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Cellular toxicity of isoniazid together with rifampicin and the metabolites of isoniazid on QSG–7701 hepatocytes

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ABSTRACT

Objective: To investigate the cellular toxicity of isoniazid together with rifampicin and the metabolites of isoniazid on cultured QSG–7701 cells lines. **Methods:** Isoniazid, rifampicin, mixture of rifampicin and isoniazid, acetylhydrazine, hydrazine were added in cultural media of QSG–7701 cells and cultured for 48 hours. The survival rate of cells was determined by MTT method. The cultural media and cells were collected and the activity of lactate dehydrogenase was detected by chromatometry. **Results:** Compared with control group, the survival rate decreased significantly and the lactate dehydrogenase released from cell increased significantly in cells treated with isoniazid, rifampicin, acetylhydrazine, hydrazine. Hydrazine, the metabolite of isoniazid produced significant damage on hepatocytes in low concentration. **Conclusions:** Rifampicin together with rifampicin and metabolites of isoniazid produce cellular toxic effects and hydrazine may be the most toxiferous metabolite.

1. Introduction

Recently, nearly ten million people are infected with tuberculosis each year, and millions of people die of tuberculosis. Asia was the region with highest morbidity and mortality. World Health Organization recommended the treatment of rifampicin together with isoniazide, but it revealed hepatotoxicity as main and serious adverse reaction[1]. So it's very important to investigate the effect and mechanism of rifampicin together with isoniazide. This experiment investigated the cellular toxicity of isoniazide,

rifampicin, acetylhydrazine, hydrazine on cultured QSG–7701 cells lines, and discussed the mechanism of increasing hepatotoxicity of rifampicin together with isoniazide.

2. Materials and methods

2.1. Reagents and instruments

Spectrophotometer (Japan Shimadzu Co.); isoniazide, rifampicin, acetylhydrazine, hydrazine(US Sigma Co.); DMEM culture medium, fetal calf serum(US Hyclone Co.); MTT; DMSO(US Amresco Co.); lactate dehydrogenase (Jiancheng Company of Nanjing); QSG–7701 cells lines from Dr. Dian–Xing Sun, Baiqiuen International Peace Hospital.

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2.2. Liver cell culture

These thawed QSG-7701 cells were stored in liquid nitrogen at 37 °C. They were centrifugated and the supernate was abandoned. Bovine serum was added in the precipitate to make suspension. After counting under microscope, they were cultivated in culture dish of 70 mm, with the density as 1×10^5 cells/mL, and volume as 5 mL. After culture for 24 h in CO₂ incubator, all liquid were changed, and it was changed it the next day. Cells were absorbed after they overgrew, and were washed with 1 mmol/L EDTA Na₂. Then EDTA Na₂ was abandoned. Cells were digested with 0.25% pancreatic enzyme digest fluid, and were in passage. After treated with medicine, they were inoculated in 6 holes or 96 holes cultural plate.

2.3. Grouping and treatment

Cells were divided into control group, rifampicin group, isoniazid group, acetyl hydrazine group, hydrazine group, and rifampicin+isoniazid group. They were cultivated for 24 h and were treated by rifampicin(20, 50, 200 mg/L), isoniazid(250, 500, 2 500 mg/L), acetyl hydrazine(125, 250, 1 250 mg/L), hydrazine(0.25, 2.5, 25 mg/L), rifampicin+isoniazid(100+50, 200+100, 300+150 mg/L), respectively.

2.4. Influence on survival rate of cells

Cells were inoculated in 96 holes cultural plate, and the density was 1×10^4 cells each hole. They were cultivated for 24 h, then placed into medicine medium and cultivated for 48 h. 20 μ L 0.5% MTT was added, and they were cultivated for 4 h. After removing cultural media, 150 μ L was added in DMSO each hole. The absorbance was read at 570 nm, and the survival rate of cells was also calculated.

2.5. Influence on lactate dehydrogenase survival rate

Cells were inoculated in 6 holes cultural plate. They were cultivated for 24 h then placed into medicine medium and cultivated for 48 h. Supernate in each hole was collected, and freeze-stored in -80 °C for measuring. Cells were washed with saline solution once and 1 mL saline solution was added in each hole. Cells were scrapped by 7 cell scraper, and cell suspension liquid was cultivated. The suspension was centrifuged at 2 000 r/min for 5 min. The supernate was abandoned. Lysis solution was added in the precipitate. They were splitted for 30 min at 4 °C, and centrifuged for 20 min at 12 000 r/min. The supernate was collected, and freeze-stored in -80 °C for measuring. The activity of lactic dehydrogenase was calculated by 2.4.0

lactate dehydrogenase and colorimetric method. The effect on cell damage and lactate dehydrogenase vitality in cells treated with drugs was analyzed by the ratio of enzyme activity of cell cultural media and cell lysis solution.

2.6. Statistic analysis

The difference between groups was analyzed by analysis of variance (ANOVA) with spss 11.5 software, and the two with Dunnett *t*.

3. Results

3.1. Influence on survival rate of cells

Compared with control group, isoniazid at 500 mg/L and 2 500 mg/L, and rifampicin at 300 mg/L significantly reduced the liver cell survival rate ($P < 0.01$); rifampicin together with isoniazid, the liver cell at middle dose and high dose reduced survival rate significantly ($P < 0.01$); hydrazine at 2.5 mg/L and 25 mg/L significantly reduced the liver cell survival rate ($P < 0.05$ or $P < 0.01$); when disposed with acetyl hydrazine, the liver cell survival rate was not significantly changed ($P > 0.05$)(Figure 1-3).

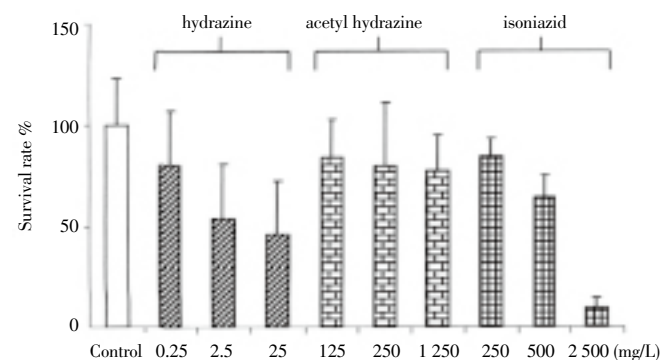


Figure 1. Effect of isoniazid, acetylhydrazine and hydrazine on survival rate of cultured QSG-7701 cells.

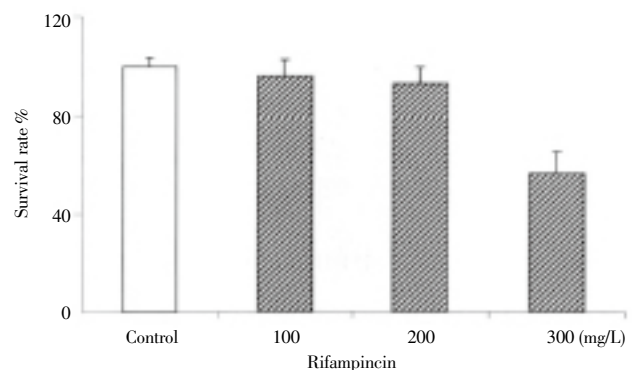


Figure 2. Effect of rifampicin on survival rate of cultured QSG-7701 cells.

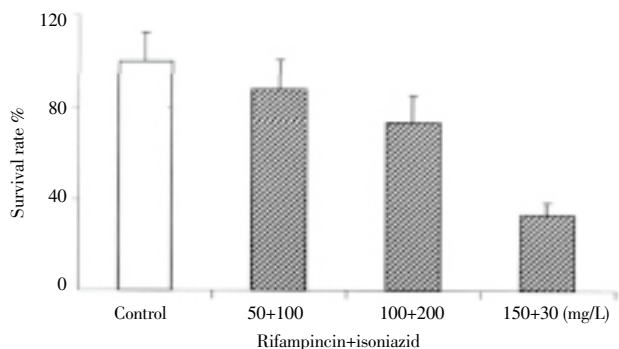


Figure 3. Effect of rifampicin and isoniazid on survival rate of cultured QSG-7701 cells.

3.2. Influence on lactate dehydrogenase survival rate

Compared with control group, isoniazid of 250, 500, 2 500 mg/L, significantly increased the LDH ratio of cells inside and outside ($P<0.01$); rifampicin of 300 m/L increased the LDH ratio of cells inside and outside by 50.3% ($P<0.05$); rifampicin together with isoniazid of 3 doses significantly increased the LDH ratio of cells inside and outside ($P<0.05$ or $P<0.01$); hydrazine of 3 doses also significantly increased the LDH ratio of cells inside and outside ($P<0.05$ or $P<0.01$); acetyl hydrazine of 250 mg/L and 1 250 mg/L increased the LDH ratio of cells inside and outside by 121.1% ($P<0.01$) and 153.1%($P<0.01$) (Figure 4-6).

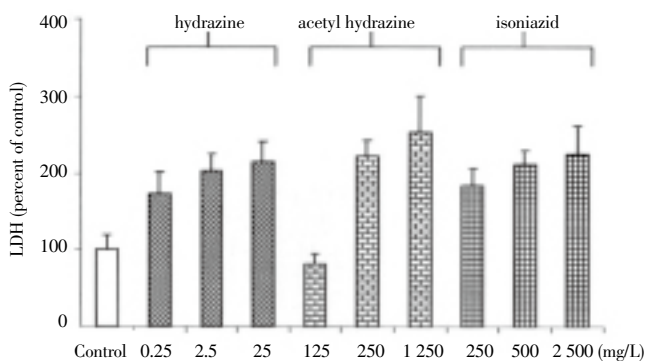


Figure 4. Effect of isoniazid, acetylhydrazine and hydrazine on LDH released from cultured QSG-7701 cells.

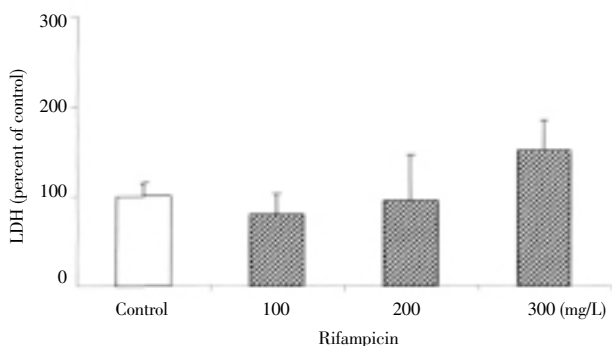


Figure 5. Effect of rifampicin on LDH released from cultured QSG-7701 cells.

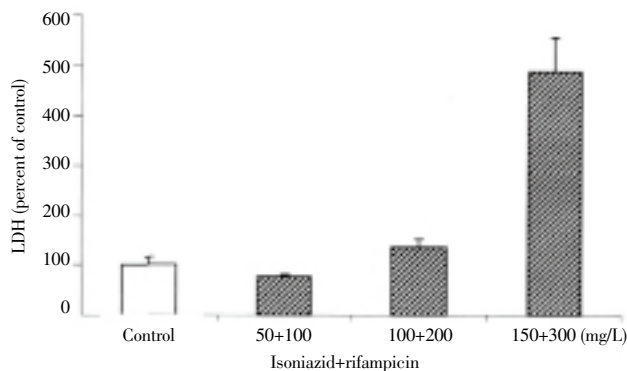


Figure 6. Effect of rifampicin and isoniazid on LDH released from cultured QSG-7701 cells.

4. Discussion

Although studies have found that new anti-tuberculosis drugs such as quinolone drugs (moxifloxacin and levofloxacin) might have a lower liver toxicity, its drug resistance and toxicology still need further research, and the high price was also a block of long-term using[2]. So rifampicin together with isoniazid is still irreplaceable. When liver-protection medicine is used together, long time using is a limit for clinical treatment. So analyzing the mechanism of rifampicin together with isoniazid is very important.

Isoniazid could directly or indirectly generate acetyl hydrazine and hydrazine by N-acetyl transferase and amide hydrolysis enzyme. They are important toxic metabolites which are connected with liver toxicity of rats or rabbit[3,4]. Rifampicin has liver toxicity and it is a kind of strong liver inducers of drugmetabolizing enzymes. When rifampicin used together with isoniazid, rifampicin could increase its liver toxicity by accelerating metabolism of isoniazid[5,6]. Research showed that except acethydrizide, isoniazid, rifampicin, hydrazine and rifampicin together with isoniazid could reduce liver cell survival rate. When rifampicin used together with isoniazid, the dose was significantly lower than either. Hydrazine (0.25 mg/L) could significantly decrease liver cell survival in a low dose, it was lower than rifampicin (300 mg/L) and isoniazid (500 mg/L). The present studies showed pathophysiology of drug-induced liver injury as (1) the breakdown of liver cells; (2) the destruction of the transporter protein; (3) cytolytic T lymphocyte activation; (4) hepatocellular apoptosis; (5)mitochondrial damage; (6) duct injury[7-10].

Lactate dehydrogenase is a kind of enzymes containing zinc molecules, and it distributes in a variety of organs and tissue in the body which provide antioxidation ability in normal cells. It can keep normal hydrosulphonyl in a

state of deoxidation to maintain activity of enzymes. Liver cells have rich lactate dehydrogenase, when liver cells are damaged. After lactate dehydrogenase is released from cells, anti-oxidation is damaged, so releasing of lactate dehydrogenase is an important index of liver cell damage^[11–19]. This experiment detected cell supernatant and the activity of lactate dehydrogenase at the same time. Comparing the ratio of activity of enzymes, the result showed that different doses of isoniazid, acetyl hydrazine, hydrazine, rifampicin, rifampicin and isoniazid could cause liver cell damage and the dose was significantly lower than the dose of causing cell survival rate decreased. Although the dose of acetyl hydrazine did not decrease liver cell survival rate significantly, it increased the releasing of lactate dehydrogenase. Acetyl hydrazine produced cellular toxicant, low dose of hydrazine (0.25 mg/L) did not affect the liver cell survival rate, but all the dose of hydrazine increased the releasing of lactate dehydrogenase. It stated clearly that hydrazine could lead to liver damage in low concentration. The experiment showed that the hepatotoxicity of hydrazine was greater than matrix isoniazid. And it also was an important metabolite which isoniazid generated hepatotoxicity by.

Conflict of interest statement

We declare that we have no conflict of interest.

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