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Composite microspheres induce the sustained release and the control of the initial release of water soluble drugs

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Although epidural analgesia may provide adequate pain relief and minimize systemic side effects, long-term, even permanent placement of epidural catheter for chronic or cancer-related pain management carries a potential risk of both superficial and deep infection. The development of antibiotics microspheres that could be dwelled in epidural drug-delivery devices is likely to achieve a significant advance allowing antibiotics given by the intradiscal route to control catheter-related infections. In the present study, the composite microspheres composed of double-walled microcapsules and PLGA were constructed for encapsulating water-soluble antibiotics, cefazolin. The results show that these microspheres could efficiently control the initial release of drug, which was only 3.0% at 2 h. Cefazolin encapsulated in the composite microspheres released gradually nearly in a constant rate in the first 16 days, and still maintained a relative fast rate in the next 14 days, indicating that composite microspheres could improve the incomplete release of entrapped drugs.

Among various approaches to manage severe pain, epidural analgesia shows its great superiority with adequate pain relief and minimal systemic side effects such as sedation, respiratory depression, hypotension, etc. However, the epidural route for analgesics administration carries a potential risk of both superficial and deep infection including neuraxial infection (Smitt et al. 1998). When epidural catheters were in the place for months even permanently in chronic or cancer-related pain patients, a high incidence of serious catheter-related infections was observed (Rathmell et al. 2006). The risk of infection in those receiving epidural therapy for more than 70 days approaches 15% (de Jong et al. 1994). Smitt et al. (1998) have performed a retrospective study of 91 patients received epidural analgesia for an average of 38 days (range, 1 day–1000 days). Technical complications and superficial infections occurred in as many as 43% of patients. Especially, a serious deep infection including epidural abscesses occurred in 13% of the patients.

The administration of prophylactic antibiotics is an appropriate treatment strategy for any type of catheter-related infections (Klessig et al. 2003). Intradiscal antibiotic administration is an attractive technique of both prophylaxis and therapy for deep epidural infection. This route may deliver high concentrations of antimicrobial agents to the site of infection. In Klessig's study, minimal recommended intradiscal antibiotic dosages for discitis could achieve more than 10-fold minimum inhibitory concentrations (MICs) determined against *Escherichia coli* B, *Staphylococcus aureus*, and *Staphylococcus epidermidis*. A prospective clinical study has also shown that none of 127 patients undergoing lumbar discography obtained discits after the administration of intradiscal cefazolin.

As antibiotics need to be used for months, sustained-release systems in the form of pellets, hydrogel, retard tablets, or microspheres could reduce the administration frequency. Considering the microenvironment of the apparatus where antibiotics are located and the intended release time, microspheres are among the most appropriate dosage forms. Encapsulation of drugs in microspheres from which they are released at a relatively slow rate over a prolonged time allows less frequent administrations, thereby increasing patient compliance and reducing the discomfort (Shirui Mao et al. 2007). Moreover, the complete release time can be regulated by altering the polymer composition and preparation conditions. However, conventional microspheres made from a single hydrophobic polymer as drug carrier such as poly(lactic-co-glycolic acid) (PLGA) have some inherent shortcomings such as high initial burst, low encapsulation efficiency for highly water-soluble drug and lack of sustained release (Giteau et al. 2008). To overcome the shortcomings of conventional microspheres, our group designed microspheres which composed of biodegradable polymer PLGA, alginate and chitosan (Zheng et al. 2004). This composite microspheres have been used to encapsulate protein, achieving high encapsulation efficiency and low initial burst (Zheng et al. 2004). In the present study, the composite microspheres were constructed with a modified approach to encapsulate antibiotics. These double-walled microcapsules are composed of hydrophilic materials chitosan and alginate which have an affinity to water-soluble drugs and thus have relative high encapsulation efficiency. Subsequently, these microcapsules are embedded in PLGA which can further prolong the release time of antibiotics from microspheres.

The mean diameter of the composite microspheres was around 60 μm and that of microcapsules dispersed in the microspheres was about 1 μm. As seen from the SEM images (Fig. 1), Chitosan coated alginate microcapsules distribute in the surface of the composite microspheres uniformly, while the conventional drug-loaded PLGA microspheres were spherical in shape with a smooth surface. The *in vitro* release profiles of these two kinds of microspheres are also quite different (Fig. 2). Firstly, for composite microspheres, the initial release was 3.0% at 2 h, 17.0% at 5 h, 34.5% at 24 h, obviously slower than that of conventional microspheres, which is 41.6% at 2 h, 43.5% at 5 h, 46.1% at 24 h (Fig. 2b). The initial burst release is caused by the rapid release of the near surface drugs and

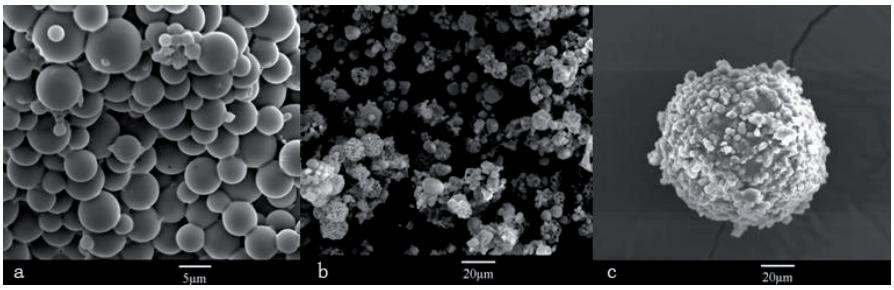

Fig. 1:
SEM images of conventional PLGA microspheres (a) alginate-chitosan microcapsules (b) and alginate-chitosan-PLGA composite microspheres (c)

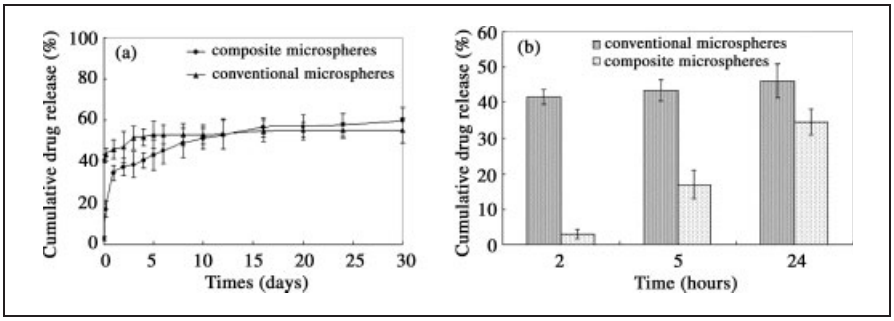

Fig. 2:
(a) Release of cefazolin from alginate-chitosan-PLGA composite microspheres and conventional PLGA microspheres. Initial burst release of Conventional microspheres and Composite microspheres. (b) Initial burst release of conventional microspheres and composite microspheres. Data are means ± SD. (n = 3)

such effect is especially prominent with encapsulation of hydrophilic small-molecule drugs since a great amount of drugs may diffuse towards the outer water phase during solvent evaporation procedures and ultimately aggregate in the surface of microspheres. Due to the severe initial burst release, adverse effect may be caused and the sustained-release time may also be shortened because of the limited drug content left after the initial burst release. In the composite microspheres, drug was first encapsulated into the microspheres before the encapsulation to the PLGA microspheres and content of near surface drugs was reduced to reduce the initial burst release. Secondly, drug released fast from the conventional microspheres in the first three days, followed by a slow release which lasted for 27 days and the cumulative release during this period only accounted for 3.7% percent of the total drug encapsulated (Fig. 2a). As for the composite microspheres, drug released gradually nearly in a constant rate in the first 16 days, and then slowed down in the next 14 days but also faster than that of the conventional ones. Thirdly, the cumulative release of 30 days of composite microspheres is 4.8% higher than the conventional ones. Crotts et al. (1997) proved that the incomplete release of entrapped drugs is related with the non-specific adsorption of the degrading PLGA surface, covalent aggregation and denaturation of drugs. It is inferred that the alginate-chitosan microcapsules give antibiotics a proper protection from the acidic microenvironment due to the degradation of PLGA (Zheng et al. 2004) while conventional PLGA microspheres lack such a protection, leading to a more complete release of encapsulated drugs.

In summary, composite microspheres are superior to the conventional ones in that the initial burst release is reduced, the release per day is more constant and the cumulative drug release is higher, thus infection may be more effectively prevented.

Experimental

1. Materials

Cefazolin was purchased from Qilu Anti Pharmaceuticals (China). Chitosan (85% deacetylation, M_W 8×10^4) was purchased from Yuhuan Oceanic Biochemistry (China). Alginate, sodiumdodecylsulfate (SDS) were obtained from Shanghai Chemical Reagent Company of Chinese Medicin (China). PLGA (lactic to glycolic acid molar ratios at 85 : 15, M_W 5.0×10^4) were purchased from Shandong Medical Instrumental Institute (China). Sorbitan Monooleate (Span 80), Polyoxyethylene sorbitan monooleate (Tween 80), iso-octane, iso-propyl alcohol, calcium chloride, and all other reagents were of analytical grade supplied by Huadong Medical (China).

2. Procedures

2.1. Preparation of chitosan-coated alginate microcapsules

Chitosan-coated alginate microcapsules were prepared just as described in the previous study with some modifications (Zheng et al. 2004). Briefly, cefazolin was dissolved in the alginate solution and Span 80 was dispersed in iso-octane, and then iso-octane was poured into the alginate aqueous solution. The mixture was emulsified and Tween 80 was added and the mixture was further stirred. Then calcium chloride solution was added dropwise. This cross-linking process lasted for 3 min. Then isopropyl alcohol was added to harden the microcapsules and the solidified microcapsules were collected by centrifugation. The alginate microcapsules prepared were dispersed into chitosan solution. Then the mixture was shaken gently for 30 min to form the alginate-chitosan complex membrane. The microcapsules were centrifuged and collected, then washed in water, and finally lyophilized.

2.2. Preparation of alginate-chitosan-PLGA microspheres

About 0.5 g PLGA was dissolved in 3 ml acetonitrile. The alginate-chitosan microcapsules were suspended in the PLGA solution and sonicated. The suspension was added into the peanut oil containing Span 80 dropwise at 600 rpm, and the emulsion was further stirred for at 800 rpm. After evaporation of the acetonitrile under reduced pressure, the solidified microspheres were centrifuged and then washed three times with petroleum ether. The resulting composite microspheres were collected by lyophilization. The morphologies of the composite microspheres were studied with a scanning electron microscopy. *In vitro* release of the composite microspheres were determined in the apparatus described by Qingguo Xu and Czernuszka (2008). The amount of cefazolin released at a certain set time was determined by UV/Vis spectroscopy at 272 nm.

2.3. Preparation of conventional PLGA microspheres by w/o/o method

Briefly, cefazolin was dissolved in 2 ml methylene chloride containing PLGA. The emulsification was carried out by sonication for 60 s to produce a s/o emulsion. The primary emulsion was then injected into 200 ml sodium dodecylsulfate (SDS) water solution with stirring and further stirred at a high speed to form the s/o/w emulsion. After evaporation of the methylene chloride under reduced pressure, microspheres were centrifuged and then washed three times with distilled water. The resulting drug loaded microspheres were lyophilized.

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Preparative isolation of oligomeric procyanidins from Hawthorn (*Crataegus* spp.)

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The oligomeric procyanidins (OPC) from Hawthorn leaves and flowers (Crataegi folium cum flore) are considered to be in part responsible for the cardiotonic clinical activity of the herbal material. Effective methods for rapid isolation of these heterogenous oligomeric clusters with defined molecular weight as reference compounds are not published until now. Therefore the water soluble fraction of an acetone/water (7 + 3) extract of Hawthorn leaves and flowers was fractionated by a combination of MPLC on RP-18 material and preparative HPLC using a diol stationary phase. This procedure resulted in the effective isolation of procyanidins with a distinct degree of polymerization (DP) from dimers DP2 up to tridecamers DP13. Exact mass measurements with negative ESI-TOF/MS were employed to confirm the respective structures of the isolated procyanidins.

Hawthorn leaves and flowers consist of the dried flower bearing branches of *Crataegus monogyna* Jacq. Emend. Lindm., *C. laevigata* (Poiret) and, more rarely, *C. pentagyna* Waldst. et Kit. Ex Willd., *C. nigra* Waldst. et Kit. and *C. azarolus* L. Pharmaceutical preparations of Hawthorn are considered as a rational based phytomedicine for treating declining cardiac performance corresponding to NYHA I and II. Flavonoids, such as flavonol and flavon derivatives, and procyanidins are considered to be the main active compounds, whereas oligomeric procyanidins (OPCs) seem to have the most marked effect (ESCOP 2003). For a recent summary of the phytochemistry and problems concerning the analysis of procyanidins from Hawthorn see Petereit and Nahrstedt (2005), Veit and Wittig (2005) and references cited therein. Because the OPCs are a highly complex heterologues series a big need is seen for isolation of the individual oligomers for quality control but also for more detailed pharmacological and pharmacokinetic studies. Therefore an acetone/water (7 + 3) extract of Crataegi folium cum flore was prepared. After removal of the organic solvent the aqueous phase was extracted with EtOAc leading to proanthocyanidins with a low degree of polymerization (DP2 to about DP4) enriched in this EtOAc phase while the longer chain oligomers are to be found in the aqueous phase beside minor amounts of DP2 to DP4 oligomers (Bicker et al., in press). The aqueous phase was further fractionated by a subsequent combination of MPLC on RP-18 material and pre-