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Research Paper

Modified Paclitaxel-loaded Nanoparticles for Inhibition of Hyperplasia in a Rabbit Arterial Balloon Injury Model

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Purpose. This study tested the possibility of localized intravascular infusion of positive charged paclitaxel-loaded nanoparticles (NPs) to better prevent neointimal formation in a rabbit carotid artery injury model.
Materials and Methods. NPs were prepared by oil–water emulsion/solvent evaporation technique using biodegradable poly (lactide-co-glycolide) (PLGA). A cationic surfactant, didodecyldimethylammonium bromide (DMAB), was absorbed on the NP surface by electrostatic attraction between positive and negative charges. NPs were characterized in such aspects as size, surface morphology, surface charges as well as *in vitro* drug release profile. Balloon injured rabbit carotid arteries were treated with single infusion of paclitaxel-loaded NP suspension and observed for 28 days. The inhibitory effects of NPs on neointima formation were evaluated as end-point.
Results. NPs showed spherical shape with a diameter ranging from 200 to 500 nm. Negatively charged PLGA NPs shifted to positive after the DMAB modification. The *in vitro* drug release profile showed a biphasic release pattern. Morphometric analyses on the retrieved artery samples revealed that the inhibitory effect of intima proliferation was dose-dependent. At a concentration of 30 mg ml^{-1}, NP infusion completely inhibited intima proliferation in a rabbit vascular injury model.
Conclusions. Paclitaxel-loaded NPs with DMAB modification were proven an effective means of inhibiting proliferative response to vascular injury in a rabbit model.

KEY WORDS: DMAB; nanoparticle; paclitaxel; restenosis; surface modification.

INTRODUCTION

Restenosis after percutaneous coronary intervention continues to be a serious problem in clinical cardiology (1). Restenosis is a complex process which is thought to be triggered by blood vessel wall injury following an intervention to relieve an arterial obstruction, and is characterized by intimal hyperplasia and vessel remodeling (1–4). Intimal growth results from vascular smooth muscle cell (VSMC) migration and proliferation into the media (5) followed by the formation of extracellular matrix (6). Currently, different routes of drug administration in restenosis therapy are under investigation. The proliferation of VSMC, the cause of restenosis development, could be inhibited by the application of radioactive (7) and drug eluting stents (DES) (8–10). However, their efficacy and safety have not been confirmed in all clinical settings, especially with regard to treating in-stent restenosis. Another important limitation of DES is the fact that the drug concentration is highest at the stent struts, where healing is most important. On the other hand, incomplete suppression of neointimal hyperplasia at the stent margins or between the struts may limit the efficacy of DES (11). It should be also noted that 30–40% of critical lesions cannot be stented, largely because they occur at branch sites or in small arteries (12). Hence, other methods for prevention of restenosis beyond drug-eluting-stents strategy are necessary. Another promising method to reduce VSMC growth and neointimal formation is local administration of a drug solution using drug delivery catheters (13). Non-stent-based local delivery of antiproliferative drugs may offer additional flexibility and efficacy in the entire range of applications. It may also deliver drugs to vessel areas not directly covered by the stent, which could be of special interest for small and tortuous vessels (14). However, the delivery efficiency and intramural retention time of infused drug solution remains rather low (15,16). Therefore, researchers have developed colloidal drug carrier systems from biodegradable polymers that may provide a local release and sustained retention of the drug in the arterial wall (2,17–20). Previous studies by Levy group (19–21)

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ABBREVIATIONS: CCA, common carotid artery; DCM, dichloromethane; DES, drug-eluting stent; DMAB, didodecyldimethylammonium bromide; EE, entrapment efficiency; HE, hematoxylin & eosin; HPLC, high performance liquid chromatography; NP, nanoparticle; PLGA, Poly(DL-lactide-co-glycolide); PVA, polyvinyl alcohol; SEM, scanning electron microscopy; TEM, transmission electronic microscopy; VSMC, vascular smooth muscle cell.

demonstrated an efficient intra-arterial localization of nanoparticles in the arterial wall, and effectiveness for the inhibition of experimental restenosis. Nanoparticles possess several advantages as a carrier system for intraarterial delivery of therapeutic agents. These advantages include their subcellular size, good suspensibility, and uniform dispersity for a catheter-based delivery, and an easy penetration into the arterial wall without causing trauma, in contrast to larger material implant such as microparticles (22). However, a low efficiency of nanoparticle retention in the arterial wall when delivered *in vivo* through an infusion catheter was noted (22). These results indicated the need for nanoparticle modifications, and new infusion techniques to enable this novel pharmaceutical dosage form to practically benefit clinical applications. Thus, previous studies by Song *et al* demonstrated modification of U-86983-loaded nanoparticles with didodecyldimethylammonium bromide (DMAB), fibrinogen, and heparin/DMAB greatly enhanced arterial retention in animal angioplasty models. Nanoparticle modified with DMAB showed at least sevenfold higher arterial wall uptake than that of the non-modified nanoparticles (23,24).

In the context of restenosis, studies conducted by various investigators have shown that paclitaxel affects development of neointimal hyperplasia in different animal models of restenosis (25,26). For example, the Taxus™ paclitaxel-eluting stent (DES) has been approved for commercial sale in the United States and other countries. However, the drawbacks of DES described above limited its application. Thus, in the present study, we investigate the hypotheses that a novel paclitaxel-loaded nanopaticle through surface modification with DMAB for the inhibition of neointimal formation after local delivery into the vessel wall of the common carotid artery (CCA) of New Zealand white rabbits.

MATERIALS AND METHODS

Materials

Briefly, paclitaxel of 99% purity was purchased from Beijing Union Pharmaceutical Factory (Beijing, China). Poly(lactide-co-glycolide) (PLGA; L/G = 50/50) with an inherent viscosity of 1.13 dl/g was purchased from Birmingham Polymers (Birmingham, AL). Didodecyldimethylammonium bromide (DMAB) was obtained from Organics (Morris, NJ, USA). Polyvinyl alcohol (PVA) (MW 30 000-70 000) was obtained from Sigma. Chemical Co (St Louis, MO). Ultra-high pure water produced by Boon Enviromental Tech. Industry Co., Ltd (Tianjin, China) was utilised for HPLC analysis. Acetonitrile used as mobile phase in high performance liquid chromatography (HPLC) was purchased from EM Science (ChromAR, HPLC grade, Mallinckrodt Baker, USA). Sodium salicylate was supplied by Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China). All other chemicals were AR grade and were purchased from Tianjin No.1 Chemical Reagent Factory.

Preparation of Paclitaxel-loaded Nanoparticles

PLGA nanoparticles loaded with paclitaxel were prepared by an oil-in-water (O/W) emulsion/solvent evaporation protocol with some modifications as described previously (19). Briefly, PLGA was dissolved in an organic mixture of dichloromethane (DCM) and acetone (9:1,v/v) at a concentration of 2% w/v. Drug powder was co-dissolved in the PLGA solution at a ratio of 30:70 (w/w) of paclitaxel to PLGA. The organic phase was poured into an aqueous solution containing PVA and this system was sonicated over an ice bath using a microtip probe sonicator (model VC-501; Sonics & Materials, Inc., Newtown, CT) at 45 W power output for 2 min to form oil-in-water emulsion. The organic solvents were allowed to evaporate for 2 h under reduced pressure by stirring over a magnetic stir plate. Nanoparticles were collected by ultra-centrifugation and washed twice with distilled water to remove free drug and PVA. The final product was dried by lyophilization for 48 h. Drug-free nanoparticles were prepared by the same method.

Nanoparticle Modification

Typically, DMAB were dissolved in distilled water at a concentration of 0.5 mg ml^{-1}. Pre-weighed nanoparticles were suspended in this solution at a concentration of 9.5 mg ml^{-1} by sonication at 45 W power output for 60 s over an ice bath, and then were collected by ultra-centrifugation. The modified nanoparticles were then lyophilized to dryness and stored desiccated under vacuum until used. For animal experiments, the product was sterilized under a dose of 15 kGy of gamma irradiation.

Characterization of Nanoparticles

Surface Morphology

Surface morphology of paclitaxel-loaded PLGA nanoparticles was visualized under a scanning electron microscopy (SEM, Jeol, JSM-5600 LV). Before imaging, freeze-dried samples were coated by platinum for 40 s. The nanoparticles were further observed by transmission electron microscopy (TEM). A drop of nanoparticle suspension was placed on copper grids (Formvar filmed). The excess fluid was removed with a piece of filter paper. The dried sample was then examined.

Size Analysis and Zeta Potential

Mean particle size of the formulations were determined by a light scattering spectrometer (BI-90Plus, Brookhaven Instruments Co.) Each sample was appropriately diluted with 0.45 μm-filtered water and the reading was carried out at a 90° angle in respect to the incident beam. Surface charge of the nanoparticles was measured on a zeta potential analyzer (model: BI-90plus, Brookhaven Instruments Co.).

Drug Loading and Entrapment Efficiency

Paclitaxel content in the PLGA nanoparticles was assayed by HPLC (Agilent LC 1100). Briefly, 5 mg particles were dissolved in 1 ml DCM under vigorous vortexing. This solution was transferred to 5 ml of a mixture of 50/50 (v/v) acetonitrile and water. Nitrogen was introduced to evaporate DCM and a clear solution was obtained for HPLC analysis.

The mobile phase of HPLC was composed of acetonitrile and water of 50/50 (v/v). The measurement was performed triplicate. The entrapment efficiency (EE) was expressed as percent of paclitaxel encapsulated in the nanoparticles.

In Vitro Drug Release

In vitro release of paclitaxel from the nanosphere was performed in a sodium salicylate solution to ensure sink condition. Stability of nanoparticles in the hydrotropic medium was investigated. Freeze-dried nanoparticles were suspended in distilled water (0.5 mg ml^{-1}) under gentle shaking, followed by sonication for 10 min to give optically clear solution. Sodium salicylate solutions of different concentration were obtained by dilution of 3.0 M stock solution with distilled water. The concentration of nanoparticles in sodium salicylate solution was fixed to 0.1 mg ml^{-1}. The change in particle size was monitored by a light scattering spectrometer (BI-90Plus, Brookhaven Instruments Co.).

In vitro drug release of paclitaxel from the nanoparticles was carried out in a double diffusion chamber using 1.0 M sodium salicylate solution as release medium. A Millipore® hydrophilic low-protein-binding polyvinylidene fluoride membrane (VVLP) (Millipore Co., Bedford, MA) with 0.1 μm pore size was placed between the two chambers. The donor chamber was filled with 4.5 ml nanoparticle-sodium salicylate suspension and the receiver chamber filled with 4.5 ml plain 1.0 M sodium salicylate solution. The chamber was placed in a 37°C shaker water bath maintained at 130 rpm rotation. The release medium in the receiver chamber was replaced periodically with fresh medium. Paclitaxel in the collected samples were extracted with 1 ml DCM to determine the amount of drug released. The analysis procedure was same as described in the determination of the EE.

Animal Experiments

New Zealand White rabbits (2.0–2.5 kg) were purchased from the Tianjin Medical Laboratory Animals Center (Tianjin, China). The Administrative Committee on Animal Research in the Institute of Biomedical Engineering, Chinese Academy of Medical Science approved all the protocols for animal experiments. Also, all the animal experiments were performed in compliance with the Guiding Principles for the Care and Use of Laboratory Animals, Peking Union Medical College, China. Rabbits were fasted overnight before all surgical procedures. The animals were sedated with intravenous infusion of sodium pentobarbital (30 mg kg^{-1}). In the supine position, a right paramedian neck incision was made, exposing the common carotid artery (CCA). Then, the distal part of the CCA was ligated. A microvascular clamp temporarily clipped onto the proximal part of the CCA, and a small arteriotomy was made 2 mm proximal to the distal ligature. Balloon injury was performed on the CCA with a 3-mm angioplasty balloon catheter (Cordis, 2×1-min inflation at 8 atm). This process was repeated two additional times to injure the intimal lining of the artery. The lumen of the balloon-injured CCA was then rinsed with normal saline. Nanoparticle suspension was injected into the balloon-injured CCA through a no. 20 gauge angiocath to keep the vessel inflated for about 30 s. Then, suck the nanoparticle suspension back into the syringe to let vessel deflated. This was repeated three times. The treated CCA was rinsed with saline for five times to remove dissociative nanoparticles before the temporary clamp was released and blood flow in the carotid segment was reestablished. The wound was closed with 4–0 nylon sutures, and the animals were returned to their cages and allowed to recover for 28 days until vessel harvest. Animals were randomly assigned to saline, drug-free nanoparticle suspension, doses of 50 mg ml^{-1}, 30 mg ml^{-1} l, 15 mg ml^{-1}, 5.5 mg ml^{-1}, 1 mg ml^{-1}, 0.3 mg ml^{-1}, 0.1 mg ml^{-1}, and 0.05 mg ml^{-1} nanoparticle suspension group, with five animals in each group.

Morphometric Analysis

Animals were given access to food and water ad libitum. After 28 days, all the animals were sacrificed by an overdose of sodium pentobarbital. The previous incision was opened, and the CCA was exposed. The carotid arteries were subsequently perfusion-fixed with 10% buffered formalin. Carotid artery sections (4 μm) were stained with hematoxylin & eosin (HE) staining and Weigert elastic fiber staining. Morphometric analysis was performed using three individual sections from the middle of each injured arterial segment by an investigator blind to the experimental procedure. Cross-sectional areas (A_{intima} and A_{media}), area ratios (A_{intima}/A_{media}), and percent area stenosis (% stenosis) were analyzed and calculated using the Scion Image System (version 4.03).

Statistical Analysis

The results are expressed as mean ± SE. Statistical comparisons between two groups were made using the twotailed, unpaired Student's *t* test, whereas comparisons among paclitaxel-treated groups were analyzed using a 1-way ANOVA followed by Duncan's post hoc test. A probability value of <0.05 was considered significant.

RESULTS AND DISCUSSION

Characterization of Nanoparticles

Nanoparticles were characterized in terms of mean size and size distribution, morphology, and surface charge. The mean size and size distribution of each batch of the nanoparticle suspension was analyzed using dynamic light scattering method. The size distribution profile showed a diameter range between 200–500 nm. The data showed that the particle size of the paclitaxel-loaded formulations increased slightly after surface modification with DMAB. The paclitaxel-loaded nanoparticles observed by SEM were spherical in shape and their size was around 300 nm. TEM images further confirmed the SEM observation. Recent studies have indicated that particle size plays an important role in the penetration and cellular uptake of particles into the vessel wall (27,28). Furthermore, particle size may even determine the biological response of the tissue to the foreign particle

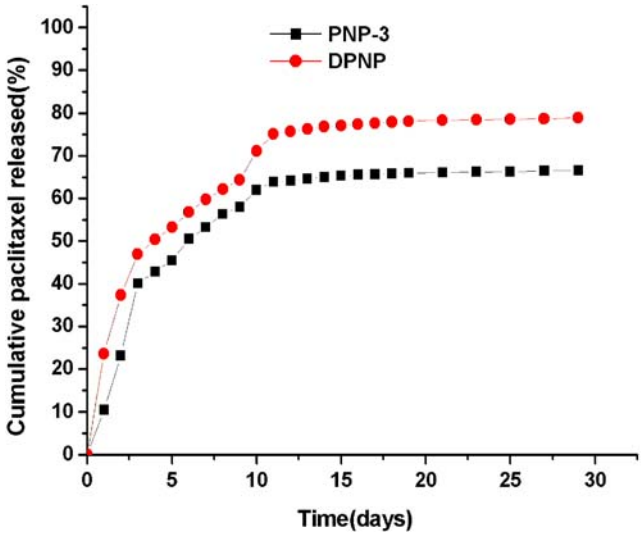

Fig. 1. The *in vitro* release profile of paclitaxel-loaded nanoparticles.

material (22,29). While inflammatory reactions with subsequent fibrosis of vascular tissue have been found when applying particles with a size of 5–10 μm (30,31), nanoparticles usually cause little or no focal inflammation. Guzman *et al.* (19) observed that fluorescent-labelled particles of 165 nm that were first deposited in the luminal, medial, and adventitial layers of the artery were found afterward in only the adventitia. They concluded that the adventitial layer acts as a reservoir for particles and eluted drugs, which then subsequently diffuse in the direction of the media. A discontinuous particle distribution has been also found by Rome *et al.* (28). They showed that different types of particles, ranging between 90–500 nm in size, tended to accumulate in the outer wall layers but not in the media. These authors suggested that the particles reach the adventitia via the vasa vasorum, but not directly by penetrating the wall. Thus, in the present study, we prepared the particles in the size range between 200–500 nm as local delivery carrier for the prevention of restenosis.

Zeta potential analysis confirmed that DMAB modification changed the nanoparticles from a negative charge of −3.57 mV to a significantly positive charge of +20.10 mV. Song *et al.* has demonstrated that modification of nanoparticles with DMAB significantly enhanced arterial wall uptake and retention compared to non-modified nanoparticles (23,24). The cationic nature of surface modified nanoparticles probably had increased ionic interactions of nanoparticles with negatively charged glycosaminglycan enriched arterial wall, thus facilitating their arterial uptake and retention (24). In addition, since DMAB is a mild surfactant, it could transiently change the permeability of the arterial vasculature and facilitate nanoparticle internalization into the arterial wall (32). Paclitaxel was found to interfere with VSMC migration and proliferation at nanomolar levels *in vitro* (26). *In vivo*, paclitaxel inhibited neointimal formation in a rat carotid artery after endothelial denudation injury. Moreover, this effect occurred at plasma paclitaxel levels about 100 to 1000 times lower than the concentrations needed to treat neoplasma (26). Thus, paclitaxel was selected as a better pharmacologic component to be formulated into nanoparticles. Together with improved efficiency of nanoparticle retention, the paclitaxel-loaded and DMAB modified nanoparticle could be a very promising approach for local therapy of restenosis.

The average recovery of the extraction and HPLC procedures is 97.85%. The data showed that the drug loading level of paclitaxel encapsulated in the PLGA nanoparticles could be as high as 25%. The drug content of the modified nanoparticles decreased slightly to 23.68%. The results revealed that the drug entrapment efficiency (EE%) of the nanoparticles formulation was more than 82%.

In Vitro Drug Release

It is difficult to conduct release experiments from polymer nanoparticles for poorly soluble drugs due to extremely low water solubility of the drugs. Therefore, maintaining the sink condition in the release experiments has been an important issue for *in vitro* release studies of poorly soluble drugs such as paclitaxel. Several methods using organic solvents or surfactants were developed for drug release (33,34), but in most cases their potential effects on release kinetics and stability were often neglected or not studied enough. Thus, there is a need to develop a better system that allows a sink condition using a small volume of aqueous media.

In this study, hydrotropic release media were explored for paclitaxel release from polymer nanoparticles. Hydrotropic agents that can solubilize paclitaxel were used to maintain the sink condition of the aqueous release media. Effect of a hydrotropic agent, sodium salicylate on the nanoparticle stability was examined by light scattering. The results revealed that sodium salicylate did not significantly affect physical stability of the nanoparticles up to a concentration of 1.25 M. Above this concentration, sodium salicylate caused dramatic increase in particle size, indicating an aggregation of nanoparticles. Thus, *in vitro* release of paclitaxel from nanoparticles were performed in an aqueous medium con-

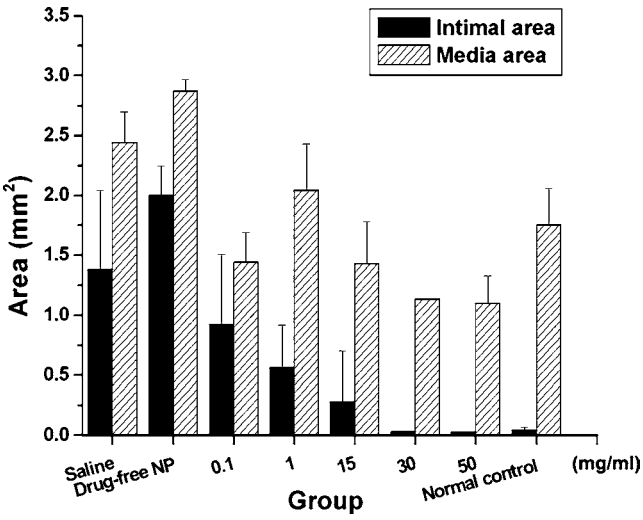

Fig. 2. Results of intimal and media cross-section area in different treatment groups ($n = 5$).

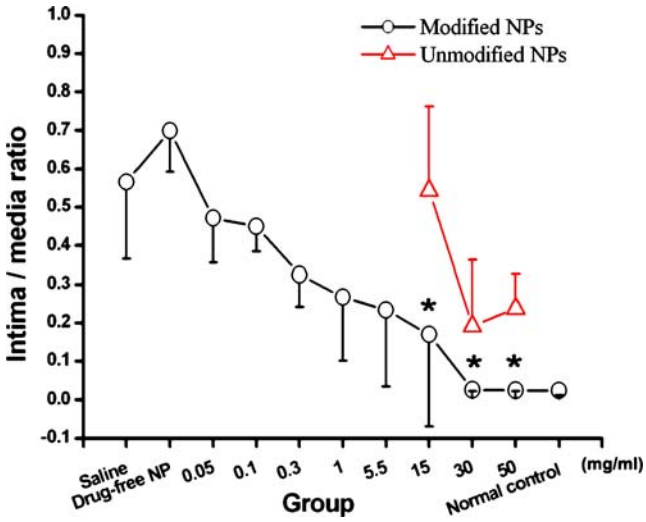

Fig. 3. Changes of the ratio of the intima/media area in different treatment groups ($n=5$). Compared with unmodified NPs, three doses (15, 30, 50 mg ml^{-1}) of modified NPs were observed to have a significant inhibition of hyperplasia (*, $P<0.01$).

taining 1.0 M sodium salicylate, where paclitaxel solubility was 34.28 μg/ml, that was more than 34 times of its original solubility in water (around 1.0 μg/ml at 25°C).

In general, drug release profile from biodegradable polymer matrices has a tri-phasic pattern (35–37): an initial burst, a second stage that is derived from diffusional release before the onset of polymer erosion, and a sudden burst resulting from swelling and disintegration of the polymeric matrix. The initial burst may have resulted from rapid dissolution of the drugs deposited on the surface. As shown in Fig. 1, both of the non-modified and modified nanoparticle formulations had similar release profiles. However, here a two-phasic release pattern was observed instead of tri-phasic pattern: an initial burst phase for the first 3 days followed by a sustained release lasted for 30 days when about 70% of drug was released out. Yasukawa *et al*. (38) revealed that the final burst might have occurred suddenly when swelling and disintegration of the polymeric matrix proceeded. Yasukawa *et al*. also found that the weight loss of PLGA followed a two-phase pattern: a lag time with no weight loss and an erosion phase with remarkable weight loss, that was accompanied by a rapid increase in drug release rate, i.e., the third burst release phase. In the present study, the *in vitro* drug release was observed for no more than 30 days, which would not reach this point, resulting in no final burst release observed. The results also revealed that the modified nanoparticles showed a faster drug release rate than the non-modified ones. The modified nanoparticles were washed by distilled water one more time than the non-modified nanoparticles in the preparation process resulting in lower amount of residual PVA. Surface-associated PVA could form a barrier to drug release from the nanoparticles (39).

In Vivo Studies

The rabbits experienced no significant changes in body weight after 28 days administration ($P>0.05$). Figure 2 shows the intimal and media cross-section area in different treatment groups. The results indicated that the degree of inhibition of neointimal proliferation was associated with nanoparticle concentration. Increasing the concentration of NP in the infusate resulted in greater inhibitory effect of neointimal proliferation. This result may be explained by our previous studies (23) that increasing the concentration of NP from 5 to 30 mg ml^{-1} significantly increased arterial NP uptake level. Compared with normal control group, 30 and 50 mg ml^{-1} nanoparticle concentration suspension group significantly inhibit neointimal proliferation ($P<0.01$). However, the therapeutic effect was not improved by administration of 50 mg ml^{-1} nanoparticle concentration suspension compared with 30 mg ml^{-1} nanoparticle concentration suspension ($P>0.05$). Our previous studies

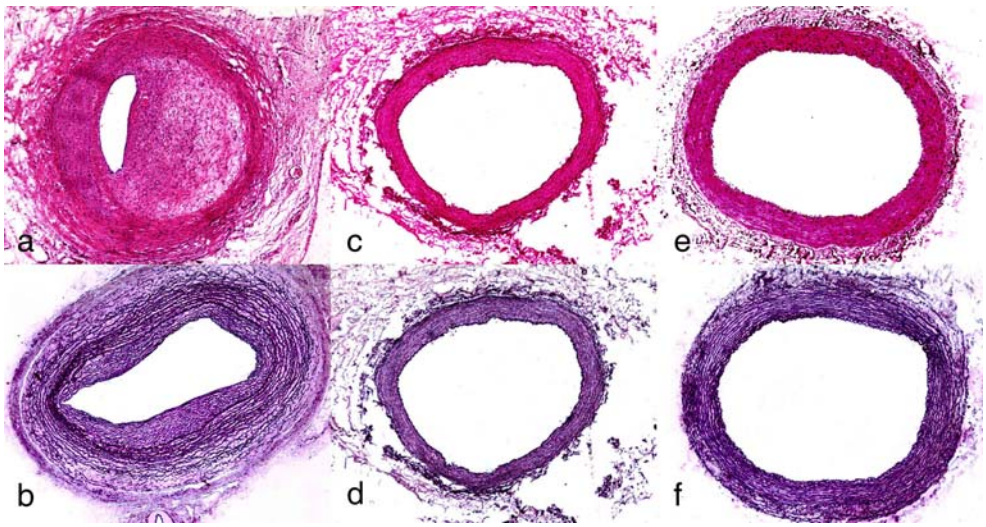

Fig. 4. Representative cross sections of rat carotid arteries taken 28 days after balloon (magnification, ×100). The *upper figure* indicates the results of HE staining, and the *lower one* is the results of elastic fiber staining. (**a**) and (**b**), Saline group; (**c**) and (**d**), 30 mg/ml nanoparticle concentration suspension group; (**e**) and (**f**), Normal control.

revealed that, from 30 mg ml^{-1} and higher, although a trend for more drug uptake with higher amounts infused was present, there was a plateau effect with very little additional benefit seen with further increases in the concentration of NP in the infusion (23). A saturation effect may also have occurred; however, this cannot be precisely established for the present studies. Thus, 30 mg ml^{-1} would appear to be an ideal NP concentration for local delivery into the vessel. Compared with the normal control group, only the saline and drug-free nanoparticle groups were observed significant media proliferation ($P<0.05$); although there was decrease in media area in the 30 and 50 mg ml^{-1} groups, the results show no significant statistical difference ($P>0.05$); the data show no significant difference in other groups ($P>0.05$). Figure 3 shows changes of the ratio of the intima/media area in different treatment groups. The results indicated that the inhibitory effects of intimal proliferation correlate positively with the concentration of the modified paclitaxel-loaded nanoparticle suspension. Compared with the drug-free nanoparticle group, the 1, 5.5 and 15 mg ml^{-1} groups were observed significant inhibition of hyperplasia ($P<0.05$); the 30 and 50 mg ml^{-1} groups were observed highly significant inhibition of hyperplasia ($P<0.01$). Compared with unmodified NPs, three doses (15, 30, 50 mg ml^{-1}) of modified NPs were observed significant inhibition of hyperplasia ($P<0.01$). As shown by Fig. 4, while saline group was observed significant intimal proliferation, the 30 mg ml^{-1} group significantly inhibits intimal proliferation of injured carotid arteries in rabbits.

It is clear from several studies that nanoparticles can be easily taken up by a variety of cells (40) and can penetrate into the connective tissue matrix (41) owing to their small size. It is known that balloon dilation during angioplasty produces endothelial denudation. Therefore, nanoparticles, when locally delivered into the intravascular lumen after balloon angioplasty, would likely penetrate the injured and denuded endothelial layer and be taken up by the subendothelial components. Furthermore, local delivery of drug-loaded NP, unlike drug-eluting stent, is not dependent on injury type, stent design or positioning (12).

When we did the local nanoparticle infusion experiments, the treated CCA was rinsed with saline for five times to remove un-adsorbed nanoparticles before reestablish blood flow in the carotid segment. Besides, the modified nanoparticles were collected by ultra-centrifugation so that free DMAB was separated and removed from the nanoparticles. Thus, there should be very few nanoparticles in systemic circulation. You *et al.* (42) reported that DMAB has lower cytotoxicity compared with other cationic surfactants. However, the toxicity will be a big issue and should be checked in animal before the system will be tested in human. Further investigations are required to test the effect of some positively charged polymers such as chitosan to see if we can eliminate low molecular cationic surfactants.

At the present time, no satisfactory catheter administered sustained action dosage form for restenosis is available. The controlled release nanoparticle holds great promise for this purpose. In the present study, nanoparticle modifications can greatly enhance arterial retention, thus helping to overcome the major challenge of nanoparticle administration within an artery in which there is the potential for arterial blood flow to wash away a significant fraction of the infused nanoparticles. From the standpoint of a controlled local delivery to a target vascular lesion, paclitaxel offers a portfolio that makes it perhaps one of the most appealing agents for this application. The key pathways that contribute to the formation of neointimal hyperplasia after arterial stenting have been broadly categorized into thrombosis, inflammation, proliferation of the smooth muscle cells (SMC) in the intima and media of the artery, migration of the medial SMC into the intima, and the secretion and organization of the extracellular matrix (ECM). Given the biochemical, cellular, and molecular complexities involved in the cascade of restenosis, localized delivery of a therapeutic entity that could target one or more of the events in the cascade is probably the most attractive, albeit challenging, approach to address the problem of restenosis (24,25). Paclitaxel was selected as the pharmacologic component for localized drug delivery system because of its ability to target the key events in the cascade of restenosis, which make its systemic delivery unfavorable but are very desirable for local delivery (24). The modified paclitaxel-loaded nanoparticles as local delivery system provide an effective means of inhibiting proliferative response to vascular injury in the rabbits. However, the efficiency was observed only 28 days, and the long-term effects of this formulation were unknown. Further studies are necessary to determine whether this formulation represents a longer delay or prevention of restenosis.

CONCLUSIONS

DMAB-modified and paclitaxel-loaded nanoparticles were prepared as unique drug carrier for intravascular drug delivery. The nanoparticles showed significantly greater effect on the inhibition of proliferative response to vascular injury in a rabbit model. The inhibitory effect of intima proliferation was dose-dependent. At 30 mg ml^{-1} concentration, the nanoparticle suspension completely inhibited intimal proliferation when delivered into the injured vessel. Local delivery of drug-loaded nanoparticles could be a promising alternative means for the prevention of the restenosis.

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