Mechanism of Testicular Interstitial Cell Damage by Heavy Metal Lead Based on Computer-aided Technology

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Abstract. Objective: To explore the toxicological effect of heavy metal lead on testicular interstitial cells by detecting the gene expression levels of enzymes related to injury healing, to provide an experimental basis for revealing the toxicological mechanism of heavy metal-induced male sexual dysfunction. Methods: The r2c cell line of testicular interstitial cell was exposed to a serum-free culture environment containing heavy metal lead. The activity of the cells was determined at 1, 3, 6, 12, and 24 h. The supernatant of the cells was collected to detect the changes in the injury healing by computer-aided technology. The RT-RCR method was used to observe the differences in the gene expressions of injury healing related enzymes. Results: CCK-8 assay showed that the viability of r2c cells was not significantly reduced after they were exposed to 100 μmol/L heavy metal lead for 12 and 24 h (P > 0.05). Computer-aided radioimmunoassay was used to detect the changes in the progesterone, and the results showed that the synthesis of progesterone was significantly reduced at 12 and 24 h (P < 0.05). After the cells were exposed to the medium containing lead, the expressions of the steroid hormone synthetase gene returned to the normal level after 12 and 24 h. However, the expressions of StAR and P450scc genes decreased significantly after exposure to lead for 3 h (P < 0.05), and the expressions of the StAR gene decreased even more dramatically after exposure to lead for 6 h (P < 0.01). Conclusions: Heavy metal lead can inhibit the synthesis of progesterone in r2c cells mainly by down-regulating the gene expression levels of cholesterol transporter StAR and rate-limiting enzyme P450scc in the injury healing pathway.

Keywords: Lead, Testicular Interstitial Cell, Star, P450scc, 3β-HSD

1. Introduction

Heavy metals such as cadmium, lead, manganese, and nickel can cause DNA damage and oxidative stress, which is closely related to male infertility. Lead is extensively used in industrial production[1-2].
Lead poisoning can lead to a series of toxicological reactions in the organism. The reproductive toxicologically to the males is presented in many aspects, including direct effects on the testis, effects on endocrine and changes in the biological function of enzymes. The report of lead toxicological effect, especially the damage to the male reproductive system \(^3\), has attracted the attention of the society and people. Biological experiments showed that the toxicological effect of lead-containing diet to male rats was shown in the reduction of hormone secretion and sexual dysfunction in different degrees. It was necessary to supplement additional hormones to alleviate the fertility deficiency caused by lead. In the survey of the non-lead occupation population, 70.2% semen samples detected lead, indicating that lead is easy to accumulate in males \(^4\). It has been found that lead can increase the number of abnormal sperm, decrease the quality of sperm and even change the chromosome structure of sperm. However, the toxicological effect of lead to Testicular interstitial cells is the lack of data \(^5\)-\(^6\).

Testicular interstitial cells are the most common swelling of the gonadal matrix. R2c, as one of its cells, is based on the testicular interstitial cells of 2-month-old rats (WFU strain). In vitro, it can still synthesize steroids in an independent hormone way, which can be considered as having the deregulated expression of the regulatory mechanism of synthetic steroids or having an unknown single regulatory mechanism. The separation process of primary interstitial cells is complicated. Now, swollen cell lines are often used to reveal the complex physiological process. R2c cells can produce progesterone continuously, including 3 β - HSD, P450scc and stAR 22r hydroxylesterol can be used as the substrate of injury healing for the observation model of heavy metal exposure in vitro.

2. Functions of testicular interstitial cells
The main function of Testicular interstitial cells is to synthesize and secrete androgen, which is a kind of steroid hormone with 19 carbon atoms, mainly including testosterone and androstenedione, etc. Androgen can produce the following effects on the male body: ① stimulate the development and maturation of male external and internal reproductive organs, and maintain their functions. It stimulates the appearance of male secondary sexual character and maintains its normal state. ② Promote the synthesis of proteins in muscles, bones, and reproductive organs. ③ There is a negative feedback mechanism to maintain androgen content in the body relatively stable.

Currently, the evaluation of male reproductive toxicological effect is mainly based on animal experiments in vivo, and the observation indexes include a single generation and multiple generations of reproductive toxicological effect and male reproductive toxicological effect. The observation endpoints of single generation and multiple generation reproductive toxicological effect experiments include fertility and pregnancy results, such as mating rate, pregnancy rate, abortion rate, live birth rate, fetal sex ratio, fetal size, fetal vicapacity, fetal deformity rate, and reproductive capacity, etc. the observation indexes of male reproductive toxicological effect include weight and histological examination of pituitary gland and sexual organ, sperm quantity and quality examination, sexual behavior, hormone level and development influence. Hence, the study of the toxicological effect on the male reproductive system mainly focuses on animal experiments and epidemiological investigation.

3. Materials and methods
3.1. Experimental materials
R2C cells were purchased from American ATCC company; trypsin, F12 medium and fetal bovine serum were purchased from American GIBCO company; RNeasy ® plus mini kit was purchased from QIAGEN, Germany; both M-MLV first-strand synthesis system and SYBR Premix Ex TaqTM were purchased from BioRad, USA; iodine 125 progesterone radioimmunoassay test kit was purchased from Northern Institute of biotechnology; CCK-8 kit was purchased from Beyotime Biotechnology; lead acetate was purchased from Alfa Aesar, UK.

3.2. Cell culture and drug treatment
1) The r2c cells were cultured in F12 medium (containing 10% FBS). The r2c cells in the logarithmic growth phase were inoculated to a 96-well plate and a 12-well plate at 9 × 10^3 cells per well and 1 × 10^4 cells per well in 12 well plate. 2) 22 r-hydroxycholesterol was dissolved in anhydrous ethanol to form a 25 mmol/L storage solution.

There are n evaluated objects u_i, u_2, ..., u_n, m indicators x_1, x_2, ..., x_m represents a multi index evaluation system, x_j = x_j(u_i)(i = 1, 2, ..., n; j = 1, 2, ..., m) represents the evaluated object u_i with the index x_j. The evaluation data matrix (decision matrix) can be expressed as follows

\[ A = \begin{bmatrix} x_{i1} & x_{i2} & \cdots & x_{in} \\ x_{21} & x_{22} & \cdots & x_{2m} \\ \vdots & \vdots & \ddots & \vdots \\ x_{n1} & x_{n2} & \cdots & x_{nm} \end{bmatrix} \]   \hspace{1cm} (1)

Where m, n ≥ 3, A represents the data in the standardized data after preprocessing.

The process of damage assessment of testicular interstitial cells by heavy metal lead is described as a general transformation

\[ y_i = f (x_{i1}, x_{i2}, \ldots, x_{in}), i \in N \]   \hspace{1cm} (2)

4. Experimental results

4.1. Determination of cell activity by cck-8
CCK-8 was used to detect the apoptosis of r2c cells exposed to 100 μ mol/L lead acetate for 1, 3, 6, 12, and 24 h. The results indicated that the growth of r2c was inhibited in different degrees with the prolongation of exposure time, as shown in Figure 1. However, compared with the control group, there was no significant inhibition of cell growth, and the difference was not statistically significant (P > 0.05).

4.2. Effect of lead acetate exposure on the injury healing capacity of r2c cells
The changes in the healing function of r2c after exposure to 100 μ mol/L lead acetate for 1, 3, 6, 12, and 24 h were determined by computer-aided technique. The results showed that the function of r2c decreased with the increase of exposure time. Compared with the control group, lead acetate inhibited the synthesis of progesterone at 12 and 24 h, and the difference was statistically significant (P < 0.05).
4.3. **RT-PCR detection**

The changes of 3β-HSD mRNA in r2c cells were detected by measuring the gene of key enzymes (star, P450scc and 3 β - HSD) in the injury healing pathway. The results indicate that lead acetate decreased the expression levels of StAR and P450scc mRNA at different time points. Table 1 shows that after 3 h of lead acetate exposure, the expression levels of StAR and P450scc mRNA decreased (P< 0.05); after 6 h of lead acetate exposure, the expression levels of StAR mRNA decreased (P< 0.01); after that, the expression levels of StAR and P450scc mRNA gradually returned to the near-normal level at 24 h.

**Table 1.** RT-PCR detection (\(\bar{x} \pm s\))

| Time (H)/group | Control group | Treatment group |
|----------------|---------------|-----------------|
|                | StAR/RPS16    | P450scc/RPS16   | 3β-HSD/RPS16 |
| 1              | 0.6114±       | 2.4796±         | 1.0965±      | 0.5579±0.0325 | 2.4111±0.1198 | 1.1307± |
|                | 0.0482        | 0.0623          | 0.1238       |               |                 | 0.1183 |
| 3              | 0.5215±       | 2.4972±         | 1.4159±      | 0.4262±0.0657*| 2.3938±         | 1.3778± |
|                | 0.0963        | 0.0656          | 0.1327       |               | 0.0673*         | 0.1369 |
| 6              | 0.5979±       | 2.4782±         | 1.3052±      | 0.3373±       | 2.4201±0.1065  | 1.3536± |
|                | 0.0766        | 0.0724          | 0.1052       |               | 0.0708**        | 0.1262 |
| 12             | 0.5985±       | 2.5767±         | 1.2804±      | 0.6569±0.0282 | 2.5246±0.0995  | 1.2146±0.939 |
|                | 0.0597        | 0.0685          | 0.0967       |               |                 | 0.1186 |
| 24             | 0.6073±       | 2.5272±         | 1.4849±      | 0.5881±0.0762 | 2.4878±0.1076  | 1.4962± |
|                | 0.0686        | 0.0976          | 0.0691       |               |                 | 0.1186 |

5. **Discussions**

The crucial first reaction in the synthesis of progesterone by r2c cells, which depends on stAR. StAR is successfully isolated from the mouse testicular interstitial cell line MA-10, playing a vital role in regulating steroid synthesis. The StAR gene transcription is regulated by cAMP-dependent PKA signaling pathway. The long-term effect of cAMP is mainly presented in regulating steroid hormone
synthetase at the gene level, which plays an essential role in maintaining the stability of P450scc mRNA and P450scc protein in testicular interstitial cells. Studies have suggested that patients with congenital adrenal lipoid hyperplasia are severely deficient in the synthesis of adrenal and steroid hormones due to the mutation of StAR gene. Meanwhile, in vitro experiments have verified that StAR protein plays a crucial role in transorting cholesterol from the outer to the inner mitochondrial membrane.

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