

Chitosan Nanospheres for Oral Mucosa Cell Permeation and Controlled Drug Release

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ABSTRACT

In this study, the effects of crosslinking on absorption properties of acetaminophen loaded chitosan (CS) nanospheres (NPs) were investigated on an in vitro model of buccal epithelium (KOSC-3 cells). Acetaminophen genipin-cross-linked CS NPs were prepared by adjusting the pH of chitosan HCl solutions to 6.4, and then mixing with acetaminophen and genipin. The samples were analyzed by transmission electron microscope (TEM) and dynamic light scattering (DLS). It was found that the size of a majority of the CS NPs ranged between 19 and 40 nm with a zeta potential between +45 and +65 mV. The cytotoxicity of CS NPs was determined by MTT assay. No observable differences in toxicity were noted on KOSC-3 cells by incubation with CS NPs of various genipin contents. CS NPs, like chitosan, possessed mucoadhesive properties. In vitro studies performed on KOSC-3 cell showed a pronounced reduction in transepithelial electrical resistance (TEER). The reduction in TEER is an indication of the opening of the cell junctions located between cells. Opening of the junctions will result in enhancement of absorption via the paracellular route. The release profile of acetaminophen from CS NPs presented that these NPs can be proposed as controlled release delivery system.

Keywords : chitosan nanospheres、KOSC-3、histological、drug release

Table of Contents

目錄 封面內頁 簽名頁 授權書 iii 中文摘要 iv 英文摘要 v 誌謝 vi 目錄 vii 圖目錄 x 表目錄 xi 1. 緒言 1 2. 文獻回顧 2 2.1 幾丁質與幾丁聚醣簡介 2 2.1.1 幾丁聚醣及其衍生物結構 2 2.1.2 幾丁聚醣顆粒之製備 3 2.1.3 幾丁聚醣顆粒於藥物控制釋放 3 2.2 架橋劑緣子素 (genipin) 簡介 8 2.3 乙醯胺酚 (acetaminophen) 簡介 8 2.4 奈米顆粒簡介 11 2.5 口腔黏膜簡介 11 2.5.1 上皮組織簡介 12 2.5.2 上皮細胞之傳遞途徑 12 2.5.3 上皮黏膜藥物傳遞系統 13 2.5.4 細胞毒性評估 20 2.6 藥物釋放評估 21 2.6.1 分光光度計偵測法 22 2.6.2 HPLC定量法 22 3.材料與方法 23 3.1 實驗架構 23 3.2 實驗器材 24 3.2.1 藥品 24 3.2.2 耗材 25 3.2.3 儀器設備 26 3.2.4 細胞株 27 3.2.5 培養基及試劑配製 27 3.3 幾丁聚醣鹽酸鹽載體製備 29 3.4 產物分析 31 3.4.1 紫外光/可見光譜分析 31 3.4.2 富立葉紅外線光譜儀試驗 31 3.4.3 TEM穿透式電子顯微鏡分析 31 3.4.4 表面電位 (zeta potential) 分析 31 3.5 細胞培養 32 3.5.1 細胞活化 32 3.5.2 繼代培養 32 3.5.3 MTT毒性試驗 33 3.5.4 細胞穿透電阻 (TEER) 試驗 33 3.5.5 Trypan blue 細胞存活率試驗 34 3.6 藥物釋放試驗 34 3.6.1 標準品配製 35 3.6.2 藥物釋放分析 36 4. 結果與討論 37 4.1 幾丁聚醣鹽酸鹽奈米懸浮顆粒之製備 37 4.1.1 紫外光/可見光光譜分析試驗 37 4.1.2 富立葉紅外線光譜分析試驗 38 4.2 穿透式電子顯微鏡觀察 41 4.3 表面電位 48 4.4 細胞培養 51 4.5 MTT試驗 53 4.6 TEER試驗 55 4.7 Trypan blue存活率試驗 57 4.8 藥物釋放試驗 61 5. 結論 64 參考文獻 66 圖目錄 圖2.1 幾丁質及其衍生物之結構式 5 圖2.2 理想狀況之藥物釋放速率曲線圖 7 圖2.3 幾丁聚醣與綠梔子素架橋後之結構式 10 圖2.4 乙醯胺酚之化學結構 10 圖2.5 口腔黏膜結構 15 圖2.6 上皮細胞穿透模式 17 圖2.7 MTT反應機制 21 圖3.1 實驗流程圖 23 圖3.2 乙醯胺酚之檢量線 35 圖4.1 各樣本之UV圖譜 39 圖4.2 各樣本之FTIR圖譜 40 圖4.3 Ch之(a) TEM圖、(b) 粒徑分析 43 圖4.4 Ch GP之(a) TEM圖、(b) 粒徑分析 44 圖4.5 Ch GP之(a) TEM圖、(b) 粒徑分析 45 圖4.6 Ch GP之(a) TEM圖、(b) 粒徑分析 46 圖4.7 Ch GP之(a) TEM圖、(b) 粒徑分析 47 圖4.8 KOSC-3細胞培養於flask之觀察 52 圖4.9 各樣本MTT試驗 54 圖4.10 5種樣本之TEER試驗 56 圖4.11 Trypan blue試驗之鏡檢圖 (a) 對照組、(b) Ch 58 圖4.12 Trypan blue試驗之鏡檢圖 (a) Ch GP (b) Ch GP 59 圖4.13 Trypan blue試驗之鏡檢圖 (a) Ch GP (b) Ch GP 60 圖4.14 幾丁聚醣奈米微球藥物載體之藥物釋放 63 表目錄 表2.1 幾丁聚醣奈米微粒製備之相關研究 6 表2.2 幾丁聚醣包覆乙醯胺酚之相關研究 9 表2.3 上皮組織分類 16 表2.4 口腔黏膜滲透促進劑 18 表2.5 黏膜黏附性高分子和傳遞系統相關研究 19 表3.1 人類口腔鱗狀細胞癌細胞株說明書 28 表3.2 KOSC-3細胞之複合培養基 28 表3.3 十倍磷酸鹽緩衝溶液配方 29 表3.4 幾丁聚醣奈米微球之組成 30 表4.1 五種樣本之平均粒徑 42 表4.2 各分散系之表面電位 49 表4.3 各樣本之表面電位 50 表4.4 各樣本之包覆效率及包覆量 62

REFERENCES

- 王長均、王仁潔、張麗姿、殷鳳儀、史中、王嘉銓、劉念先、馬國興、趙壯飛、徐佳福、鄭瓊娟、蔡元榮、吳慶祥。2006。ROSS組織學。第98-144頁。合記圖書出版社。台北，台灣。
- 行政院衛生署。2000。中華藥典第五版。第1-3頁。衛生署。台北，台灣。
- 徐世昌。2001。生物高分子-幾丁質與幾丁聚醣之介紹與應用。化工資訊 15(2):36-45。
- 張安華。2005。實用奈米技術。第233-235頁。新文京開發出版股份有限公司。台北，台灣。
- 陳慶源。2002。脈衝列電磁場刺激對骨母細胞生物活性的影響:18-28。中原大學醫學工程研

究所碩士論文。中壢，台灣。6.梁晃千。2000。以天然交聯劑 genipin 交聯明膠的藥物制放微粒載體：體外與體內性質評估：11-15。國立中央大學化學工程研究所碩士論文。桃園，台灣。7.莊景光。2004。離子鍵結行奈米微粒製備與其對小腸上皮細胞滲透能力之探討：1-50。國立清華大學化學工程學系碩士論文。新竹，台北。8.魏育慧。2000。利用動物細胞進行抗癌藥物篩選。食品工業 32(11):27-35。9.蔡宏銘。2002。中藥對骨細胞活性的評估：7-27。中國醫藥學院中國醫學研究所碩士論文。台中，台灣。10.謝慰親。雷射光散射儀。德芮克國際股份有限公司。11.Aksungur, P., Sungur, A., Unal, S., ?skit, A. B., Squier, C. A. and ?enel, S. 2004. Chitosan delivery systems for the treatment of oral mucositis: in vitro and in vivo studies. *Journal of Controlled Release*. 98:269-279. 12.Amir, H. S. 1998. Buccal mucosa as a route for systemic drug delivery : A review. *J Pharm Pharmaceut Sci.* 1(1):15-30. 13.Aungest, B. J. and Rogers, N. J. 1988. Site dependence of absorption promoting action of Laureth-9, Na salicylate, Na2EDTA, and Aprotinin on rectal, nasal, and buccal insulin delivery. *Pgarm. Res.*5: 305-308. 14.Bogdan, I. F., Maya, T., Hans E. J., Gerrit B. 2006. Enhancement of bronchial octreotide absorption by chitosan and N-trimethyl chitosan shows linear in vitro/in vivo correlation. *Journal of Controlled Release* 110: 353 – 361. 15.Boonsongkirt, Y., Mitrevej, A. and Mueller, B. W. 2006. Chitosan drug binding by ionic interaction. *European Journal of Pharmaceutics and Biopharmaceutics*.62: 267-274. 16.Bumgardner, J. D., Yuan, Y., Chesnutt, B.M., Utturkar, G., Haggard, W.O., Yang, Y. and Ong, J.L. 2007. The effect of cross-linking of chitosan microspheres with genipin on protein release. *Carbohydrate Polymers* 68 : 561-567. 17.Desai, K. G. H. and Park, H. J. 2005. Preparation and characterization of drug-loaded chitosan-tripolyphosphate microspheres by spray drying. *Drug Development Research* 64:114-128. 18.Eirheim, H. U., Bundgaard, C. and Nielsen, H. M. 2004. Evaluation of different toxicity assays applied to proliferating cells and to stratified epithelium in relation to permeability enhancement with glycylcholate. *Toxicol. Vitro* 18: 649-657. 19.Falk, B., Garramone, S., Shivkumar, S. 2004. Diffusion coefficient of paracetamol in a chitosan hydrogel. *Materials Letters* 58:3261-3265. 20.Gandhi, R. and Robinson, J. 1992. Mechanisms of penetration enhancement for transbuccal delivery of salicylic acid, *Int. J. Pharm.*, 85:129-140. 21.Harris, D. and Robinson, J.R. 1992. Drug delivery via the mucous membranes of the oral cavity, *J. Pharm. Sci.* 81: 1-10. 22.Hirokazu, T., Richer, C., Hirokazu, O., and Kazumi, D. 2005. Acetaminophen particle design using chitosan and a spray-drying technique. *Chem. Pharm. Bull.* 53(1)37-41. 23.Hoogstraate, A. J., Senel, S., Cullander, C., Verhoef, J., Junginger, H. E., and Bodde, H. E.,1996. Effect of bile salts on transport porcine buccal epithelium in vitro, *J Control. Rel.*,40 : 211-221. 24.Kockisch, S., Rees, G. D., Tsibouklis, J. and Smart, J. D. 2005. Mucoadhesive, tricosan-loaded polymer microspheres for application to the oral cavity:preparation and controlled release characteristics. *European Journal of Pharmaceutics and Biopharmaceutics*. 59:207-216. 25.Lin, Y. H., Mi, F.L., Chen, C. T., Chang, W. C., Peng, S. H., Liang, H.F. and Sung, H. W. 2007. Preparation and characterization of nanoparticles shelled with chitosan for oral insulin delivery. *Biomacromolecules* 8, 146-152. 26.Maestrelli, F., Garcia-Fuentes, M., Mura, P. and Alonso. M. J. 2006. *European Journal of Pharmaceutics and Biopharmaceutics*. 63:79-86. 27.Maestrelli, F., Zerrouk, N., Chemtob, C., Mura, P. 2004. Influence of chitosan and its glutamate and hydrochloride salts on naproxen dissolution rate and permeation across Caco-2 cells. *International Journal of Pharmaceutics* 271: 257-267. 28.Manganaro, A.M. and Wertz, P.w. 1996. The effects of permeabilizers on the in vitro penetration of pro pranolol through porcine buccal epithelium, *Mil. Med.*, 161:669-675. 29.Mi, F. L., Sung, H. W., Shyu, S.S., Su, C.C., and Peng, C. H., 2003. Systhesis and characterization of biodegradable TPP/genipin co-crosslinked chitosan gel bads. *Polymer*.44: 6521-6550. 30.Nguyen, T. T. B., Hein, S., Ng, C. H., Stevens, W. F. 2007. Molecular stability of chitosan in acid solutions stored at various conditions. *Journal of Applied Polymer Science*, Vol.107, 2588-2593. 31.Olivier, P., Testard, P., Marzin, D. and Abbott, D. 1995. Effect of high polyol concentrations on the neutral red absorption assay and tetrazolium-MTT test of rat hepatocytes in primary culture. *Toxicol. Vitro* 9(2): 133-138. 32.Shojaei, A.H. and Li, X. 1997. Determination of transport route of acyclovir across buccal mucosa, *Proceed. Int. Symp. Contril. Rel. Bioact. Mater.*, 24:427-428. 33.Siegel, I.A. and Gordon, H.P. 1985. Durfactant-induced increase of permeability of rat oral mucosa to non-electrolytes in vivo, *Arch. Oral Boil.*, 30:43-47. 34.Takahashi, H., Chen, R. Okamoto, H., Danjo, K. 2005. Acetaminophen particle design using chitosan and a spray-drying. *Chem. Pharm.Bull.*53 (1) : 37-41. 35.Varum, K. M., Egelalndal, B. and Ellekjær, M. R. 1995. Characterization of partially N-acetylated chitosans by near infra-red spectroscopy. *Carbohydrate. Polymers*. 28:187-193. 36.Veronique. P., Anne des, R., Virginie, F., Marie G., Yves-J, S. 2006. Nanopaticles as potential oral drlicery systems of proteins and vaccines : A mechanistic approach. *Journal of controlled release*. 116: 1-27. 37.Xiaoyan. A., Jun, Y., Min, W., Haiyue, Z., Li, C., Kangde, Y., Fanglian, Y. 2008. Preparation of chitosan-gelatin scaffold containing terandrine-loaded nano-aggregates and its controlled release behavior. *International Journal of Pharmaceutics* 350: 257-264. 38.Zhou, H.Y., Chen, X. G. 2008. Characteristics and degradation of chitosan/cellulose acetate microspheres with different model drugs. 39.Zhou. H. Y., Chen. X.G., Meng. X. H. 2006. Release characteristics of three model drugs from chitosan/cellulose acetate multicrosphere. *Biochemical Engineering Journal* 31: 228-233.