

Cytotoxicity of hollow silica nanoparticles loaded with photosensitizers on huh-7 cells

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Abstract: To observe the cytotoxic effect of the photodynamic therapy mediated by the traditional photosensitizer polyhematoporphyrin (C₃₄H₃₈N₄NaO₅, Photosan-II) Photosan-II was loaded into HSNP by one-step wet chemical, PS and hollow silica nanoparticles (HSNP) loaded PS on Huh-7 cells and compare the cytotoxic effects. -based synthetic route. The cellular viability was determined by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. Apoptotic and necrotic cells were measured by flow cytometry. The suitable drug concentrations of free PS and HSNP loaded PS on Huh-7 cells were 20mg/L and 5mg/L respectively, and the suitable incubation time were 4 h and 2 h respectively. Under the same drug concentration, the survival rates of free PS and HSNP loaded PS were 62.46%±1.93% and 6.54%±1.24% (P<0.05). Under the same drug concentration and incubation time, the total rates of apoptosis necrosis caused by free PS and HSNP loaded PS mediated PDT were respectively 22.00%±2.24% and 87.23%±2.1% (P<0.05). PS-loaded HSNP mediated PDT can inhibit proliferation of cancer cells and induce apoptosis more quickly and effectively. As the loading system of PS, HSNP can make the photosensitizer have stronger solubility and drug concentration efficiency, which can significantly improve the therapeutic effect of PDT.

Keywords Photodynamic therapy, Photosan-II, hollow silica nanoparticles, Huh-7, hepatocellular carcinoma

INTRODUCTION

PDT has shown a good application prospect in the diagnosis, prevention and therapy of tumors. For the therapy of liver cancer, compared with the traditional treatments such as chemotherapy, radiotherapy, and operation, PDT is a targeted technology with minimal invasion and it can reduce the harm to normal tissues when it kills cancer cells, which provides a new strategy for the therapy of liver cancer.

A variety of drug delivery nano-systems have been developed to deliver photosensitizers, such as liposomes (Oh *et al.*, 2001), polymer carriers, cremophor emulsion (Soncin *et al.*, 1995), microspheres and nanoparticles (Wieder *et al.*, 2006), which can improve the properties of photosensitizers. However, most of the designed drug delivery nano-systems need releasing of the loaded drug, which will slow down the seepage velocity of photosensitizers to tumors tissues. Nanotechnology-based drug delivery system has become one of the most extensive studied fields in nanomedicine in the recent decade (Hatakeyama *et al.*, 2011), which is a designed drug delivery nano-system needn't releasing of the loaded photosensitizers. The new photosensitizer can improve the selectivity of photosensitizer through crosslinking with the all kinds of carriers to promote the absorption of tumors to the photosensitizer and improve the cytotoxic effect of PDT on tumor cells.

Currently, there are some reports about the photodynamics therapy on tumors using the nanotechnology combined with photosensitizers, however, there is no related literature report about researches of the application of nanosized photosensitizer mediated PDT on liver cancer.

MATERIALS AND METHODS

Preparation of hollow silica nanoparticles loaded with photosensitizers

PS-loaded HSNP was prepared by one-step wet chemical-based synthetic route, which is similar with the study of Deng *et al.* (2013). Briefly, 0.615mg of Polyacrylic acid (or Sodium polyacrylate) and 1mg Photosan-II were dissolved in 4ml ammonia, and then 100ml anhydrous ethanol was added rapidly and the system was stirred in the dark until it became clear (about 0.5h). After that, 1.395g TEOS was added into the system slowly in 10h at a time interval of 2 h under vigorous magnetic stirring. After the reaction, the products were centrifuged and washed above three times in deionized water and anhydrous ethanol, and dried at 60°C in a vacuum drying oven (Deng *et al.*, 2013).

Cell line and cell culture

The Huh-7 human hepatoma cell line was purchased from Chinese academy of sciences in Kunming. Huh-7 cells were cultured in high glucose DMEM containing 10% fetal calf serum in an incubator under 37°C with 5% CO₂, and cells in the logarithmic phase were selected for

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experiments. Huh-7 cells were then digested with pancreatic enzyme and prepared into the cell suspension with the concentration of 2×10^5 . And the cell suspension was inoculated into the 96-well culture plate with 200ul per hole and the plate was then placed into the constant temperature incubator. The cells were divided into traditional photosensitizer PS group and HSNP-PS group. Each group was then divided into control group (neither light nor photosensitizer was added), pure photosensitizer group (only different doses of photosensitizers were added with no light), pure light group (only different doses of light were added with no photosensitizer) and experimental group (both photosensitizer and light were added). And the nanoparticles suspension was diluted into the solution with the concentration of 0, 1.25, 2.5, 5, 10 and 20 mg/L with the culture medium without serum. The cell culture fluid was abandoned and the cells were washed with PBS for 2 times. After 200ul Photosan culture medium was added in the dark condition, the cells were cultured in the incubation for 1, 2, 4 and 6h respectively. After the incubation, 200ul common culture medium was added and the cells were exposed in the laser with the wavelength of 630 nm and light energy of 2.5, 5 and 10 J/cm^2 respectively. The nanosized photosensitizer was used in the same steps to treat the cells.

MTT array

The treated cells were incubated sequentially for 24h and 20ul MTT was added into each hole. After they were incubated for 4 h, the culture solution in each hole was abandoned and 150ul DMSO was added. A blank hole was added with 150ul DMSO as the zero adjustment standards and oscillated on an oscillator for 10min to fully dissolve the crystal substances. The wavelength of 57 nm was selected to determine the OD value of each hole on the enzyme-linked immune detector and the results were recorded. The cell survival rate = OD value / OD value of the control group $\times 100\%$.

Flow cytometry

Based on the experiment results determined by MTT, the appropriate function parameters of the two photosensitizers were screened to treat the cells. The cells were divided into group A (blank control group), group B (the appropriate function parameters of HSNP-PS were used by free PS) and group C (HSNP used its own appropriate function parameters). The cells in each group were collected for flow cytometry apoptosis detection (Annexin V-FITC&PI apoptosis detection kit, Biouniquer Company).

STATISTICAL ANALYSES

The SPSS statistical software package was used to assess the experimental data by univariate analyses of variance.

RESULTS

MTT array

After PDT, the proliferation of Huh-7 cells in the two groups were significantly inhibited with the increase of light dose in a certain range ($P < 0.05$), and when the light dose reached to 10 J/cm^2 , the cells could not be significantly inhibited by the increasing light dose ($P > 0.05$), as shown in fig. 1. After PDT, the proliferation of Huh-7 cells were significantly inhibited with the increase of the concentration of photosensitizer and incubation time ($P < 0.05$). When the concentrations of photosensitizer reached to 20mg/L (Photosan group) and 5 mg/L (nanosized Photosan group), and the incubation time reached to 4 h (Photosan group) and 2h (nanosized Photosan group), the cell proliferation could not be significantly inhibited by the increasing light dose or incubation time ($P > 0.05$), as shown in figs. 2 and 3.

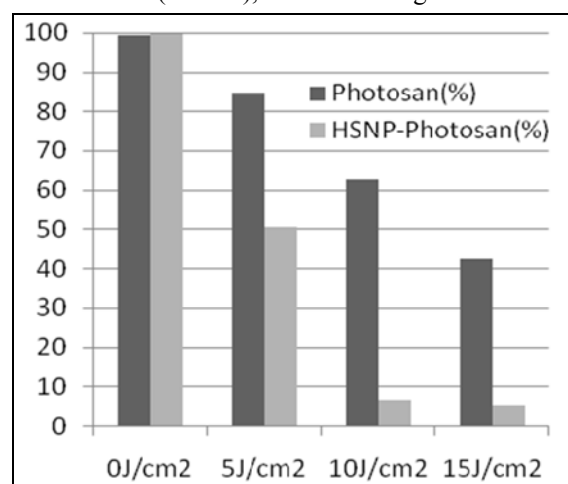


Fig. 1: The impact of light dose on PDT therapeutic effects (cell viability).

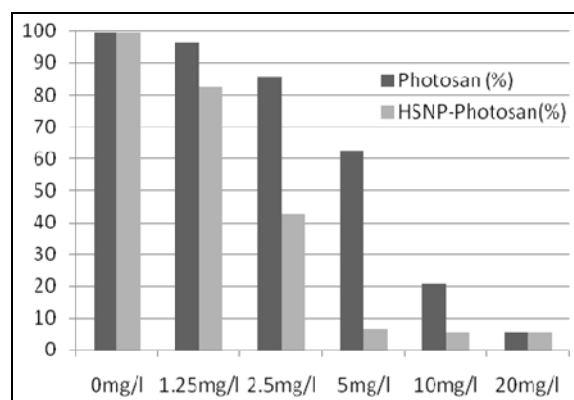


Fig. 2: The impact of concentrations on the therapeutic effects of PDT (cell viability).

Flow cytometry

After PDT, the apoptosis rates of three groups were significantly different ($P < 0.05$). The apoptosis rate of group C (nano Photosan, used its own appropriate function parameters) was 88.2%, which was significantly

higher than 22.0% of the group B (Photosan, used the suitable function parameters of nanosized Photosan) ($P < 0.05$), as shown in fig. 4.

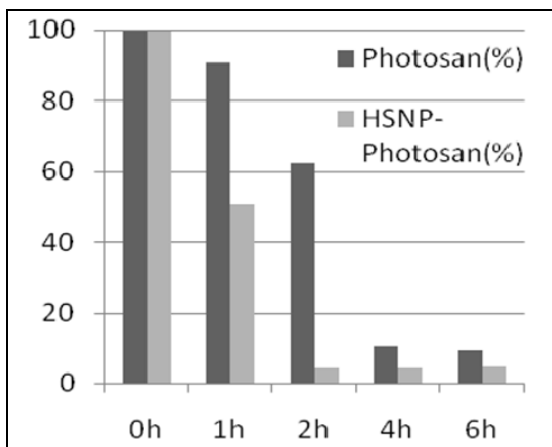


Fig. 3: The impact of incubation times on the therapeutic effects of PDT (cell viability).

DISCUSSION

PDT is a nonsurgical therapy for tumors using photosensitizers and photodynamic reaction. As a kind of cold light chemical reaction, PDT mainly relies on the interaction between the photosensitizer and light (laser was commonly used), and it is affected by the oxygen concentration in tissues, therefore, the photosensitizer is an important factor influencing the PDT effect. The clinical application of PDT in the treatment of liver cancer has been restricted compared to other superficial tumors

on body surface or in cavities. Because liver has high pigmentation, which makes the laser with treatment wavelength cannot effectively penetrate the diseased tissue; At the same time, normal liver cells can also absorb and save most of the photosensitizers, which makes the photosensitizer cannot achieve the high selectivity in liver cancer cells (Solban *et al.*, 2006). Because in clinical practice, the action site of optical fiber can be inserted into the cancer tissue through the channel established under the guide of ultrasound or CT, a more efficient photosensitizer with higher selectivity of tumor tissues is needed for the clinical PDT therapy of liver cancer, which can fully exert the oxygen excitation ability of the laser with fixed wavelength. Therefore, a more effective new photosensitizer will be a breakthrough for the clinical PDT therapy on hepatocellular carcinoma.

Compared with the traditional photosensitizers, nanosized photosensitizers have characteristics of small particles, big relative surface area, high surface reactivity, more activity centers, high catalytic efficiency, and strong adsorption ability. The combination of photosensitizers and nanoparticles has been confirmed to have abilities to reduce drug toxicity, increase drug solubility in the medium (Kennedy *et al.*, 1990), and enhance the photodynamic effect of photosensitizer (Peng *et al.*, 1997). Some studies also showed that it could improve the targeted combination ability of photosensitizers on specific tissues (Ortel *et al.*, 1998). Many traditional photosensitizers (e.g. Photosan) have the characteristic of hydrophobicity, which inevitably leads to the following problems: the transport efficiency of photosensitizers in the blood circulation is not high and the photodynamic

Table 1: Flow cytometry analyses of groups A, B, and C

	A	B	C
Apoptosis and necrosis rate (%)	2.67±0.577	21.960±2.244*	87.233±2.095* ^Δ

*Group A compares with group B and C, $P < 0.05$; ^Δ Group B compares with group C, $P < 0.05$.

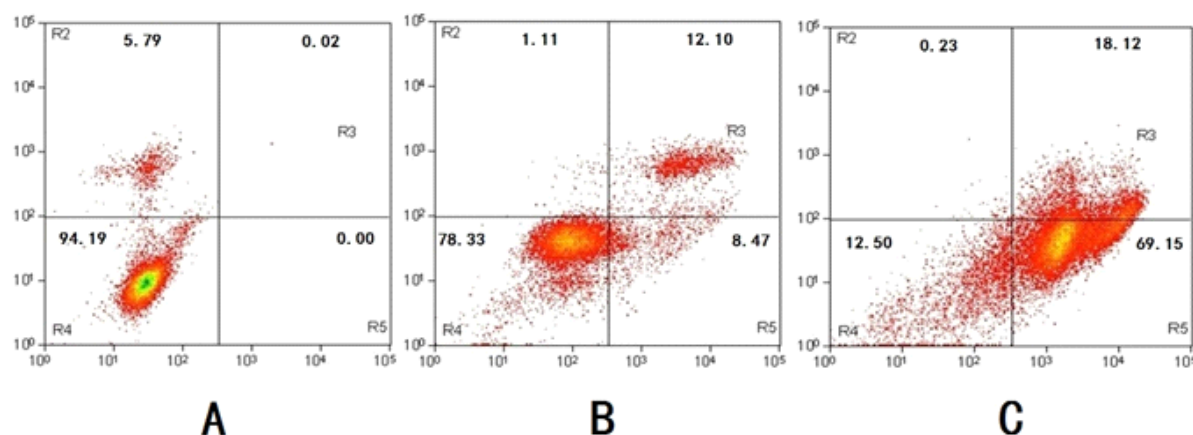


Fig. 4: Flow cytometry analyses of groups A, B, and C. The lower left quadrant represents normal cells, the lower right quadrant represents apoptotic cells, the upper right quadrant represents necrotic or late apoptotic cells, and the upper left quadrant represents mechanically damaged cells.

effect is weakened by the aggregation function of photosensitizers. However, in recent years, the research progress of nanosized drug provides a way to solve this problem. Although people have developed a variety of nanosized drug carriers, which can carry photosensitizers, e.g. liposomes (Gaullier *et al.*, 1997), polymer carrier (Casas *et al.*, 2001), cremophor emulsion, microspheres and nanoparticles (Redmond *et al.*, 2006). They can all improve the properties of photosensitizers, but they all need to have a process to release the loaded drug, which will slow down the rate of tumor cells to absorb photosensitizers and extend the time of photosensitizers to reach the effective drug concentration (Moan *et al.*, 1991).

HSNP has characteristics of high controllability of the shape and size, good water solubility, stable property and high biological compatibility, therefore, HSNP has obvious advantages in loading photosensitizers compared with other traditional nanosized carriers which need to release the loaded photosensitizers (Moan *et al.*, 2001). In addition, compared with the solid silica nanoparticles loading system, HSNP is easier to penetrate than small molecules such as singlet oxygen, and the singlet oxygen is the key molecule effector of PDT. HSNP, therefore, is likely to be a kind of ideal nanosized carrier system which can improve the photodynamic effect of photosensitizers. After the determination of the survival rate of Huh-7 cells after PDT by MTT, HSNP-PS with the concentration of 5 mg/L and incubation time of 2 h can reach the cytotoxic effect as the same as the traditional photosensitizer free PS with the concentration of 20 mg/L and incubation time of 4h. And the cytotoxic effect of HSNP-PS is significantly higher than free PS while they are set at the same concentration and incubation time. Due to the larger cavity of HSNP, the drug loading ability is stronger than the solid silicon, and the effect of shortening the time of the photosensitizer to achieve the required effective concentration is more significant (Xue *et al.*, 2001).

CONCLUSION

Compared with the free PS, PS-loaded HSNP mediated PDT can inhibit the proliferation of cancer cells and induce apoptosis more quickly and effectively. HSNP, as the PS loading system, can make photosensitizer have stronger hydrophilicity and efficiency of drug concentration without affecting the photometrics, which can significantly improve the therapeutic effect of PDT.

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