Estudio experimental sobre la apoptosis de las células de cáncer de próstata inducidas por la cápsula de Huazheng

Experimental Study on Apoptosis of Prostate Cancer Cells Induced by Huazheng Capsule

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Resumen
Este artículo estudia el efecto inductor de la apoptosis de Huazheng Capsule en la célula de cáncer de próstata PC-3. Se usó el método MTT para determinar el valor IC50 de la cápsula Hua Zheng para las células PC-3. El método de detección cuantitativa se utilizó para estudiar la apoptosis de las células PC-3 inducidas por la cápsula Hua Zheng, como la electroforesis en gel de ADN y el análisis de contenido de ADN de citometría de flujo. Resultados: la cápsula de Huazheng podría inducir la apoptosis de las células PC-3, como obviamente se inhibió el crecimiento celular, se ralentizó la proliferación celular, las células se volvieron redondas gradualmente, se redujo el volumen celular, etc. Debajo de la membrana nuclear, algunos orgánelos como mitocondrias y endoplasmáticos el retículo se hinchó y se formaron cuerpos apoptóticos. Mientras tanto, la cápsula Hua Shi también podría inducir cambios bioquímicos apoptóticos en las células PC-3, como la extracción de ADN y el análisis de electroforesis en gel de agarosa. Los resultados mostraron que la cápsula Hua Shi podría inducir la fragmentación del ADN de las células PC-3 y formar un patrón de electroforesis en escalera de ADN apoptótico. Aparecen células en fase GL. Hubo una cierta correlación entre la proporción de apoptosis y la concentración de drogas. Conclusión: la cápsula de Huazheng puede inducir la apoptosis de las células PC-3, y su efecto está relacionado con la concentración del fármaco.

Palabras clave: Concentración de drogas; Tumor; Apoptosis; Celda PC-3

Abstract
This paper studies the apoptosis inducing effect of Huazheng Capsule on prostate cancer cell PC-3. MTT method was used to determine the IC50 value of Hua Zheng capsule for PC-3 cells. The method of quantitative detection was used to study the apoptosis of PC-3 cells induced by Hua Zheng capsule, such as DNA gel electrophoresis and flow cytometry DNA content analysis. Results: Huazheng capsule could induce apoptosis of PC-3 cells, such as cell growth was obviously inhibited, cell proliferation was slowed down, cell became round gradually, cell volume was reduced, etc. Under the nuclear membrane, some organelles such as mitochondria and endoplasmatic reticulum were swollen and apoptotic bodies were formed. Meanwhile, Hua Shi capsule could also induce apoptotic biochemical changes in PC-3 cells, such as DNA extraction and agarose gel electrophoresis analysis. The results showed that Hua Shi capsule could induce DNA fragmentation of PC-3 cells and form apoptotic DNA ladder electrophoresis pattern. GL phase cells appeared. There was a certain correlation between the ratio of apoptosis and the concentration of drugs. Conclusion: Huazheng capsule can induce apoptosis of PC-3 cells, and its effect is related to drug concentration.

Key words: Drug concentration; Tumor; Apoptosis; PC-3 cell

1. Introduction

Prostate cancer is one of the most common tumors in western countries, and also one of the main causes of death for men in European and American countries[1]. Because the prostate is a male internal reproductive organ with special anatomical site, it is difficult for drugs to enter and spread to the surrounding area, so the treatment of traditional Chinese and Western medicine is very difficult[2-3]. At present, western medicine has many treatment methods, such as surgical operation, hormone therapy and chemical medicine therapy, but none of them can significantly improve the survival rate of prostate cancer patients[4]. Therefore, it is necessary to explore the pathogenesis and treatment of TCM from the perspective of molecular biology and genetics.

In recent years, with the deepening of the research on the molecular regulatory mechanism of apoptosis and the further elucidation of the relationship between apoptosis and tumor development, people have gradually realized that inducing apoptosis of tumor cells is not only one of the mechanisms of radiotherapy, chemotherapy,
hyperthermia and some biotherapy, but also can be used as a new tumor treatment strategy alone, and may form To develop a new tumor treatment method that can intervene the imbalance of apoptosis by targeting apoptosis related genes or protein products in apoptosis signaling pathway, that is, aiming at the blocked targets in apoptosis signaling pathway, to increase the sensitivity of tumor cells to apoptosis induction by stimulating apoptosis promoting factors and / or inhibiting anti apoptosis genes To accelerate the apoptosis of tumor cells and achieve the purpose of tumor treatment[5-6]. At the same time, apoptosis inducing therapy can also be used in combination with other tumor treatment methods. According to different tumors, the combination of inhibiting tumor cell proliferation, inducing tumor cell differentiation and inducing tumor cell apoptosis can be considered comprehensively to optimize the clinical treatment plan and improve the effect of tumor treatment[7]. The phenomenon of apoptosis provides a new way of thinking for the treatment of tumor with traditional Chinese medicine, and the in-depth study of the mechanism of this process will better guide the clinical practice of traditional Chinese medicine in the treatment of tumor.

2. Materials and methods

2.1 Experimental materials

2.1.1 Cell, serum, medium, enzyme and reagent

PC-3 cell line of human prostate cancer was purchased from typical culture preservation center of Wuhan University; rpmI-1640 medium and calf serum were purchased from geBco / BRL company; MTT (tetramethylazazolium blue) reagent and propidium iodide (PI) were purchased from American sigma company; NRase A and protease K were purchased from Promega company; other biochemical and molecular biological reagents were purchased from Huamei bioengineering company.

2.1.2 Huazheng capsule

The compound Chinese medicine chemical capsule is provided by Hubei Provincial Traditional Chinese Medical Hospital. It is composed of toad spider skin, centipede, Solanum nigrum, woodlouse bug, and rat killer. It is dark brown and PH6.5.

2.1.3 Instrument

Ultra clean bench, carbon dioxide incubator, automatic enzyme plate, ihactih800 transmission electron microscope, FACScan flow cytometer

2.2 Experimental method

2.2.1 Cell culture

PC-3 cells were cultured in RPMI-1640 medium containing 10% calf serum, 100 U / M I of cyan and 100 U / M I of streptomycin respectively. The cells were incubated in 5% CO2 incubator at 37 ℃, and then digested and subcultured with EDTA trypsin mixture.

2.2.2 MTT assay of Huazheng capsule

The cells with logarithmic growth were inoculated on 96 well plate with 1x102 · L⁻¹, each well was 100ul, and then the compound traditional Chinese medicine Huazheng capsule was added according to a certain concentration, each concentration was added with 8 holes, each hole was 100ul, the control group was added with the same amount of culture solution, the zero adjustment group was only added with 100ul culture solution, after incubation for 48h, each hole was added with 50ulmit (2G · L⁻¹) for further incubation for 4h, then all the liquid was removed, and DMSO was added into each hole (dimethylsulfoxide) 150ul, the a (OD) value of each pore was measured by enzyme-linked immunosorbent assay (ELISA) at 490nm wavelength, and the survival rate [\text{sR} = \frac{a \text{(OD) value of the drug group}}{a \text{(OD) value of the control group X100%}}] and the killing rate of the drug to the cell (\text{CR} = 1-\text{sR}) were calculated.

2.2.3 Detection of apoptosis

2.2.3.1 Electron microscope observation

PC-3 cells were incubated in 1640 medium together with the appropriate concentration of Huazheng capsule, and morphological observation was made after a certain period of time.

2.2.3.2 TEM observation

PC-3 cells were incubated with 0-0.4 mg / ml Huazheng capsule for 72 hours, then the cells were collected and the transmission electron microscope samples were prepared respectively to observe the ultrastructure of PC-3 cells before and after the drug treatment. The preparation of electron microscope specimens was entrusted to the electron microscope Department of Wuhan University Medical College. The preparation process is as follows:

① The cells were centrifuged (1000 PRM, 10 min) and the supernatant was discarded.
2. Add 4% glutaraldehyde fixative solution which is precooled (4 °C) into cell precipitation, and fix 20 min at 4 °C.

3. Remove the fixative, add 10% calf serum and let it stand for 2 min to make the cells coagulate.

4. Remove calf serum, add 4% glutaraldehyde fixative again, and fix it at 4 °C for 24 hours.

5. Take out the cell clot and cut it into 1 mm³ small pieces, and rinse twice with PBS.

6. Fixed 2 hours after routine 2% osmic acid, washed twice with PBS, 15 minutes each time.

7. Use 70%, 80%, 90% and 100% acetone gradients to dehydrate successively for 15 minutes each time.

8. Dehydrate 100% acetone again for 20 min.

9. Immerse the cell sample with the embedding agent (acetone: epon812) for 1H.

10. The slices were fished on the copper net covered with fomrvar membrane, stained with uranium acetate for 20 min, and washed twice with double steaming water.

11. Lead nitrate staining for 20 min, double steaming and washing twice.

12. Observe and take photos under hitachi 800 transmission electron microscope.

2.2.3.3 Extraction of DNA and analysis of agarose gel electrophoresis

After incubating 72 hr with PC-3 cell and 0–0.4 mg/mL syndrome capsule, the cells of the control group and the control group were collected. DNA was extracted and analyzed by agarose gel electrophoresis. The method of DNA extraction is as follows:

1. Cells were collected, washed twice by PBS (2000 rpm, 3 min), and the supernatant was discarded.

2. 400 μl of cell lysate (10mm HCl, 150mm NaCl, 10mm EDTA, 1% SDS, ph8.0) and 15 μl of protease K (20mg / ml) were added to the cell precipitation, and the mixture was kept at 55 °C for 1H.

3. Phenol, phenol / chloroform (25:24), phenol / chloroform / isoamyl alcohol (25:24:1) were extracted once respectively.

4. Take the supernatant, add 2.5 times volume of cold anhydrous ethanol and 1 / 10 volume of 3mnaac (ph5.2), mix well, and precipitate at -20 °C for 24 hours.

5. After centrifugation (12000 rpm, 10 min), the supernatant was discarded and DNA was washed twice with precooled 70% ethanol.

6. Naturally dry the DNA precipitation, add 50 μ l te solution containing 20mg / ml RNase A, digest at 37 °C for 2 hr, and store at -20 °C for standby.

The DNA samples extracted from 15 L were electrophoretic on 1.5% agarose gel and observed after EB staining.

2.2.3.4 Detection of DNA hypodiploid peak by flow cytometry

After incubation of PC-3 cells with Huazheng capsule (0–0.4 mg / ml) for 72 hours, flow cytometry was used to analyze the cells before and after the drug treatment, and the formation of DNA hypodiploid peak was detected.

1. 5x10⁵ ~ 1x10⁶ cells were collected from each sample and washed twice with cold PBS.

2. The cells were fixed overnight at 4 °C with precooled 70% ethanol.

3. Centrifugation (1000 rpm, 8 min), discarding the fixed solution, PBS washing twice, PBS re suspending cells.

4. Add nrasea to the final concentration of 50 μ g / ml and incubate at 37 °C for 30 min.

5. Add pi to the final concentration of 50 μ g / ml, and stain at room temperature without light for 30 min. Detect the content of DNA in cells with facean flow cytometer (Beeton Dickinson company of the United States) by standard program, and analyze the data with clelquest software.

3. Results

3.1 Cytotoxic effect of Huazheng capsule

3.1.1 Curve of survival rate and drug concentration

It can be seen from the curve of cell survival rate and drug concentration that the survival rate of PC-3 cell decreases with the increase of drug concentration, and the killing rate of Huazheng capsule can reach more than 50% at the concentration level of 50 μ g / ml (Table 1).

<table>
<thead>
<tr>
<th>Drug concentration (μg/mL)</th>
<th>12.5</th>
<th>14.3</th>
<th>16.7</th>
<th>20</th>
<th>25</th>
<th>33</th>
<th>50</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival rate</td>
<td>74.70%</td>
<td>72.75%</td>
<td>71.80%</td>
<td>59.10%</td>
<td>57.91%</td>
<td>57.20%</td>
<td>49.53%</td>
<td>27.80%</td>
</tr>
</tbody>
</table>

3.1.2 Killing effect on prostate cancer cell PC-3
Different concentrations of Huazheng capsule can inhibit the growth of PC-3 cells to a certain extent. When the concentration is 0.1mg/ml, the inhibition rate of Huazheng capsule to PC-3 cells is 72.2%. It is speculated that the IC$_{50}$ of Huazheng capsule to PC-3 cells is 0.0498mg/ml, so it can be considered that Huazheng capsule has a certain killing effect on PC-3 cells. See Table 2.

Table 2. Killing effect of Huazheng Capsule on PC-3 cells

<table>
<thead>
<tr>
<th>Huazheng capsule (mg/ml)</th>
<th>OD value</th>
<th>Tumor inhibition rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.99525</td>
<td>0</td>
</tr>
<tr>
<td>0.1</td>
<td>0.270625</td>
<td>72.2</td>
</tr>
<tr>
<td>0.05</td>
<td>0.492875</td>
<td>50.47</td>
</tr>
<tr>
<td>0.033</td>
<td>0.569125</td>
<td>42.8</td>
</tr>
<tr>
<td>0.025</td>
<td>0.576375</td>
<td>42.09</td>
</tr>
<tr>
<td>0.02</td>
<td>0.58725</td>
<td>40.99</td>
</tr>
<tr>
<td>0.0167</td>
<td>0.7145</td>
<td>28.2</td>
</tr>
<tr>
<td>0.0143</td>
<td>0.7215</td>
<td>27.5</td>
</tr>
<tr>
<td>0.0125</td>
<td>0.7435</td>
<td>25.3</td>
</tr>
<tr>
<td>IC50 (mg/mL)</td>
<td></td>
<td>0.0498</td>
</tr>
</tbody>
</table>

3.2 Morphological observation on apoptosis of PC-3 cells induced by Huazheng capsule

Under electron microscope, PC-3 cells of each treatment group were treated with 0.1 - 0.04mg/ml Huazheng capsule for 2-3 days, and then gradually appeared the disappearance of cell synapses, cell roundness, cell body shrinkage, nucleolus pyknosis, cracking and even disappearance, and then gradually died. The morphological changes of apoptotic cells take place in the ultrastructure[8]. The typical changes of cell structure in different stages of apoptosis can be clearly observed by transmission electron microscopy, which is one of the most reliable indexes to judge apoptosis. In this study, the ultrastructures of the cells and the control cells after the effect of Huazheng capsule were observed under the transmission electron microscope[9-10]. It can be seen that the chromatin of the control cell nucleus was uniform and the nuclear membrane was complete (Fig. 1, a). However, the cells after the effect of Huazheng capsule showed typical morphological changes of apoptosis (Fig. 1, B-D): including irregular morphology of the nucleus, pyknosis and condensation of chromatin, forming a round or oval electron density high homogeneity Block (see Fig. 1, b); the nuclear membrane loses integrity, the nucleus is severely folded and deformed, and dense chromatin blocks are gathered under the nuclear membrane in a ring (see Fig. 1, c); some organelles have morphological changes, such as abnormal mitochondrial structure, which is characterized by swelling, steep disorder, unclear structure, low density, and the formation of apoptotic bodies can be seen (see Fig. 1, c); apoptotic bodies contain not only dense chromatin blocks, but also swollen mitochondrial somaclonal organelles (Fig. 1, d).

Figure 1. Ultrastructural changes of PC-3 cells induced by Huazheng capsule

3.3 Chromosome DNA fragmentation of PC-3 cells induced by Huazheng capsule

It is another classical method to detect the characteristic "DNA ladder" band of apoptosis. In this study, 72hr cells were collected from different concentrations of PC-3 capsules after treatment with different concentrations of capsules. The cells after treatment and untreated cells were collected. DNA and 1.5% agarose
gel electrophoresis were used to extract the cells[11]. As shown in Figure 2, the DNA of the cells treated with 0.3mg/ml and 0.4mg/ml Huazheng capsule shows a typical "trapezoid" band (swimlane 3, swimlane 4) with a size of about 180-200bp and an integral multiple (based on the DNA molecular weight standard of swimlane m). However, the cells treated with 0.1mg/ml Huazheng capsule (swimlane 2) and the cells treated with 0.3mg/ml Huazheng capsule (swimlane 1) only have a large one near the electrophoresis sample hole. There is no "DNA ladder" in genomic DNA. It can be seen that Huazheng capsule can induce the characteristic biochemical changes of apoptosis in PC-3 cells, namely DNA fragmentation.

Figure 2. DNA fragmentation of PC-3 cells induced by Huazheng capsule

3.4 Pharmacological Capsules Induced DNA Diploid Peaks in PC-3 Cells

Flow cytometry to detect cell cycle DNA content is the most widely used specific method for quantitative detection of apoptosis. When apoptosis occurs, flow cytometry can detect a characteristic subdiploid karyotype peak, i.e., an apoptotic peak, produced by apoptotic cells. In this study, cell DNA after different concentrations of capsules were stained with PI and subjected to flow cytometry. The results showed that the sub-diploid DNA peaks below the GO/G1 phase DNA peak appeared in the PC-3 cells after the action of the capsule, and the apoptotic rate increased with the increase of the drug concentration: the concentration was 0.1 mg/mL, 0.2mg/mL, 0.4mg/mL, the apoptotic rate was 8.02%, 15.6% and 22.3%, respectively, while the apoptosis rate was not detected in the untreated control cells.

4. Discussion

With the deepening of apoptosis research, the laboratory detection method of apoptosis has also been developed. At present, the detection methods for apoptosis are mainly based on the morphological and biochemical changes of apoptosis[12-14]. Therefore, researchers may choose to use some of these methods to qualitatively identify apoptosis or according to different needs. Quantitative analysis.

Genetic studies have shown that although the signals that induce apoptosis vary, some characteristic morphological and biochemical changes occur when apoptosis occurs. A variety of apoptosis detection methods were established for the special morphological changes of apoptotic cells, including: observation of typical morphological changes of apoptotic cells by various microscopy techniques such as optical microscopy, electron microscopy, phase contrast microscopy, and laser confocal microscopy; Membrane permeability specific DNA-binding dyes such as Hoechst 33342, Hoechst 33258, DAPI, etc., stained DNA can observe nuclear morphological changes of apoptotic cells under a fluorescence microscope. At present, morphological observation is still the most classic and reliable method for judging apoptosis, and it is the basis for judging whether apoptosis occurs. In the present study, we used the above method to detect the morphological changes of PC-3 cells induced by Huayu Capsule: the characteristic ultrastructural changes of PC-3 cells were observed by transmission electron microscopy[15].

The results of this study showed that Huayu Capsule can induce DNA fragmentation of PC-3 cells, and it is a "DNA ladder" band on agarose gel electrophoresis; DNA diploid peak appears, which proves at the molecular level. The ability of Huayu Capsule to induce apoptosis, it may become an ideal anti-tumor preparation of traditional Chinese medicine, especially for the treatment of chemotherapy-resistant patients with recurrence or metastasis after treatment will achieve the desired effect[16].
The characteristics of traditional Chinese medicine treatment of tumors are mainly through the methods of strengthening the righteousness and eliminating evil spirits, dispersing detoxification and other methods, so as to fully adjust the resistance of the motive body itself, thereby improving the symptoms, improving the quality of life of patients or even "surviving with tumors". Most patients who are often seeking treatment of traditional Chinese medicine are those with advanced or recurrent disease, and some have even had extensive metastasis of the lesions, and most of them have undergone surgery, radiotherapy, chemotherapy, and any treatment to achieve tumor lesions. It is more difficult to completely disappear, and the biggest problem to be solved in treatment is how to alleviate the pain, improve the quality of life and prolong the survival period. Therefore, it also determines the efficacy of traditional Chinese medicine in the treatment of tumors, is to stabilize the tumor, or even reduce and eliminate the tumor, improve symptoms to achieve the purpose of "tumor survival." The biggest difference between the efficacy characteristics of effective cases and modern medical treatment is that the former, the tumor shrinkage is not obvious, but the survival period is prolonged, and the symptoms are obviously improved; the latter, the tumor may shrink significantly in a short time, but relapse quickly. The tumor increased, the survival period did not significantly prolong, and the quality of life rapidly decreased. Taking lung cancer treatment as an example, small cell lung cancer as a chemotherapy-sensitive tumor, after intense chemotherapy, some patients can achieve complete remission (CR) effect, but the recurrence rate is very high, the 5-year survival rate is still less than 10%; In cell lung cancer, in addition to the long-term survival of a small number of patients with early surgery, there is still no effective treatment method, and due to the toxic side effects of chemotherapy, the patient's quality of life is rapidly reduced due to the severe impact on the body's immune function[17-18]. According to incomplete statistics, a large number of patients with advanced stage III–IV tumors have turned to dialectical treatment of traditional Chinese medicine, and some patients have benefited from treatment, achieved certain effects, and improved the quality of life. For example, Pan's treatment of 80 cases of primary bronchogenic carcinoma with lung compound, Park's 185 cases of advanced lung cancer with lung tumor cream, its survival and quality of life are better than chemotherapy group. Liu's use of nourishing yin and health, benefit temperature Yang method for the treatment of advanced primary lung adenocarcinoma, compared with the chemotherapy group, has the effect of prolonging the survival of the patient, alleviating and stabilizing the lesion, improving the quality of life, and adjusting the immune function of the body. Another example is the recurrence of esophageal cancer, the main factor is still local recurrence, especially after radiotherapy; the case of chemotherapy drug treatment is effective, the shortest time of recurrence is only 2 months, even if the clinically limited esophageal cancer is operated or irradiated After treatment, there are still quite a few cases of recurrence or death from distant spread, and the overall 5-year survival rate is around %. The combination of traditional Chinese medicine with radiotherapy, chemotherapy, surgery, etc. can significantly prolong the survival period and improve the quality of life. The combination of traditional Chinese medicine and western medicine combined with Fuzheng Peizhi prescription and chemotherapeutic esophageal cancer in 233 cases, the 1, 3, and 5 years survival rates were respectively 42%, 8.3%, 7.1%, the curative effect was significantly better than the chemotherapy alone group. With the combination of Ziyin Yangwei method and radiotherapy, the treatment effect of the treatment group and the control group was also significantly different, the former was better than the latter. Modern medicine has made great achievements in the research and treatment of tumors, but there have also been some shortcomings and defects. Traditional Chinese medicine has rich experience in the prevention and treatment of this disease. The modern scientific mechanism of traditional Chinese medicine for treating cancer is becoming more and more clear, and the treatment methods are becoming more and more abundant. It gradually shows its unique advantages and great potential. We should make full use of modern scientific and technological means to carry out Clinical and experimental research on medical treatment of tumors. It is believed that strengthening research on the apoptosis and mechanism of tumor cells induced by Chinese medicine will undoubtedly provide new ideas and ways for the prevention and treatment of tumors.

5. Conclusion

In summary, through the application of a series of qualitative and quantitative methods of apoptosis, we detected that PC-3 cells after the action of capsules showed a variety of morphological changes and biochemical changes. However, we conclude that the capsule can induce apoptosis in PC-3 cells.

References


