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Effect of Metformin on Lactate Metabolism in Normal Hepatocytes under High Glucose Stress in Vitro

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ABSTRACT

Objective To study the effect of metformin on lactate metabolism in hepatocytes in vitro under high glucose stress. In vitro LO2 cells, liver cells were randomly divided into blank control group, 25 tendency/L glucose solution, 27 tendency/L glucose solution, 29 tendency/L glucose solution, 31 tendency/L glucose solution, 33 tendency/L glucose solution, 35 tendency/L glucose solution treatment group, the optimal concentration of 31 tendency after L, use 30 tendency for L metformin solution, and then divided into blank control group, the optimal concentration of glucose solution, normal liver cells + metformin solution normal liver cells. The optimal concentration of glucose solution normal liver cells + metformin solution respectively in the 12 h, 24 h, 48 h on cell count plate to calculate the number of liver cells, and using lactic acid determination kit the optimal concentration of glucose solution + normal liver cells and normal liver cells + the optimal concentration of glucose solution + metformin solution respectively in the 12 h, 24 h, 48 h of cell cultures of lactic acid value. There was no significant change in the lactic acid concentration but significant increase in the number of surviving hepatocytes in the high-glycemic control group compared with that in the high-glycemic control group without metformin. Metformin has no significant effect on lactic acid metabolism of hepatocytes under high glucose stress in vitro, and has a protective effect on hepatocytes under high glucose stress. Based on this, it is preliminarily believed that metformin is not the direct factor leading to diabetic lactic acidosis.

1. Introduction

With the change of people's lifestyle and habits, the incidence of diabetes has been on a straight rise. At present,

1.01 million new diabetes patients are newly diagnosed in China every year. It is predicted that the number will reach 59 million in 2025, and more than 90% of the patients will be type II diabetes mellitus (T2MD)^[1-3]. China

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has the largest number of diabetic patients^[4]. Because the occurrence and development of diabetes involve a variety of factors, the pathogenesis is complex, and there is a variety of damage mechanism interaction, so far there is no cure.

Clinically, traditional hypoglycemic drugs have played a good role in controlling blood glucose and delaying the occurrence of complications, but there are still some limitations and adverse reactions. Metformin is an oral metformin antidiabetic drug, which is widely used in clinic because of its significant hypoglycemic effect and multi-faceted clinical application value. Metformin is also regarded as the preferred hypoglycemic drug in the world. Home and DDP studies have demonstrated the safety and efficacy of metformin in the treatment of diabetes^[5]. However, with the increasing application of metformin in clinical practice, some side effects have been gradually shown in the use of metformin as a hypoglycemic agent, among which lactic acidosis (LA) has been a concern of clinicians. Patients taking metformin caused by lactic acidosis, called metformin correlation lactic acidosis (MALA), MALA is the use of metformin treatment in the process of unusual and severe adverse reactions, is due to the metformin hindered the pathways of the lactic acid to glucose in the mitochondria, the cause of lactic acid in the body to generate too much or too little, caused the body to produce the metabolic disorders, the mortality of the disease is very high^[6-7]. Studies have shown that the lactic acidosis caused by metformin may be related to the severe diseases of diabetic patients^[8], which is mainly related to renal insufficiency, cardiac insufficiency and hypoxia of patients. Based on this, this paper will elaborate the effects of metformin intervention on lactic acid metabolism of normal liver cells under high glucose stress in vitro, and then explore whether metformin has a direct correlation with lactic acid metabolism, so as to provide reference for the clinical treatment of diabetes and the rational use of metformin.

2. Materials and Methods

2.1 Main Reagents and Instruments

Reagents: LO2 liver cell line, DMEM medium, trypsin, penicillin-streptomycin double antibody mixed solution, metformin, 10% fetal bovine serum, lactate assay kit (all purchased from Haikou Ruike Biological Technology Co., Ltd.) were selected.

Instruments: microscope, ultra-clean biological platform, CO₂ cell incubator.

2.2 Experimental Methods

2.2.1 Cell culture

LO2 cells with 10% fetal bovine serum DMEM culture completely, at 37 °C and 5% CO₂ incubator in the breeding, cultivation to the cell wall, routine cell culture, 1 ~ 2 days in a fluid, when the culture cell coverage was 80% ~ 90%, subculture, repeated operation, will have a bottle of the represented the cells cryopreserved, in order to avoid accident cause lack of LO2 liver cells to experiment.

2.2.2 Group Model Establishment Control Experiment

2.2.2.1 Establishment of High Glucose Model

Before the beginning of this experiment, LO2 liver cells were pretreated with high Glucose, and the optimal concentration of Glucose (G) was tested. The concentration gradient of G was set as 0 mmol/L, 25 mmol/L, 27mmol/L, 29mmol/L, 31 mmol/L, and 33 mmol/L. The preexperiment obtained 31 mmol/L as the optimal concentration, and the lactic acid content was measured with the lactic acid kit.

2.2.2.2 Establishment of Metformin Model

In this experiment, metformin hydrochloride tablets were diluted to 30mmol/L into cell culture medium to pretreat LO2 hepatocytes with metformin for 12 h, 24 h and 48 h.

2.2.2.3 Establishment of Intervention Model

In this experiment, 30mmol/L of metformin hydrochloride culture solution and 31mmol/L of glucose solution were added into the culture flask. After culture for 12 h, 24 h and 48 h, the lactic acid content was measured with the lactic acid kit.

2.2.3 Hepatocyte Proliferation was Determined by Cell Count Method

Trypsin was used to decompose the cells, and after digestion for a period of time, the culture solution was added to stop digestion, and then the culture solution was transferred to the counting plate with pipetting gun, and the cells were counted in strict accordance with the cell counting rules.

2.2.4 Lactic Acid in Samples was Detected by Kit Method

In addition, metformin solution and high-glucose treated cell solution were added and put into carbon dioxide cell incubator for culture for 12 h, 24 h and 48 h, and the lactic acid value in the cell culture medium was measured by the lactic acid kit respectively, so as to judge the effect

of dimethyldiastema on cells under high glucose.

2.3 Statistical Methods

Statistical analysis SPSS25.0 statistical software was used for data processing. Sample t test was used for sample mean and one-way analysis of variance was used for multiple mean. $P < 0.05$ was considered statistically significant.

3. Results and Analysis

3.1 Selection of the Most Suitable Liver Cells with High Glucose Concentration

After this experiment, the most suitable concentration of G in LO2 liver cells under high glucose stress was explored, as shown in Table 1. When the concentration of G was 31 mmol/L, the most appropriate concentration was

found.

3.2 Effects of Metformin on Lactic Acid Metabolism of Normal Liver Cells under High Glucose Stress

30 mmol/L metformin was prepared in the medium, and the control group and the experimental group were set. Normal liver cells, high glucose + normal liver cells, metformin + normal liver cells, and high glucose + metformin + normal liver cells were designed respectively. The changes of lactic acid concentration measured at 12 h, 24 h and 48 h of culture were shown in Table 2.

According to the experimental group and control group, the metformin itself does not cause liver cells to produce lactic acid, under the environment of high sugar, according to the results of the experiment concerning group contrast, before adding metformin, the concentration of lactic

Table 1. Selection of the most suitable liver cells with high glucose concentration (normal liver cells)

The concentration of added glucose (mmol/L)	25	27	29	31	33	35	0
Number of surviving liver cells (12h)	1.12×10*7	1.06×10*7	1.02×10*7	0.98×10*7	0.88×10*7	0.40×10*7	1.30×10*7
Number of surviving liver cells (13h)	2.04×10*7	1.97×10*7	1.95×10*7	1.92×10*8	1.72×10*8	1.25×10*8	2.60×10*7
Number of surviving liver cells (14h)	3.99×10*7	3.92×10*7	3.79×10*9	3.76×10*9	3.63×10*9	3.19×10*9	5.20×10*7
Number of surviving liver cells (15h)	1.68±0.29	2.46±0.79	3.27±1.38	3.48±1.42	4.63±1.63	5.34±2.26	0
Number of surviving liver cells (16h)	3.89±1.42	4.79±1.68	5.24±2.17	5.89±2.64	6.23±2.79	8.98±2.86	0
Number of surviving liver cells (17h)	6.72±2.33	8.43±2.56	9.14±2.99	9.28±3.11	10.87±3.34	11.27±3.39	0

Table 2. Effects of metformin on lactic acid metabolism of normal liver cells under high glucose stress

	Group 1 (liver cells in normal group)			Group 2 (normal + high glucose)		
	12h	24h	48h	12h	24h	48h
Add glucose concentration mmol/L	/			31		
Add metformin concentration (mmol/L)	/			/		
Lactic acid concentration (mmol/L)	0	0	0	3.48±1.42	5.89±2.64	9.28±3.11
Number of surviving liver cells (1)	1.30×10*7	2.60×10*7	5.20×10*7	0.98×10*7	1.92×10*8	3.76×10*9
	Group 3 (normal group + metformin)			Group 4 (hepatocytes + high glucose + metformin)		
	12h	24h	48h	12h	24h	48h
Add glucose concentration mmol/L	/			31		
Add metformin concentration (mmol/L)	30			30		
Lactic acid concentration (mmol/L)	0	0	0	3.30±1.39	5.83±2.63	9.17±3.08
Number of surviving liver cells (unit)	1.30×10*7	2.60×10*7	5.20×10*7	1.15×10*7	2.24×10*7	4.79×10*7

acid in culture medium of high glucose + normal liver cells increased from 1.42 mmol/L to 3.48 mmol/L in 12 h, and the highest value increased to 5.89 mmol/L after 24 h. After 48 h, the increase rate of cell number began to decrease significantly, and the lactic acid concentration also increased to 9.289 mmol/L, and after adding metformin, There were no significant changes in lactate concentration and cell number compared with those under high glucose stress alone.

4. Discussion

A large number of studies have shown that lactic acidosis is a rare and serious complication of diabetic patients, most of which occur in patients who take guanidine drugs and are accompanied by liver and kidney insufficiency, heart failure, etc.^[9-10]. In recent years, studies have suggested that Metformin Lactate Acidosis (MALA) caused by normal therapeutic doses of Metformin is rare, but it may also lead to elevated plasma lactic acid levels and even Lactate Acidosis (LA) if it is improperly used in clinical practice^[11]. Liver is an important organ of glucose metabolism. Liver can absorb and use glucose to reduce blood sugar, and can convert glucose into liver glycogen for storage. Patients with cirrhosis have increased insulin resistance, which will affect glucose metabolism and cause hepatogenic diabetes. Diabetes can also affect the liver, especially patients with type 2 diabetes who are prone to liver function damage and non-alcoholic fatty liver disease^[12]. As a traditional hypoglycemic agent, metformin can promote the metabolism of glucose, increase its anaerobic colysis, improve the level of lactic acid and lead to lactic acidosis. In addition, metformin can inhibit the utilization of lactic acid by the liver and muscle and inhibit gluconeogenesis, thus reducing the production of glucose, and thereby increasing the risk of lactic acid poisoning by accumulation of lactic acid^[13-15].

In this study, it was found that metformin had little effect on the metabolism of lactic acid in liver cells under high glucose environment, and there was no significant difference in the content of lactic acid measured between the experimental group and the control group after adding different levels of metformin, but different levels of metformin could promote cell proliferation. High glucose environment can inhibit the proliferation of liver cells, the reason may be that high glucose induces the expression of STC2 in liver cells, and overexpression of STC2 can further enhance the proliferation inhibition ability of liver cells induced by high glucose^[16]. In addition, high glucose has also been shown to promote the secretion of inflammatory cytokines such as TNF - α , IL - 6, and regulate the expression of apoptosis-related molecules

B lymphoma 2 and Bax, thereby inducing apoptosis of hepatocytes^[17]. Metformin promotes the proliferation of hepatocytes, possibly because it inhibits the secretion of inflammatory cytokines and the activity of nuclear factor- κ B (NF- κ B) through AMPK-dependent pathways, so as to promote cell proliferation^[18].

In conclusion, metformin has no significant effect on lactic acid metabolism of hepatocytes in high glucose environment, but different concentrations of metformin have a protective mechanism for hepatocytes and can promote cell proliferation.

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