行政院國家科學委員會專題研究計畫　成果報告

光動力及高溫對於細胞胞飲作用的影響

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中　華　民　國 94 年 10 月 31 日

一、中文摘要

光動力治療是一種新興的癌症治療方式，主要應用於治療固態癌症，其作用機轉係以雷射光源或是其它非雷射光源激發細胞內的光感藥物，光感藥物受到光子的刺激逐步產生光化學作用，形成單相氧分子或自由基並破壞細胞內微細結構如粒腺體，因而導致細胞死亡。我們利用新的光動力應用：光化學內化作用（photochemical internalization，PCI）作為本計畫研究主題技術。高溫腫瘤治療在臨床上由於會破壞癌細胞的細胞的膜構造而抑制其生長。本計畫將研究光化學內化及高溫對於細胞胞飲作用效率的影響。

根據先前的研究，我們利用奈米載體 PAMAM-FITC 作為標的物質，用來探討光動力及高溫對於細胞胞飲作用的影響，研究經過處理後是否能增進 PAMAM-FITC 進入細胞的效果，或是增加細胞胞飲作用，研究結果對於奈米載體應用將有所幫助。

關鍵詞：光化學內化、高溫、細胞吞噬、胞飲作用、樹枝狀高分子

二、英文摘要

Photodynamic therapy (PDT) is a promising new treatment for cancer that has been recently accepted in the clinic. PDT involves the localization of a light-sensitive drug in the target tissue prior to illumination using an appropriate wavelength. Exposure of tissue containing a sufficient concentration of photosensitizer to irradiation of adequate wavelengths initiates photodynamic activation to generate single oxygen free radicals and other reactive species, causing cellular damage and tissue necrosis. It is a procedure having the potential to selectively destroy malignant tissues without causing serious damage to the adjacent normal structures if light dosimetry can be delivered with care. PCI is a novel method that can release macromolecules from endocytic vesicles by introducing photochemical damage to the membranes of these vesicles.

In this project, the effects of PCI combine with hyperthermia on cellular uptake or endocytosis will be studied by PAMAM-FITC monitoring.

Keyword: photochemical internalization, hyperthermia, cellular uptake, endocytosis, dendrimer

三、前言

PAMAM dendrimers are a new class of highly branched spherical polymers that had been successfully employed as a substrate for the attachment of antibodies, contrast agents, and radiopharmaceuticals for applications in a number of different areas of biology and medicine. Photochemical internalisation (PCI) is a novel technique using the principle of photodynamic therapy (PDT) for releasing large, biologically active molecules at specific sites in living tissue. Hyperthermia may be another approach to enhance the endocytosis of the tumor cells.

四、研究目的

In this study, we studied if endocytosis cnould be enhaced by PCI or by hyperthermia. We believe this study could possess big impact on the combined modality for clinical cancer

treatment.

五、文獻探討

Nanotechnology has developed to a stage that makes it possible to produce, characterize and specifically tailor the functional properties of nanomaterials for clinical applications in recent years. Dendritic polymers (dendrimers) are a novel class of nanomolecules, distinguished from linear and randomly branched polymers by the inclusion of precisely one branch point per repeat unit[1]. PAMAM dendrimers are a new class of highly branched spherical polymers that are highly soluble in aqueous solution and have a unique surface of primary amino groups[2-4]. The defined structure and large number of surface amino groups of PAMAM dendrimers have led these polymers to be employed as a substrate for the attachment of antibodies, contrast agents, and radiopharmaceuticals for applications in a number of different areas of biology and medicine[4-6]. Furthermore, the evidences of antibody/dendrimer conjugates in previous *in vitro* and *in vivo* studies have been shown to be nontoxic to the animals and are able to target biologic agents to specific cells [4-6].

Photochemical internalisation (PCI) is a novel technique using the principle of photodynamic therapy (PDT) for releasing large, biologically active molecules at specific sites in living tissue. It is known that most membrane-impermeable macromolecules are taken up by cells through the process of endocytosis.[7] In some cases, the endocytosed macromolecules then stay confined within the cell in organelles such as endosomes and lysosomes until they are degraded by hydrolytic enzymes located in these organelles, so they never get released into the cytosol of the cells. The concept of PCI is to break down these organelles to release much greater amounts of the introduced molecules into regions of the cell where they can become biologically active.[8-10] Some PDT photosensitisers, like aluminium disulphonated phthalocyanine ($AlPcS_{2a}$) localise on the membranes of these organelles and when activated by exposure to light of an appropriate wavelength (670-675nm for $AlPcS_{2a}$), break down these membranes, so releasing the macromolecules.[8-10] Hyperthermia is heat treatment for medical application. The temperature of the tissue is elevated artificially with the aim of receiving therapeutic benefits. But if the hyperthermia could enhance the endocytosis of tumor cells is not clear.

Therefore, the PAMAM dendrimer was choose as a model of drug nanocarrier in this study and the effects of PCI and hyperthermia on cellular endocytosis was investigated.

六、實驗方法

- Cell line: Ca9-22
- Hyperthermia – 39, 41, 43℃
- PAMAM-FITC　final conc.:13.5ug/ml
- Fluorescence microscopy and flow cytometer

七、結果與討論

Fig. 1 shows that the cellular uptake of PAMAM dendrimer by hyperthermia treatment in Ca9-22 cells by flow cytometric monitoring. It is obvious that the cellular uptake of

PAMAM-FITC was inhibited at 43℃ and there are no apparent different of cellular uptake with heat treatment below 41℃ for short term monitoring. The fluorescent measurement of cellular uptake in the presence or absence thermal treatment was shown in Fig. 2. It is conspicuous that the uptake of PAMAM-FIC was inhibited with thermal treatment. But for long term measuring, the results of cellular uptake of PAMAM-FITC with thermal treatment at 37℃ and 43 ℃ after 18h were similar (Fig. 3). It may due to the time delay of cellular response after thermal treatment. The acute response of cells with thermal treatment was started for short term monitoring, and then the cellular protective mechanism was activated and the PAMAM-FITC uptake was enhanced.

For PCI treatment, the uptake of PAMAM-FITC was enhanced at 30 min monitoring, but that was similar to the observation without PCI at 3h (Fig. 4). The fluorescence intensity was also quantified by flow cytometer (data not shown).

八、結論

1. The cellular uptake of PAMAM-FITC was inhibited for short term thermal treatment but could be enhanced by long term treatment.
2. PCI approach could enhance the uptake of PAMAM-FITC and that could be supported by our observation by fluorescence microscopy and flow cytometer. .

九、計畫成果自評

The changed uptake of PAMAM-FITC with thermal treatment could be considered the thermal effect of endocytosis. The enhanced uptake of PAMAM-FITC may be due to the cellular membrane acutely destroyed by PCI approach and that could be repaired. Therefore, the detail mechanism of PCI effect on cellular uptake (or endocytosis) needs to be investigated.

十、參考文獻

1. Buleier, E., Wehner, W., and Vogtle, F. *Synthesis*. 1978, 155-158.
2. Tomalia, D. A. & Durst, H. D. *Techniques in Current Chemistry*, ed. Weber, E. (Springer, Berlin), 1993: 193–245.
3. Tomalia, D. A., Naylor, A. M. & Goddard, W. A., III, *Angew. Chem., Int. Ed. Engl.* 1990: 29, 138–175.
4. Wu, C., Brechbiel, M. W., Kozak, R. W. & Gansow, O. A., *Bioorg. Med. Chem. Lett.* 1994: 4, 449–454.
5. Barth, R. F., Adams, D. M., Soloway, A. H., Alam, F. & Darby, M. V., *Bioconjugate Chem.* 1994: 5, 58–66.
6. Singh P., Moll, F., III, Lin, S. H., Ferzli, C., Yu, K. S., Koski, R.K., Saul, R. G. & Cronin, P. , *Clin. Chem.* 1994: 40, 1845–1849.
7. Sorkin A, von Zastrow M. Nat Rev Mol Cell Biol 2002;3:600-614
8. Berg K, Selbo PK, Prasmickaite L, Tjelle TE, Sandvig K, Moan D, Gaudernack G, Fodstad O, Kjolsrud S, Anholt H, Rodal GH, Rodal SK, Hogset A. Cancer Res 1999;59:1180-1183
9. Prasmickaite L, Hogset A, Tjelle TE, Olsen VM, Berg K. J Gene Med 2000;2:477-488
10. Selbo PK, Sandvig K, Kirveliene V, Berg K. Biochimic Biophysic Acta-General

Subjects 2000;1475:307-313
11. Wolkers WF, Looper SA, Fontanilla RA, Tsvetkova NM, Tablin F, and Crowe JH, Biochimica et Biophysica Acta 2003; 1612: 154– 163

附圖

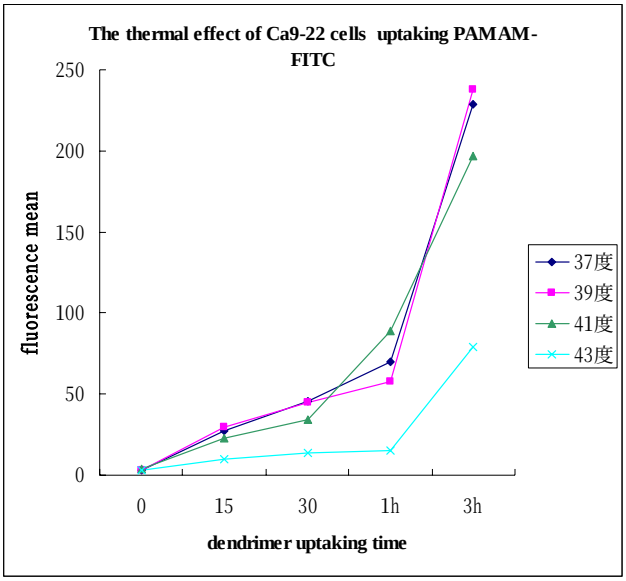

Fig. 1

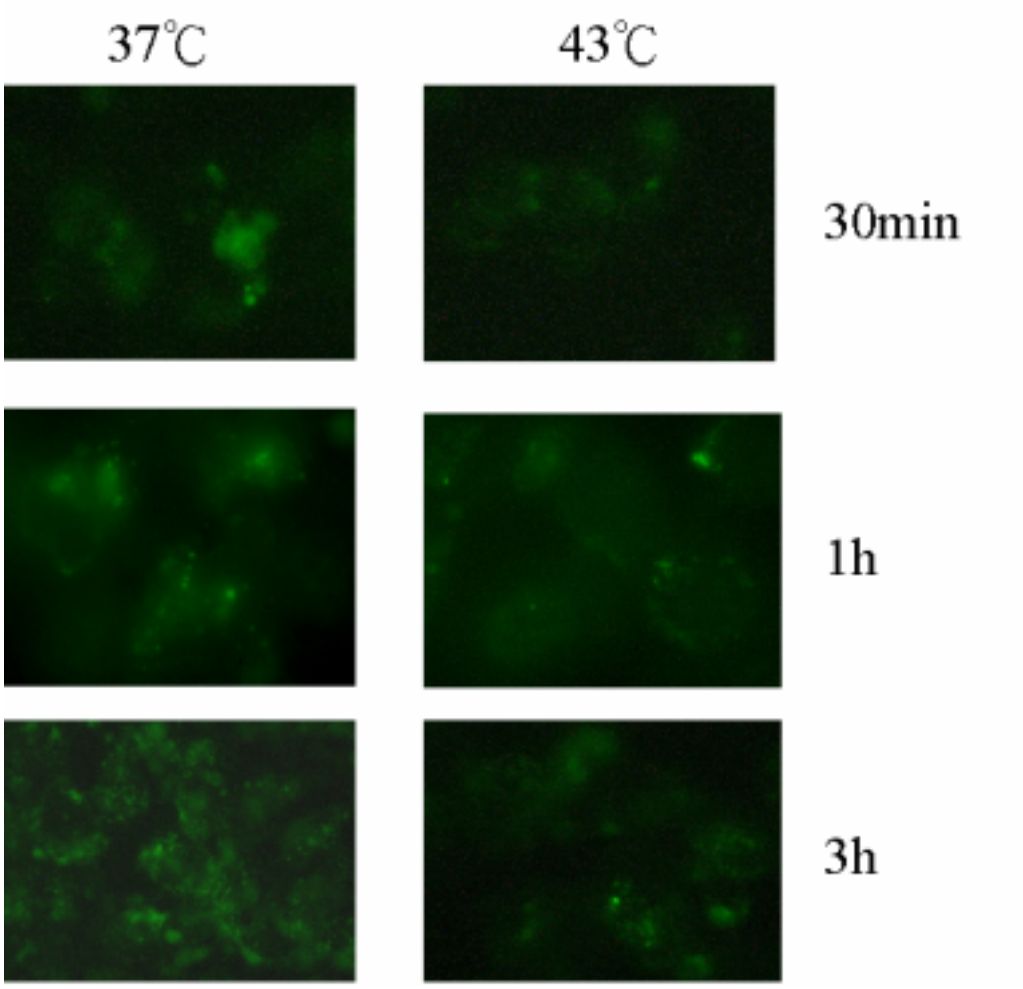

Fig. 2

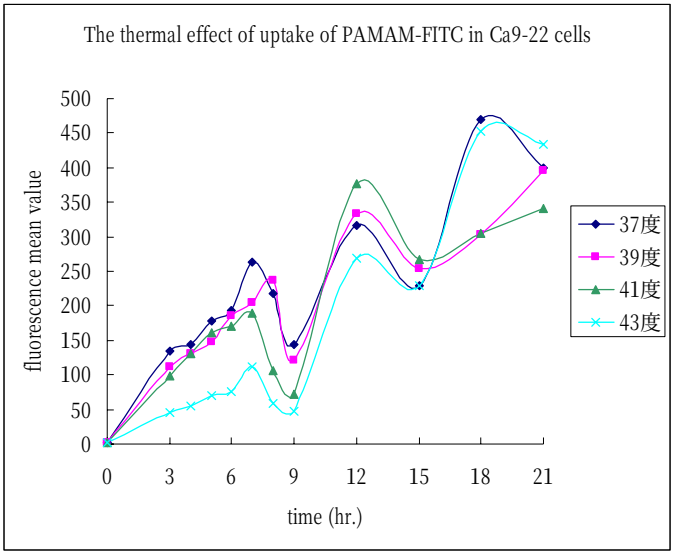
The thermal effect of uptake of PAMAM-FITC in Ca9-22 cells
fluorescence mean value
500
450
400
350
300
250
200
150
100
50
0
0
3
6
9
12
15
18
21
time (hr.)
37度
39度
41度
43度

Fig. 3

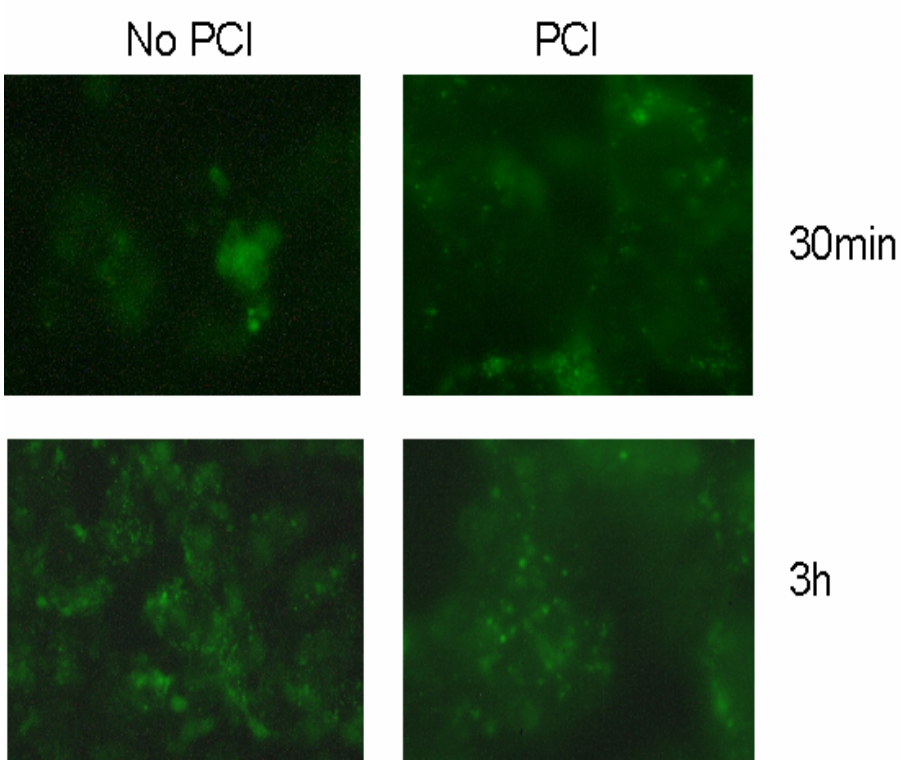
No PCI
PCI
30min
3h

Fig. 4