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

Jianhao Wu[1] Qinnan Zhang[1] Yubiao Yang[1] Chunyue Wang[1] Xindi Yue[1] Zhilu Li[2] Pingping Yan[3*]

1. School of International Education, Hainan Medical University, Haikou, Hainan, 570100, China
2. The First Clinical College of Hainan Medical University, Haikou, Hainan, 570100, China
3. Clinical Skills Center, Hainan Medical University, Haikou, Hainan, 570100, China

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ABSTRACT

To study the effect of metformin on lactate metabolism in hepatocytes in vitro under high glucose stress. Method: LO2 hepatocytes was cultured in vitro, hepatocytes were randomly divided into blank control group, 25 mmol/L glucose solution, 27 mmol/L glucose solution, 29 mmol/L glucose solution, 31 mmol/L glucose solution, 33 mmol/L glucose solution, 35 mmol/L glucose solution treatment group, after determining the optimal concentration as 31 mmol/L, use 30 mmol/L metformin solution, and then divided into blank control group, normal hepatocytes + the optimal concentration of glucose solution, normal hepatocytes + metformin solution , normal hepatocytes+. The optimal concentration of glucose solution normal hepatocytes + metformin solution, calculate the number of hepatocytes on cell count plate respectively in the 12 h, 24 h, 48 h, and use the lactic acid kit to determine the lactic acid value of the cell culture medium of normal liver cells + optimal concentration glucose solution and normal liver cells + optimal concentration glucose solution + metformin solution at 12 h, 24 h, and 48 h, respectively. Results: 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. Conclusions: 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 is on the rise. At present ,1.01 million new diabetic patients are added every year in China. Of the 59 million people projected to reach 2025, more than 90 per cent are II diabetes (T2MD) [1-3]. China has the largest number of diabetics [4]. Because the development of diabetes involves many factors, the pathogenesis is more complex, and there is a variety of damage mechanisms interaction, can not be cured. Traditional antidiabetic drugs play a good role in controlling blood glucose and delaying

**Corresponding Author:*
Pingping Yan,
Clinical Skills Center, Hainan Medical University, Haikou, Hainan, 570100, China;
Email: 1289762133@qq.com

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complications, but there are still some limitations and adverse reactions. Metformin is an oral antidiabetic drug of biguanidine. Because of its remarkable antidiabetic effect and clinical application value, metformin is widely used in clinic. Metformin is also regarded as the first-line antidiabetic drug in the world. HOME and DDP experimental studies have demonstrated the safety and efficacy of metformin in the treatment of diabetes [5]. However, with the increase of clinical application of metformin, some side effects have been gradually shown during its use as an antidiabetic drug, among which lactic acidosis (LA) is a concern of clinicians. Patients with lactic acidosis caused by taking metformin, is called as Metformin-related lactic acidosis (MALA),MALA is a rare and severe adverse reaction during metformin treatment because metformin hindered the pathway of lactic acid to glucose in mitochondria, causing Too much lactic acid produced or too little enimated in the body, leading to metabolic disease, which has a very high mortality rate [6-7]. Studies have shown that metformin-induced lactic acidosis may be associated with severe disease in diabetic patients themselves [8]. It is mainly related to renal insufficiency, hypoxia and so on. Based on this, this paper will explain the effect of metformin on lactic acid metabolism in normal hepatocytes under in vitro high glucose stress, and then explore whether metformin has a direct correlation with lactic acid metabolism. To provide reference for clinical treatment of diabetes and the rational use of metformin.

2. Materials and Methods

2.1 Main Reagents and Instruments

Reagent: Cell selected LO2 hepatocyte strain, DMEM medium, trypsin, penicillin-streptomycin double antibody mixed solution, metformin, 10% fetal bovine serum, lactate assay kit (all purchased from Haikou Ruike Biotechnology Co., Ltd).

Instruments: Microscope, Ultra-clean Biological Table, CO_2 Cell incubator.

2.2 Experimental Methods

2.2.1 Cell Culture

LO2 cells were used in a DMEM complete culture medium of 10% fetal bovine serum,Cultured in 37℃ , 5% CO_2 incubator. The culture box was cultured to the cell wall, and the conventional cell culture was changed once ~2 days. When the cell coverage reached 80% and 90%, take a transgenral culture, repeat operation. Freeze a bottle of subcultured cells to avoid accidental lack of LO2 hepatocytes.

2.2.2 A Comparative Experiment 1.2.2 Grouping Model

Establishment of high sugar model

High glucose pretreatment of LO2 hepatocytes was performed before the experiment, was performed (Glucose, G)the optimal concentration test, G concentration gradient is set as 0 mmol/L, 25 mmol/L, 27 mmol/L, 29 mmol/L, 31 mmol/L, 33 mmol/L, 31 mmol/L calculated out as optimal experimental concentration, The lactic acid content was measured by lactic acid kit.

Establishment of the metformin model

Metformin hydrochloride tablets were diluted to 30 mmol/L to cell culture medium and pretreated LO2 hepatocytes for, the culture time is 12 h, 24 h, 48 h.

Establishment of intervention model

The metformin hydrochloride medium with 30 mmol/L concentration and glucose solution with 31 mmol/L concentration were added to the culture bottle. After 12 h, 24 h, 48 h of culture, the lactic acid content was calculated by lactic acid kit.

2.2.3 Proliferation of Hepatocyte by Cell Count

The cells were decomposed by trypsin, digested for a period of time and then added to the culture medium to terminate the digestion. Then the culture medium was removed to the counting board with the liquid transfer gun, and the cells were counted strictly according to the rules of cell counting.

Table 1. Optimal Sugar Concentration (Normal hepatocytes)

Concentration of glucose added (mmol/L)	25	27	29	31	33	35	0
12 h(surviving hepatocytes)	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
13 h(surviving hepatocytes)	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
14 h(surviving hepatocytes)	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
15 h(surviving hepatocytes)	1.68 Soil 0.29	2.46 Soil 0.79	3.27 Soil 1.38	3.48 Soil 1.42	4.63 Soil 1.63	5.34 Soil 2.26	0
16 h(surviving hepatocytes)	3.89 Soil 1.42	4.79 Soil 1.68	5.24 Soil 2.17	5.89 Soil 2.64	6.23 Soil 2.79	8.98 Soil 2.86	0
17 h(surviving hepatocytes)	6.72 Soil 2.33	8.43 Soil 2.56	9.14 Soil 2.99	9.28 Soil 3.11	10.87 Soil 3.34	11.27 Soil 3.39	0

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2.2.4 Hepatocyte Proliferation was Determined by Cell Counting Method

After adding metformin solution and high sugar treated cell solution into carbon dioxide cell incubator for 12 h, 24 h, 48 h, the lactic acid value in cell culture medium was determined by lactic acid kit, so as to judge the effect of dimethyl double strand on cells with high sugar.

2.3 Statistical Methods

The statistical analysis uses SPSS25.0 statistical software to process the data. The sample mean is uesd by sample t test, and the multiple mean is analyzed by single factor variance to. $P<0.05$ was statistically significant.

3. Results and Analysis

The G concentrations of the most suitable hepatocytes LO2 high glucose stress were explored after this experiment, as shown in Table 1, and the optimum experimental concentrations were obtained when the G concentration was 31 mmol/L.

The control group and experimental group were equipped with 30 mmol/L of metformin in culture medium. Normal hepatocytes, high glucose + normal hepatocytes, metformin + normal hepatocytes, high glucose and metformin + normal hepatocytes were designed respectively. The changes of lactate concentration measured in 12 h, 24 h, 48 h of culture were shown in Table 2.

Metformin itself does not produce lactic acid in hepatocytes. In high glucose environment, according to the experimental results of 2 and 4 groups, before metformin was added, The concentration of lactic acid in the culture medium of high glucose + normal hepatocytes increased from 1.42 mmol/L to 3.48 mmol/L, then increased to 5.89 mmol/L after 24 h, The increase rate of cell number began to decrease significantly after 48 h, lactic acid concentration also increased to 9.289 mmol/L. After metformin was added, there was no significant change in lactate concentration and cell number compared with those under high glucose stress.

4. Discussion

A large number of studies have shown that lactic acidosis is a rare and serious complication of diabetes, most of which occurs in patients with biguanidine and accompanied by liver and kidney insufficiency, heart failure and so on [9-10]. Recent studies have found that the association of lactic acidosis (Metformin Lactate Acidosis,MALA) caused by metformin with a normal therapeutic dose is rare, but improper clinical use may also lead to elevated plasma lactate levels and even lactic acidosis (Lactate Acidosis,LA).1 [11]. The liver is an important organ of glucose metabolism. The liver can absorb and use glucose, then reduce blood glucose and can convert glucose into liver glycogen and store it. Increased insulin resistance in cirrhotic patients affects glucose metabolism and causes hepatogenic diabetes. Diabetes also affects the liver, especially in patients with type 2 diabetes, and is prone to liver damage and nonalcoholic fatty liver disease [12]. Metformin, as a traditional antidiabetic agent, can promote glucose metabolism, increase its anaerobic fermentation, increase the level of lactic acid and lead to lactic acidosis. In addition, metformin can inhibit the utilization of lactic acid by liver and muscle, inhibit gluconeogenesis, thus reducing glucose production, thus increasing the risk of lactic acid poisoning [13-15].

In this study, metformin had little effect on lactate metabolism in hepatocytes in high glucose environment. After adding different levels of metformin to the experimental group and the control group, there was no significant difference in lactate content. However, different levels of metformin can promote cell proliferation.The high glu-

Table 2. Effects of metformin on lactate metabolism in normal hepatocytes under high glucose stress

	Group 1 (normal hepatocytes)			Group 2(normal group + high glucose)		
	12h	24 h	48 h	12h	24 h	48 h
Concentration mmol/L of glucose added	/			31		
Add metformin concentration (mmol/L)	/			/		
Concentration of lactic acid (mmol/L)	0	0	0	3.48 Soil 1.42	5.89 Soil 2.64	9.28 Soil 3.11
Number of surviving hepatocytes (m)	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(hepatocyte + high glucose + metformin)		
	12h	24 h	48 h	12h	24 h	48 h
Concentration mmol/L of glucose added	/			31		
Add metformin concentration (mmol/L)	30			30		
Concentration of lactic acid (mmol/L)	0	0	0	3.30 Soil 1.39	5.83 Earth 2.63	9.17 Soil 3.08
Number of surviving hepatocytes (m)	1.30×10*7	2.60×10*7	5.20×10*7	1.15×10*7	2.24×10*7	4.79×10*7

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cose environment can inhibit the proliferation of hepatocytes, which may be due to the STC2 expression induced by high glucose, and the overexpression of STC2 can further enhance the inhibition ability of hepatocyte proliferation induced by high glucose [16]. Moreover, studies have shown that high glucose can promote the secretion of TNF-α, IL-6, and other inflammatory cytokines and regulate the expression of apoptosis-related molecules B lymphoma 2 and Bax, thus inducing hepatocyte apoptosis [17]. Metformin can promote the proliferation of hepatocytes, probably because it can inhibit the secretion of inflammatory cytokines and the activity of nuclear transcription factor κB (NF-κB) through AMPK dependent pathway, so as to promote cell proliferation [18].

To sum up, metformin has no great effect on lactate metabolism in hepatocytes in high glucose environment, but different concentrations of metformin have protective mechanism on hepatocytes and can promote cell proliferation.

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