for terminal collection of large blood volumes. The SAALs were determined by the Fully Automatic Biochemical Analyzer (FA-300, Clindia Systems B.A.B.V, Belgium).

5. Histological examination

Preparation of histological slides consisted of fixing, processing, embedding, sectioning and staining. First, tissues were fixed and dehydrated with 10% formaldehyde for 48 hours. Second,

the tissue was placed in warm paraffin wax which filled the spaces that have water in them. Tissue-Tek VIP 5 Jr. (Sakura LLC, Japan) was used to dehydrate the tissue. Third, the tissue was trimmed and mounted for cutting by a DSC 2 microtome (Leica Biosystems, USA). Thin sections were cut for subsequent staining and mounting on microscope slides. Fourth, the tissue was stained by hematoxylin and eosin. Fifth, slides were viewed with a light microscope (Olympus, USA) and photos were captured by an MU 500 5.1MP camera (AmScope, USA).

6. Statistical analysis

Data are presented as the mean and standard deviation (SD). Comparative results between group 1 with other groups were produced by a Student's t-test. The data analysis was performed using SPSS (version 18.0). A p-value of 0.05 was considered statistically significant.

Results

1. Serum α -amylase level with AP

The injection through the tail vein protocol of caerulein (0.4 mL/hour) for groups 2, 3 and 4 was completed four times at one-hour intervals to induce AP in the rats. The mild AP was confirmed by SAAL results and pancreatic histology evaluation. SAAL was significantly ($p < 0.02$) increased in group 2 rats compared to group 1. SAAL was 1347.10 ± 10.76 units (U)/L in group 1 and 1804.50 ± 134.32 U/L in group 2 twelve hours after the last dose of caerulein. After AP was confirmed, the treatment was started.

SAAL was 2005.73 ± 110.69 U/L in group 2, 2352.45 ± 15.36 U/L in group 3, and 1953.77 ± 96.04 U/L in group 4 on the third day of experiment. On the fifth day, the health condition of rats in group 3 was poor as their movement was slower than other days, they had decreased appetite and the rats died because of breathing and heart dysfunction. During the cardiac puncture the blood viscosity of group 3 was higher than normal and the blood color was like bistre. The macrostructure of the pancreas did not distinguish from fatty tissue, and additionally rats had dedicated hydrothorax, cardiac hypertrophy, and pneumonia. The health condition rats in group 4 was mild, but from the fifth day, the experiment treatment was stopped. For group 2, on the fifth day of the experiment, SAAL was decreased (1787.17 ± 74.25 U/L) when compared to the third day of the experiment

(1804.50 ± 134.32 U/L), but the change was not significant ($p > 0.05$). In contrast, SAAL was statistically decreased in group 3 (591.15 ± 88.61 U/L, $p < 0.001$) and group 4 (983.40 ± 27.16 U/L, $p < 0.001$) between the fifth and third day of experiment (Figure 1).

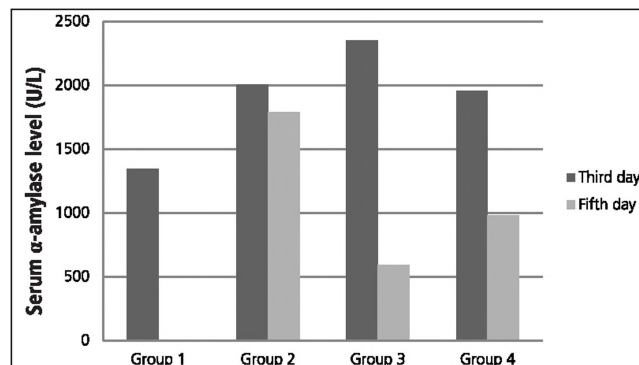


Figure 1. The serum α -amylase levels on the third and fifth day of experiment.

2. Histological evaluation of pancreatic damage

The observed change in our study resembles a mild form of AP, characterized by acinar cell adhesion caused by apical pole enzyme secretion of acinar cells (Figure 2A). The ducts lumen was empty between acinus (Figure 2B) and the veins were engorged (Figure 2C).

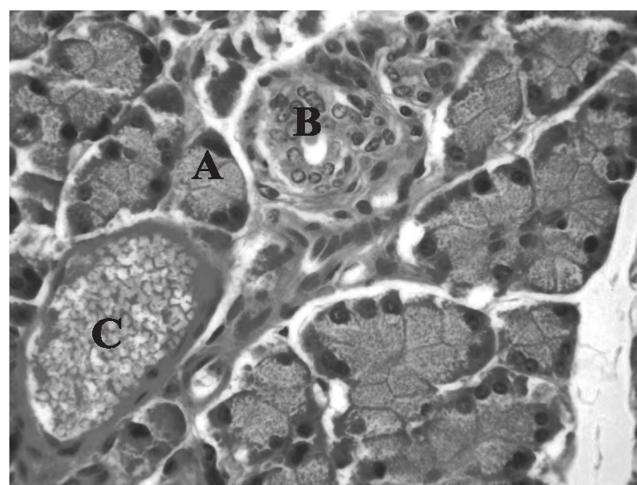


Figure 2. Pancreatic microstructure of a section with AP stained by hematoxylin and eosin at 400x magnification. Observed is: (A) acinar cell adhesion by apical pole, (B) empty duct between acinus, and (C) engorged veins between acinus.

The histological evaluation of the pancreas was completed for three rats from each group (2, 3, and 4) on the third day of the experiment. Dilated acinar cells pressed on the peripheral vessels and intermediate tissue was filled with enzyme in group 2 (Figure 3C). The acinar cells were filled with enzymes, and ducts of the pancreas were invisible since they were narrowed in group 3 (Figure 3A). Additionally, intermediate fluid accumulation (edema) was observed with peripheral vascular stenosis (Figure 3A). In group 4, the pancreatic exocrine cells were filled with enzymes, and intermediate tissue edema was lower compared with group 3 (Figure 3B). The peripheral vascular circulation was normal (Figure 3B).

On the fifth day of the experiment, the health condition of rats in group 3 was poor. Maladjustment and inactivity modification were observed and three rats died. Therefore, blood was collected and the pancreas was examined histologically. The

histological examination of the pancreas in group 3 rats showed that: (1) the acinar cell configuration was changed to be located near the vessels and the cell nucleus was pressed as a result of edema (Figure 4A), (2) the acinar cell configuration was changed and the intermediate fluid accumulation (edema) was increased (Figure 4B), (3) the histological change was similar with (1) and (2) and additionally microvascular strokes were observed (Figure 4C).

Due to the rat health condition and the pancreas histological results, treatments were stopped from the fifth day of the experiment. Also, inflammation of the lungs, infiltration of the spleen and sticky pancreas tissue was observed. After stopping treatment six rats were selected from each group on the sixth day for histological examination. The changed shape of the acinar cell was revived and vascular dilatation was observed in group 3.

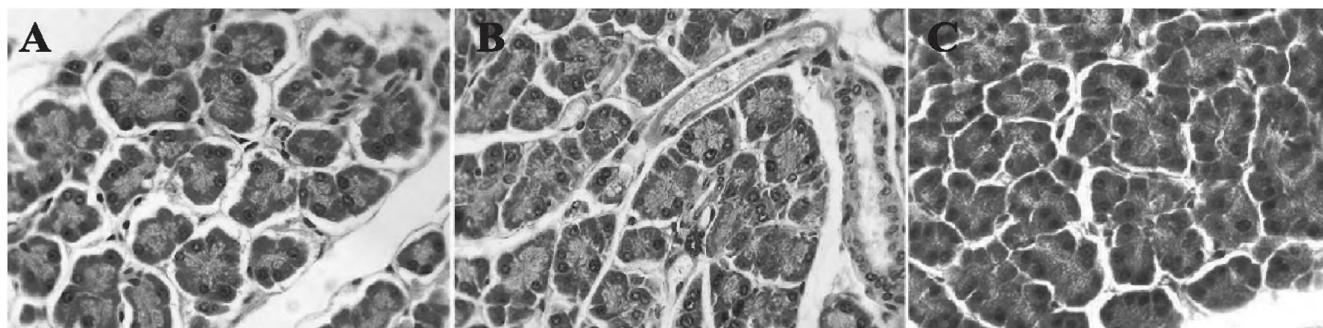


Figure 3. Pancreatic microstructure on the third day of the experiment for a section stained by hematoxylin and eosin at 400x magnification. Observed is: (A) pressed nucleus and edema in group 3, (B) lower edema and normal microvascular structure in group 4, (C) acinar cells filled with enzymes leading to pressed vessels and intermediate tissue in group 2.

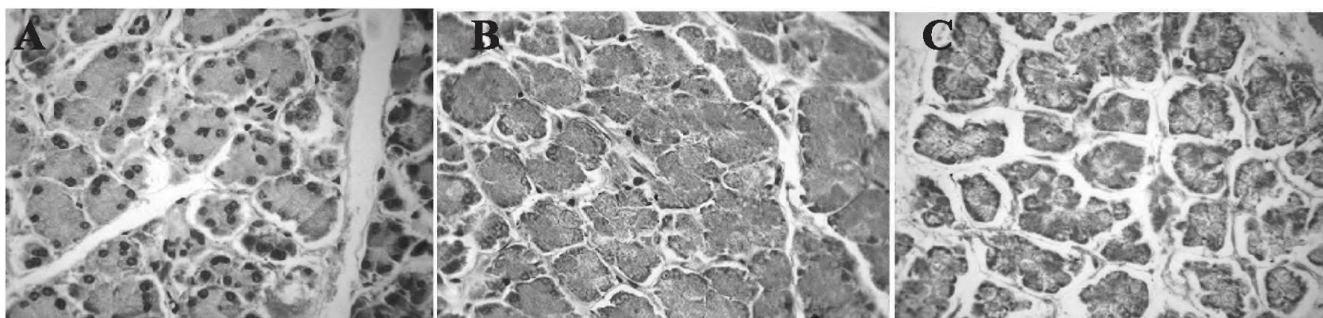


Figure 4. Pancreatic microstructure on the fifth day of the experiment for group 3 for sections stained by hematoxylin and eosin at 400x magnification.

Discussion

Our study is the first study to use a MBF suspension in the AP rat. Although Mongolian people have been using MBF suspension for treatment of pancreatitis (acute or chronic pancreatitis is not clear) for many years, there have been no scientific-based results confirming its efficacy. Therefore, the main purpose of our basic study was to determine the efficacy of MBF suspension in treating pancreatitis. These study results might be a source of a novel medication for pancreatitis in the future but not AP.

In this study, we confirmed AP using SAAL. Matull et al. showed that amylase level is one of the biochemical markers of AP and is the most commonly used in the clinical practice to confirm AP [8]. Caerulein was effective at inducing mild AP as confirmed by the SAAL in the rats. SAAL was not significantly different in group 2 between the third (2005.73 ± 110.69 U/L) and fifth (1787.17 ± 74.25 U/L) days of the experiment. In both treatment groups, SAAL was significantly decreased on the fifth day of the experiment. In group 4, acinar cell damage was relatively lower than group 3. The lower damage of acinar cell might be related to the sandostatin (octreotide) having beneficial effect in the treatment of severe AP [9]. Interestingly, the pancreatic cell advanced damage (microvascular strokes, intermediate fluid accumulation, acinar cell configuration and autolysis) was observed in group 3. Additionally, the health condition of group 3 rats was poor and spleen and lung tissue damage was detected by histological examination. Secretion of pro-inflammatory mediators such as interleukin 1 (IL), tumor necrosis factor- α , and IL-6 lead to pancreatic cell necrosis and cell death [10-12].

Regarding results related to MBF, Purevdorj et al. determined chemical composition of MBF. The study results showed linoleic acid at 33.11% in MBF and that it is different between marmot lamb and infants [13]. Furthermore Dugarsuren and Dagdanbazar and Nyamdorj et al. found lymphoid cells in MBF [14, 15]. The researchers suggest MBF might have an immune activity function [14, 15].

The importance of our study is that it for the first time MBF suspension was used with caerulein-induced AP in rats. MBF was important for early Mongolian people and is important today as it is still being used in medical treatment. However, our current study was limited to only investigating treatment of AP and not chronic pancreatitis due to several reasons. Capturing

marmot must be done under license of slaughter by the Ministry of Nature, Environment, and Green Development of Mongolia and marmot can carry plague, their activity is dependent upon season, and their purchase price is expensive.

In conclusion, our study results suggest that the MBF suspension might be stimulating pancreatic juice secretion. Therefore, the suspension is not beneficial in the treatment of AP. Future medical study should clarify MBF suspension dose and effects of chronic pancreatitis.

Conflict of Interest

The authors state no conflict of interest.

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