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Effect of Different Isorhamnetin of Different Density Cultured Rat C6 Glioma Cells in Vitro

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ABSTRACT

Objective To explore the influence of different concentrations of isorhamnetin on C6 glioma cell morphology. **Methods** Set the blank control group, blank solvent control group and reagent group of four concentration, the growth of cells were observed under microscope; MTT assay was used to test the effect of isorhamnetin on cultured C6 glioma cells, as well as calculate the cell inhibition rate and survival rate; flow cytometry was used to check the detection peak and detection rate of Isorhamnetin group and negative control apoptosis group, and analyzed the relationship between different concentrations of isorhamnetin and C6 glioma cell apoptosis rate; total protein was extracted from cells, and used Western blotting to detected total AKT protein and Ser473 AKT protein loci in cells; used SD rats to construct brain glioma model, feed isorhamnetin plain to them for five days, and then used HPLC to detect plasma, liver, brain tissue content. **Results** Under the observation of inverted microscope and image analysis, after using Isorhamnetin, tumor cells appear apoptosis and necrosis change. Display with different Isorhamnetin MTT colorimetric method shows that the higher the concentration of added Isorhamnetin, the worse the growth rate of C6 glioma cells in vitro, and the higher the Inhibitory rate, the lower survival rate. The flow cytometric

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detection shows the C6 glioma cells which is added 40 ug/ul Isorhamnetin have the highest rate of apoptosis. After adding 80 μg/μl concentration of the isorhamnetin, C6 glioma cells have the lowest survival rate. Western blot test shows the AKT protein and Ser473 total site AKT protein density is in reverse proportion to the increase of the concentration of the isorhamnetin. High performance liquid chromatographic method has determined that there are isorhamnetin in both the rat plasma and brain tissue, which shows that the plasma and tissue all have different isorhamnetin distribution, and isorhamnetin mainly exist in the brain tissue. **Conclusion** Low concentration of isorhamnetin can induce apoptosis of C6 glioma cells, and high concentration of isorhamnetin can lead to apoptosis and necrosis of C6 glioma cells in vitro, which has obvious inhibitory effect on the growth of glioma cells, and the mechanism is closely related to PI3K/AKT pathway, and in SD rat brain glioma model , the high performance liquid chromatography was used to detect the content of plasma and brain tissue, which indicated the isorhamnetin has target in brain tissue, which provided experimental evidence for the development and utilization of isorhamnetin in mice.

0 Introduction

In recent years, some antineoplastic, such as those derived from artemisinin, have developed well. Plant-derived drug monomers or compounds have great potential for discovery of new drugs, such as providing innovative ideas for the design of an ideal new drug structure, as well as being used as a precursor to the creation of new drugs. In recent years, Isorhamnetin (ISO), which belongs to flavonoids, has been found to not only have the function of relieving cough and eliminating phlegm, reducing digestion, activating blood circulation and dispersing blood stasis, but also can better resist myocardial hypoxia, antiarrhythmic, anti-oxygen free radicals, ischemia, relieve angina pectoris, lower serum cholesterol and other cardiovascular effects, but in current clinical, they only used for cough and phlegm, digestion of stagnation, blood circulation and scattered stasis. In recent years, some studies have found that Isorhamnetin has significant effect on some tumor cells [1-2], at the same time, it also has selective inhibitory effect on the tumor[3], such as inhibiting the growth of A549 and MCF-7 cells in vitro, inducing apoptosis and affecting the differentiation of lung and gastric cancer cells. [4-5] But it has no effect on the proliferation and differentiation of PC3, K562 and AD293. Leining and other people [6] had mentioned the relationship between rat Isorhamnetin and mouse glioma cells in the experiment, but the author had consulted a large number of literatures and had found that no detailed test and mechanism of the effect of rat isorhamnetin on glioma cells have been reported yet. This study explored the effects of different concentrations of isorhamnetin on rat C6 glioma cells in vitro, and provided an experimental basis for the study of Isorhamnetin in vivo and in vitro.

1 Materials and methods

1.1 Materials

Cell lines for the experiment: C6 glioma cells are purchased from the CAS Shanghai Institute of Cell.

Rats in the experiment: 20 SD rats, half male and half female, 300g for each one averagely, provided by Laboratory Animal Center of our hospital (SPF level).

Test reagent of cell: DMEM Medium purchased from GIBCO Company, 10% Fetal bovine serum (Four Seasons Biological Co., Ltd.), dimethylsulphoxide (Chongqing Chemical reagent Factory), Isorhamnetin (Shenzhen Tong Tian Biochemical Technology Co.,

Ltd.), 0.25% Trypsin (Hyclone company), MTT assay Kit for cell proliferation and cytotoxicity, Annexin V-FITC/PI assay kits for amphophilic cell apoptosis were purchased from Shanghai Yanbin Co., Ltd., AKT and Ser473 AKT protein Ⅰ resistance (cell signaling company, import), cell culture dish is purchased from Hangzhou Bio-Tech Co., Ltd. and the Tissue Protein extraction kit from Beijing Baitaike Company.

Main instruments in the cell experiment: Flow cytometry (SL), high-speed refrigeration centrifuge (Universal 32R, Germany), inverted microscope (Olympus, Japan), UV spectrophotometer (Auriud CE2401, UK), Gel image analysis System (touching 995gel Document System, Tiancheng technology company, electrophoresis instrument (FC101, Dalian), ultra-low temperature refrigerator (-80℃) (Sanyo, Japan), Incubator (Nuaire,usa), ultra-pure water Production (MILLI-Q).

1.2 Method

Preparation of different concentrations of rat Isorhamnetin solution: Isorhamnetin (Produced by Shenzhen Tong Tian Biochemical Technology Co., Ltd.), white powder-like solid, showing purity of 98%, 10mg branch. The drug is dissolved into the mother liquor with the 0.1% NaOH solution, and then diluted with DMEM complete culture solution, to make the final concentration reaches 1 μmol/μl, and after filtration and degerming, the drug is placed in 4 ℃ to be used.

Drug treatment and cell morphological observation: The rat C6 glioma cells which showed a nice growth have diamond-shape, strong stereoscopic sense, little small intracellular particles, clear edge and structure, and strong refraction. The typical rat C6 glioma cells were inoculated into the six-orifice plate (2ml solution per hole), after the cell density of the six-orifice plate reached 80%, it was divided into three groups: the blank control group, the DMSO solvent control group, and the drug experimental group, and treated them by 10, 20, 40, and 80mg/l isorhamnetin, respectively, and after the cells were cultured in 48 hours, the growth and morphological changes of the cells in the control and other groups were observed under microscope.

The inhibition rate of growth was measured by MTT method: The blank 0.1% NaOH control was set. In the blank control group, the equivalent 0.1% NaOH and DMEM complete culture liquid were added in the 37℃,5%c02 culture box. Pick some rat C6 glioma cells in logarithmic growth period, and adjust the number of cells to 5×10^4/l inoculation in 96-hole culture plate, each hole 100μl, and set 3 complex holes. In the experimental group, concentration of 10, 20, 40, 80mg/L isorhamnetin were given, and 10%DMEM complete culture liquid added as a blank control and solvent control, with complete medium dilution MTT, the final concentration of s5mg.ml^{-1}, each hole added 10μl, and put the cell back to 5%COZ incubator and incubated for 4 hours, then the medium with MTT was absorbed and the 150μl solution was added to each pore, and the absorption value of each hole was measured at 490 nm by the enzyme labeling instrument. The inhibitory rate of the treated cells was calculated by 100% control.

Flow cytometry to detect apoptosis: after given drugs for 3 times and culturing for 72h, suction out of the culture solution was sucked out, and washed by PBS and added 0.25% trypsin for digestion for 1min. Blow and beat it into the suspension and sucked in the centrifuge tube, 1200rpm centrifugation for 3min. Discarded supernatant, added 2mlPBS and flushed, 1200rpm centrifugation for 3min, and repeat again. Discarded the supernatant after centrifugation, added the binding liquid 400μl in the cell apoptosis detection kit, and then add the reagent Annexin-v-FITC staining solution 5μl to each tube. After 15min incubation in 4℃ in dark, l0μl iodide (P1) staining solution (20μg.m1-1) 10μl was added and incubated in 4 ℃ in dark for 5min. The apoptosis was detected on the flow cytometry.

Western blotting method was used to detect the total AKT protein and Thr473 AKT protein in C6 glioma cells: The control group's and other groups' cells after treatment of 24 hours (more than 2×10^5), washed by cold PBS for 2 times, and added the pyrolysis fluid on the ice bath to crack the for 30min in 4℃, 12500rpm centrifugal for 5min, collected supernatant and determined protein concentration. Isolated protein by 10% SDS-PAGE, and used the half dry method to turn it on the nitrocellulose membrane. After adding 1:2000 dilution and reacting for 2.5h, wash it by TBST for 2 times, then add 1:1000 dilution AKT and Thr473 AKT for incubation for 1 hour. Finally ECL will show and exposed the result.

The rat model of brain glioma was divided into groups, and drugs are given according to the groups: after 4 days after the mold was modeled, the animals were randomly divided into 3 groups, and the first group is normal control group, the second group is the saline control group, and the 3rd group is the rat Isorhamnetin group, the drug was given the day before the rats had been fasting for 24h, and was irrigated into stomach (according to the standard of 50mg/kg) respectively after 3d, 5d, 7d, 10D, 15D, totally 5 times.

Tumor inhibition rate calculation: calculated according to the time after rat model was set, and after the rat model of the 3rd day, 5th days, 7th days, 9th days, on the 12th day, a tumor-removing tissue from the Isorhamnetin group was executed to measure the longest diameter (L), the shortest diameter (L), the widest diameter (W), the narrowest diameter (W) and the thickness (H) of the transplanted tumor, and the average length diameter (L) and Width diameter (W), and calculate the size of the transplanted tumor according to the formula: V (volume) =V (volume) = L ×W×H √2. According to the formula: the inhibition rate%=[(tumor volume in the negative control group － tumor volume in Isorhamnetin group) ／tumor volume in the negative control group] ×100%, and the macroscopic isorhamnetin inhibition rate was obtained.

Pathological observation of tumor specimen: After12h the drug was given in the last time (the 12th day), the rats were executed by decapitation. Removed the transplanted tumor, and picked half of it quickly into the -80 refrigerator, to be done in the determination of isorhamnetin content of rats. The other half of the tumor was fixed in 10% formalin fixed fluid, and was gradually dehydrated, the tissue was treated with 4% PBS poly formaldehyde liquid in cardiac perfusion fixation, conventional dehydration, paraffin embedding was taken as a coronal section of thick 4mm. After HE staining, the HE staining slice was observed by light microscope.

Determination of the content of isorhamnetin in brain tissue: a transplanted tumor in the blank control group and in the Isorhamnetin group were picked, and according to Isorhamnetin HPLC detection, determined the distribution of rat isorhamnetin, and determined the isorhamnetin distribution in the plasma and brain tissues of SD rats in vivo.

1.3 Statistical treatment

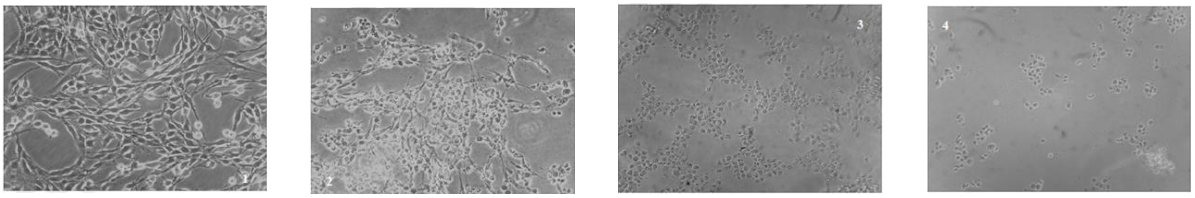
The spss13.0 statistical software was used for analysis, measurement data was demonstrated by $\bar{x}\pm s$, counting data was demonstrated in terms of rate or percentage. The comparison between groups was based on the sample mean t test or X2 test, and the correlation analysis of single factor variance analysis and correlation analysis was used in many groups, $p<0.05$ means the difference has statistical significance.

2 Results

2.1 Effects of rat isorhamnetin of different concentrations on cell morphology

The growth of C6 brain glioma cells with good recovery and passage: Cell activity is strong, can be adherent after 6h, and adherent cells changed into

triangles or long ribs, they grow so quickly that after 48h, they can be full of bottles, that is, come into the platform period. After 72h, we can see that some tumor cells were piled up, and there was no contact inhibition growth, and there were a lot of dead exfoliated cells in the culture fluid after 96h. Under the optical microscope, the morphology of monolayer cells is triangular, pattern-shaped and polygonal. The cells showed long ribs and irregular polygons by HE staining (Fig. 1).



Normal Rats　20 μg/ml Isorhamnetin　40 μg/ml Isorhamnetin　80 μg/ml Isorhamnetin

Figure1. Effects of rat isorhamnetin of different concentrations on macro form of C6 glioma cells（×400）

2.2 Adopt MTT assay to detect survival rate of cells

With the increase of the concentration of Isorhamnetin, the survival rate of C6 glioma cells decreased gradually and the growth inhibition IC50 was gradually increased. When isorhamnetin was 10μg/μl, 20μg/μl, 40μg/μl, 60μg/μl and 80μg/μl, the cell survival rate (%) was 87.81, 79.80, 65.57 and 64.02, respectively. It is indicated that the effect of isorhamnetin on inhibition of C6 glioma cell growth relies on concentration, the higher the concentration, the more obvious the inhibition function. Draw a figure with the concentration of Isorhamnetin as the horizontal axis, and the inhibitory rate of C6 glioma cell as the vertical axis, we can get when the isorhamnetin effect C6 glioma cell for 48h, then the IC_{50} is about 76.32μg/μl, (n=3,*t test, $p<0.05$).

dosage（μg/ml）	Average OD	Survival rate of cells （%）	IC_{50}（μg/μl）
80	0.331	64.02±2.4	
40	0.339	65.57±2.7	
20	0.413	79.80±3.5	76.32
10	0.454	87.81±4.1	
0	0.517	100.00	

Table 1. Effects of rat isorhamnetin of different concentrations on survival rate of C6 glioma cells and IC_{50} figures which are calculated

2.3 Apoptosis rate and apoptosis peaks detected by cell apoptosis test

As shown in Fig. 2, the apoptosis rate of the tumor cells in the blank control group was 5.83%, and the normal growth rate of tumor cell area was 88%. The apoptosis rate of 20μg/μl isorhamnetin apoptosis was 14%, and the survival rate of normal tumor cells was 80.4%. The apoptosis rate of 40μg/μl isorhamnetin apoptosis was 16.7%, and the survival rate of normal tumor cells was 76.7%. The apoptosis rate of 80μg/μl isorhamnetin apoptosis was 19.3%, and the survival rate of normal tumor cells was 75.4%. The results showed that with the increase of the concentration of isorhamnetin, the apoptosis rate of glioma cells in rat was increased.

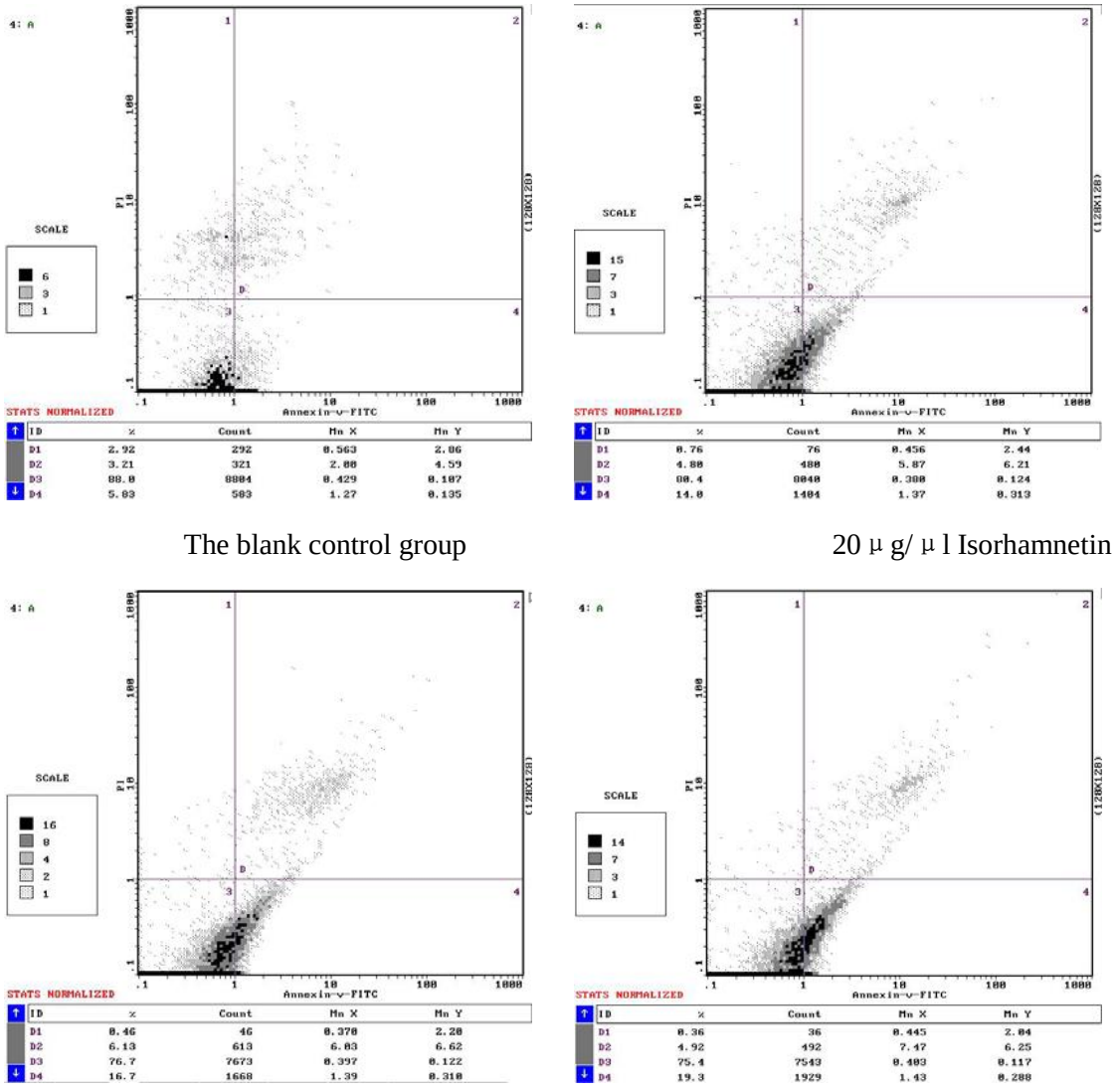


Figure 2. Effects of rat isorhamnetin of different concentrations on apoptosis of C6 glioma cells

Among them，D1 area was tumor cell area for normal growth, D2 area was for necrotic tumor cell， D3 area was for necrotic and apoptotic tumor cell, D4 area was for apoptotic tumor cell

2.4 SDS-PAGE Western blotting analysis

As shown in Fig. 3, the blank control group was 0.1% NaOH solvent control group, pAKT（Ser473）protein strips was the deepest among the four groups above, and PAKT (Ser473) expression is the highest; and protein strips of the pAKT（Ser473）of the protein group which was added 10μg/μl isorhamnetin was lighter than 2, and pAKT（Ser473）expression was stronger; protein strips of the pAKT（Ser473）of the protein group which was added 20μg/μl isorhamnetin was lighter than 2; protein strips of the pAKT（Ser473）of the protein group which was added 40μg/μl isorhamnetin was also light; protein strips of the pAKT（Ser473）of the protein group which was added 80μg/μl isorhamnetin was also light. The results showed that the pAKT（Ser473） protein was a gradually weakening trend with the increase of the concentration of Ser473AKT in rats, and the total AKT protein bands in the cells had no difference in expression.

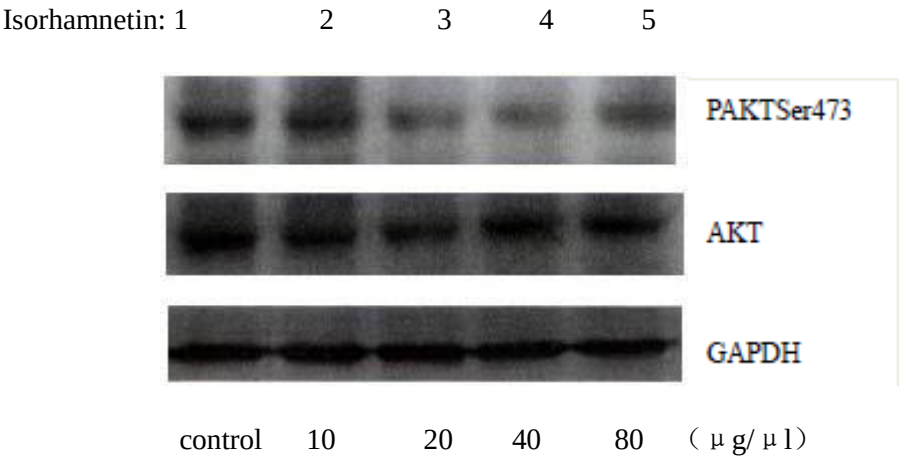
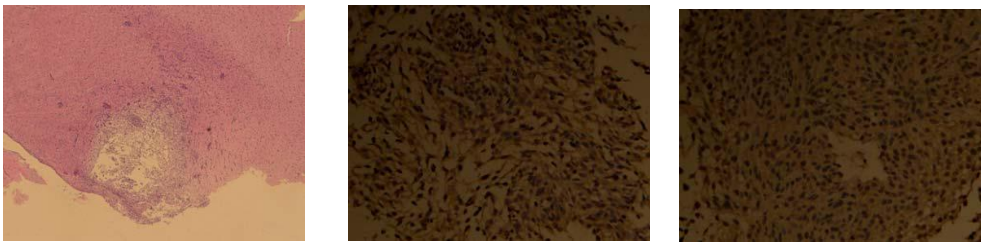


Figure 3. Effects of rat isorhamnetin of different concentrations on AKT protein and pAKT (Ser473) protein expression of C6 glioma cells

2.5 Brain tissue slices of rat model

As shown in Fig. 4, in the experimental group, HE staining slice in dark red was tumor tissue in good condition, and the hole in middle was atrophic tumor tissue, and light red one was normal brain tissue. In the blank control group, we can see obvious intercellular connection, and index of refraction of cell nucleus is good, cell form is clear: they were in polygon, triangle and climbing-flake shape. The refractive index of rat models after continuous feeding of isorhamnetin are obviously lower than the blank control group, but there were some cavities, there were no obvious filaments between the cells, the cell shape became round and the cell margin was not obvious.



C6 rat model which are given drugs blank control group fed isorhamnetin continuously

Figure 4. Observation of tumor tissue under the light microscope

2.6 HPLC graph of isorhamnetin in plasma and brain tissue in rats

As shown in table 2, the peak time of isorhamnetin control product was at 13.71min, and the peak area was 384.46; the rat plasma sample was isorhamnetin, the peak time was close to 15.00min, it is more accurate to say that it was complex compound of isorhamnetin and certain carriers of the plasma, and a small peak behind it may be flavonoids degraded by isorhamnetin in the plasma; the peak time of isorhamnetin in rat's brain tumor tissue was 13.91min, the peak area is 13.98; the test result of Kidney sample was that no peak in 15min, and it may be the isorhamnetin metabolism to other monomer; in E. Liver test results, there was no obvious peaks in 15min.

	1	2	3	4	Average data
Peak area of isorhamnetin in the control product	384.46	400.31	396.92	371.52	388.30
Peak area after 15min in brain tissue	13.98	7.09	9.73	11.24	10.51
Peak area after 15min in plasma tissue	0.63	2.14	1.15	4.05	1.99
Peak area after 15min in kidney	0.00	0.00	0.00	0.00	0.00
Peak area after 15min in liver	0.01	0.00	0.02	0.01	0.01
Percentage of plasma distribution					2.7%
Percentage of brain tissue distribution					0.51%

Table 2. Use HPLC detection to test the peak area of isorhamnetin in rat's plasma and brain tissue

3 Discussion

The development of chemotherapeutic drugs, killing tumor cells, causing apoptosis and necrosis of tumor cells are of great significance to the treatment of tumors, and apoptosis is the initiative and programmed death of gene control. Apoptosis is irreversible death, such as DNA fragment rupture, nuclear membrane rupture, protein denaturation degradation, enzyme inactivation, and apoptosis-inducing drugs may also have effects on normal cells, but apoptotic cells do not relapse, and can also inhibit from cell proliferation, and to solve the drug resistance of the mechanism of apoptosis. Therefore, it is important to study the relationship between the drug and tumor, and to explore its mechanism in order to seek the treatment of tumor. The experimental results showed that the survival rate of C6 glioma cells in vitro could be significantly reduced when the concentration of isorhamnetin was bigger than 40μg/μl. In inducing apoptosis experiment, we can observed in the inverted microscope that the nucleus condensed into a group, the cell volume became smaller, the apoptotic body (containing organelles and fragments) was formed, and the nucleus fragmentation, cell rupture and the number of cells decreased significantly. In the experiment of the effect of MTT colorimetric assay on the survival rate of C6 glioma cells cultured in vitro after Isorhamnetin was added, the results showed that the survival rate of C6 cells decreased with the increase of isorhamnetin concentration in rats. The apoptosis of C6 glioma cells was detected by flow cytometry after 48h treated by Isorhamnetin. The results showed that the cells of one phase moved to four phases with the increase of the concentration of rat, and the proportion of normal proliferative cells decreased, the apoptosis cells increased in four phases and the necrosis cells of the two phases increased. A result was obtained by using different experimental methods: With the increase of the concentration of rat, the survival rate of glioma cells in vitro was decreased, the proportion of apoptosis increased, and a certain dose was correlated.

The genes that regulate the normal cycle of cells are divided into the original oncogene and the tumor suppressor gene, the gene expressing the 3-phosphate kinase (phosphoinositide3-kinase， PI3K) family belongs to the original cancer gene, and the gene expression product can activate 3,4,5-three phosphate. Used as the second messenger within the cell, it is the protein kinase PKB (AKT) from the cytoplasm to the cell membrane activated signal factor. Cell proliferation and apoptosis are directly related to the P13K/Akt signaling pathway, which can lead to the occurrence, metastasis and invasion of tumors [7]. The PI3K activated product--PIP2 and PIP3--were combined with AKT platelet-white cell c kinase substrate homologous structural region (pleckstrin homology，PH) to activate two sites of AKTSer473 and Thr308 and alter the original conformation, to achieve the ultimate goal of Akt activation, the Akt is then transported from the cytoplasm to the cell membrane, and then the downstream pathway is activated. AKT activation can activate multiple target sites downstream, such as: Bad、caspase-9 precursor, mammalian target of rapamycin (mTOR), forkhead transcription factor Family (FKHR/AFX /FOX), Glycogen synthase kinase -3 (GSK-3), p21cipl and Raf [7].

The activated AKT transmits signals to downstream factors, regulates information transcription and cell cycle, and PI3K/AKT signaling pathways play an extraordinary role in cell survival and cell metabolism. The results showed that the expression of p- AktSer473 in rat C6 glioma cells was also different after adding different concentrations of rat isorhamnetin, and the expression of p- AktSer473 decreased with the increase of the concentration of isorhamnetin, and showed that when the concentration of 80μg/μl was added, the expression of p- AktSer473 in rat C6 glioma cells was the lowest, which indicated that p-

AktSer473 was activated in the rat C6 glioma cells. And the difference of expression suggested that the expression of isorhamnetin P-Akt Ser473 could be inhibited after the addition of isorhamnetin. The occurrence and development of the tumor, more directly, is that the rat Isorhamnetin has inhibitory effect on the PI3K/Akt signaling pathway, and the expression of p- Ak tSer473 protein is decreased and the rate of apoptosis is increased in rat C6 glioma cells.

In recent years, the emphasis on artemisinin and other important herbal medicines has increased, and the isorhamnetin belong to flavonoids, in recent years, the study found that in addition to the role of relieving cough and eliminating phlegm, activating blood and dispersing blood stasis, isorhamnetin also has many pharmacological effects, such as dilation of coronary artery, antiplatelet aggregation and anti-tumor [8-9]. Many literatures have reported that isorhamnetin has inhibitory effect on various cultured tumor cells in vitro, however, the effect of brain glioma has not been reported, and this study shows that when the concentration of isorhamnetin is greater than 40μg/μl, the inhibitory effect on the rat C6 glioma cells is obvious, and the apoptosis is induced, featuring cell contraction, membrane folds, the volume becomes small, forms the apoptotic small body (contains the apoptotic nucleus fragment). Detection of the growth inhibition and survival rate of rat C6 glioma cells by MTT colorimetric assay shows that after 48h of isorhamnetin, the value of C6 glioma cells decreased and the survival rate was significantly reduced, and the data showed that the concentration of isorhamnetin had a certain quantitative relationship with C6 brain glioma. The flow cytometry test shows that the cell survival rate of rat C6 glioma cells was 75.4% after 48h, and the apoptosis rates were 19.3%, compared with 15% in the negative control group, which indicated that isorhamnetin had great potential in promoting rat C6 glioma cells. Western blotting experiment showed that with the increase of concentration of isorhamnetin, the brightness of AKTSer47 protein stripe decreased and the protein expression reduced, which indicated that isorhamnetin could inhibit the activation of AKT protein and enhance the apoptosis-promoting effect of rat C6 glioma cells. At the same time, rat brain glioma in vivo model experiment showed that after 5 days of feeding the rat isorhamnetin drug, the rat isorhamnetin monomer was detected in the plasma and brain tissues of rats, and it was indicated that isorhamnetin had a certain target to glioma, which provided a new basis for isorhamnetin as a therapeutic drug.

When the concentration of isorhamnetin was 40μg/μl and 80μg/μl, no matter in MTT assay and the flow cytometry, the results are the same: isorhamnetin inhibited the C6 glioma cells in rats, and the cell apoptosis was the most obvious in the experimental group at these two concentrations. When the concentration of isorhamnetin was 80μg/μl, the cell membrane rupture, the edge of cells were not clear, the intact cell structure was lost, and the nucleus was shrunk or disappeared. The results showed that the apoptosis of rat C6 glioma cells was obvious under the action of a certain concentration of rat isorhamnetin, and the necrosis of C6 glioma cells could be caused when the concentration of the isorhamnetin was high. Of course, this is a commonality. In the process of tumor cell death caused by many antitumor drugs, small doses cause apoptosis, and when the concentration reached a certain threshold, necrosis will be caused, and high concentrations will generally lead to coexistence of apoptosis and necrosis [10]. The reason is that in late period of apoptosis, when the macrophage load is large enough and unable to quickly remove the phagocytosis of the cell fragments, then it can no longer devour the apoptotic body, and the cell will occur secondary necrosis [11-12]. From the point of view of treatment, if the compound or monomer which can induce apoptosis can be used for clinical treatment, then from the observation and analysis of the inducing effect can show that the isorhamnetin can inhibit the

growth of C6 glioma cells and induce apoptosis, and the higher the concentration, the stronger the effect, which set an experimental basis for developing a new type of antitumor drug from Rat Isorhamnetin was established [13-15]. In addition, we should note that in the rat brain glioma model experiment, isorhamnetin was found in plasma and brain tissue detection, and the distribution of it in the brain is also considerable, which indicating a problem that the targeting of isorhamnetin brain tissue is still worth affirming, and it is worth believing that in the near future, isorhamnetin can have a further development as a drug for brain glioma.

In this paper, in culturing rat C6 glioma cells in vitro, the growth status of rat C6 glioma cells was observed by adding different concentrations of isorhamnetin, and the cell proliferation rate was detected by MTT colorimetric assay, and the apoptosis was detected by the flow cytometry. By detecting the expression of total AKT protein and Ser473 AKT protein in intracellular target protein, the relationship between isorhamnetin and PI3K/AKT signaling pathway in effecting rat C6 glioma cells was further studied, and the application value of isorhamnetin in the treatment of glioma was studied. The conclusions are as follows: The survival rate of C6 glioma cells in rats was affected by different concentrations of isorhamnetin, with the increase of isorhamnetin concentration, the survival rate of rat C6 glioma cells decreased gradually, and the correlation between mass concentration and inhibition ratio was shown. The apoptosis of C6 glioma cells was induced by isorhamnetin, and the apoptosis rate of rat C6 glioma cells was different in different concentrations of isorhamnetin, and the proportion of apoptosis increased with the increase of the concentration of isorhamnetin. The detection of protein expression showed that total AKT protein and Ser473 AKT protein content in the cells decreased with the addition of isorhamnetin, which was inversely proportional to the tolerance. It is also shown that the mechanism of the effect of isorhamnetin on C6 glioma cells in rats is closely related to the mechanism of PI3K/AKT signaling. After the isorhamnetin entered into mice, it was mainly distributed in the brain tissue, and the brain tissue was the main target tissue of isorhamnetin action.

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