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Formulation and cytotoxicity of doxorubicin nanoparticles carried by dry powder aerosol particles

Shirzad Azarmi [a,b,c], Xia Tao [a,c], Hua Chen [a], Zhaolin Wang [d], Warren H. Finlay [d], Raimar Löbenberg [a,*], Wilson H. Roa [c]

[a] *Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alta., Canada T6G 2N8*
[b] *Research Center for Pharmaceutical Nanotechnology, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran*
[c] *Department of Oncology, Cross Cancer Institute, University of Alberta, 11560 University Avenue, Edmonton, Alta., Canada T6G 1Z2*
[d] *Department of Mechanical Engineering, University of Alberta, Edmonton, Alta., Canada T6G 2G8*

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Abstract

Regional drug delivery via dry powder inhalers offers many advantages in the management of pharmaceutical compounds for the prevention and treatment of respiratory diseases. In the present study, doxorubicin (DOX)-loaded nanoparticles were incorporated as colloidal drug delivery system into inhalable carrier particles using a spray-freeze-drying technique. The cytotoxic effects of free DOX, carrier particles containing blank nanoparticles or DOX-loaded nanoparticles on H460 and A549 lung cancer cells were assessed using a colorimetric XTT cell viability assay. The mean geometric carrier particle size of 10 ± 4 μm was determined using confocal laser scanning microscopy. DOX-loaded nanoparticles had a particle size of 173 ± 43 nm after re-dissolving of the carrier particles. Compared to H460 cells, A549 cells showed less sensitivity to the treatment with free DOX. The DOX-nanoparticles showed in both cell lines a higher cytotoxicity at the highest tested concentration compared to the blank nanoparticles and the free DOX. The cell uptake of free DOX and DOX delivered by nanoparticles was confirmed using confocal laser scanning microscopy. This study supports the approach of lung cancer treatment using nanoparticles in dry powder aerosol form.

Keywords: Cytotoxicity; Doxorubicin; Nanoparticles; Powder inhalation; Spray-freeze drying; Lung cancer cells

1. Introduction

Pulmonary inhalation systems have been used to deliver pharmaceutical compounds for the prevention and treatment of respiratory diseases (Sharma et al., 2001; Wang et al., 2003). For sufficient drug deposition the particle size of the aerosol has to be between 1 and 5 μm (Finlay, 2001). Nanoparticles are generally too small for pulmonary delivery due to their small mass median aerodynamic diameter (MMAD). The MMAD determines the deposition location of particles within the lungs. We recently developed inhalable carrier particles, which were loaded with empty nanoparticles (Sham et al., 2004). In this work it was demonstrated that the carrier particles dissolve after coming in contact with the aqueous medium and release the nanoparticles. Carrier particles can be made with an appropriate MMAD to optimize alveolar deposition. Such an approach can be used for the local delivery of drug-loaded nanoparticles to the lungs.

Lung cancer is the second most common cancer and the most common cause of cancer related death in both men and women (Jemal et al., 2004). In the present study the anticancer agent doxorubicin (DOX) was used as a model drug. Its incorporation into poly(butylcyanoacrylate) nanoparticles by an emulsion polymerization process is well established (Gulyaev et al., 1999). Recent investigations of the translocation of nanoparticles in the alveolar region indicate that surfactants coated polystyrene particles translocated across the alveolar capillary barrier while uncoated particles did not (Kato et al., 2003). Therefore, in the present study we used polysorbate 80 coated nanoparticles which were loaded with DOX.

The cytotoxic effects of free DOX, carrier particles containing blank nanoparticles or DOX-loaded nanoparticles on H460 and A549 lung cancer cell lines were assessed using a colorimetric XTT cell viability assay. The cell uptake of free DOX and

* Corresponding author. Tel.: +1 780 492 1255; fax: +1 780 492 1217.
E-mail addresses: rloebenberg@pharmacy.ualberta.ca, RAIMAR@UALBERTA.CA (R. Löbenberg).

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DOX delivered by nanoparticles was confirmed using confocal laser scanning microscopy.

2. Materials and methods

2.1. Materials

Dextran 70,000 and polysorbate 80 were supplied by Sigma (Ontario, Canada). *N*-Butylcyanoacrylate monomer (Lot. 02GD9236) was a gift from Loctite Ltd. (Dublin, Ireland). DOX was purchased from Pfizer Canada Inc. (Ontario, Canada). Lactose monohydrate was obtained from FMC (Philadelphia, USA). Human non-small cell lung cancer H460 and A549 cell lines were obtained from American Type Culture Collection (ATCC, Rockville, MD, USA). Cytotoxicity was analyzed using a cell proliferating XTT kit (Roche Molecular Biochemical, Laval, Que., Canada).

2.2. Preparation of DOX-loaded nanoparticles

DOX-loaded nanoparticles were prepared by an emulsion polymerization method (Gulyaev et al., 1999). Briefly, 100 μL of *n*-butylcyanoacrylate monomer was added to 1% dextran solution in 10 mL of 0.01 M HCl under constant stirring at 600 rpm. After 30 min DOX was added to the mixture to obtain a final concentration of 0.2 mg/mL. The particles were purified by centrifugation at 80,000 × *g* for 30 min (Beckman L8-M ultracentrifuge, CA, USA) and washed three times using de-ionized water, followed by the addition of 0.5% (v/v) polysorbate 80 under stirring at 400 rpm for 1 h and re-suspended in PBS.

2.3. Determination of drug loading on nanoparticles

Drug loading was calculated from the difference between the initial DOX concentration and the superfluous DOX determined in the supernatant liquids (Gelperina et al., 2002). The drug concentration was determined using a spectrophotometer (Beckman DU 7400 spectrophotometer, California, USA) at the maximum wavelength of 233 nm.

2.4. Spray freeze-drying procedure

Spray freeze-drying was previously described by Sweeney et al. (2005). In brief, 720 mg of lactose, as carrier, was mixed with DOX-loaded nanoparticles containing 1000 μg DOX in 6 mL aqueous solution. A two-fluid nozzle (Spraying Systems Co., Wheaton, IL, USA) utilizing gaseous nitrogen at a flow rate of 0.6 scfm was employed to atomize the suspensions, which were supplied at a flow rate of 37 mL/min using a peristaltic pump (CTP-A, Chem-Tech, Punta Gorda, FL, USA). The nozzle was placed ∼15 cm above a 600 mL flask containing 300–400 mL of liquid nitrogen. Following spraying, the flask contents were transferred into a Pyrex vacuum beaker, and the liquid nitrogen was allowed to evaporate. The vacuum container was attached to a freeze dry system (Freezone 4.5, Labconco Corp., Kansas City, MO, USA) operating at 0.004 mbar with the collector at −52 °C. The powder in the flask was held at subzero temperature for the initial 7 h, followed by 41 h at 23 °C. After 48 h, the powder was collected and stored in a sealed vial at 4 °C.

2.5. Particle size analysis of nanoparticles

The particle size of the nanoparticles was determined by photon correlation spectroscopy using a Zetasizer, model HSA 3000 (Malvern, Worcestershire, UK). For particle size analysis, 100 μL of the nanoparticle suspension was dispersed in 4 mL de-ionized water. Measurements were carried out at 25 °C. The mean particle size and polydispersity index were determined.

2.6. Measurement of mass median aerodynamic diameter

Mass median aerodynamic diameter (MMAD) of the powder was measured using a Mark II Anderson Cascade Impactor (Graseby Anderson, Smyrna, GA, USA) with effective cut-off points recalibrated at 60 L/min. A passive dry powder inhaler that utilizes cyclonic action as well as mechanical impaction to disperse powder particles was used to deagglomerate and deliver the powder (Finlay and Wang, 2003). The flow rate was monitored with a pneumotachometer (PT 4719, Hans Rudolph Inc., Kansas City, MO, USA).

2.7. Activity testing

A colorimetric XTT assay system was utilized to determine the cytotoxicity of the free DOX, empty nanoparticles and DOX-loaded nanoparticles on the H460 and A549 cancer cell lines. Cells were grown in a humidified 5% carbon dioxide atmosphere at 37 °C on a 96-well microplate, with each well containing 5000 cells immersed in 100 μL of 10% fetal bovine serum, and 1% penicillin/streptomycin. The cells were allowed to adhere for 14 h. The cells were then incubated with free DOX (doxorubicin hydrochloride injection, Novopharm, Toronto, Canada), blank nanoparticles (powder form) or DOX-nanoparticle (powder form) at various concentrations for 48 h. Following incubation, 50 μL XTT labeling mixture was added to each well. The microplate was incubated for a further 4 h. A Benchmark microplate reader (Bio-Rad Laboratory, Mississauga, Ont., Canada) with a 492 nm optical filter and a 650 nm reference wavelength was utilized to measure the absorbance of each well. The fraction of viable cells was calculated by subtracting the optical density fraction of treated cells from the untreated cells. Each arrangement had a minimum of two measurements.

2.8. Cell culture

H460 (HTB-177) and A549 (CCL-185) lung cancer cell lines were used for the cell uptake studies. These cells are adherent cells, which grow as a monolayer. For the propagation of H460 cells ATCC, complete growth medium (RPMI 1640 medium with 2 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, 10 mM HEPES, and 1.0 mM sodium pyruvate, 90%; fetal bovine serum, 10%) was used. For the propagation of A549 cells ATCC complete growth medium (Ham's F12K medium with 2 mM L-glutamine adjusted to con-

tain 1.5 g/L sodium bicarbonate, 90%; fetal bovine serum, 10%) was utilized. The cells were grown in 75 mL flasks (Corning, Acton, MA, USA) in an atmosphere of 5% CO_2 and 100% relative humidity and subcultured two to three times per week.

2.9. Cell uptake study

Approximately 10^4 cells were grown in a Lab-Tek II Chamber Slide™ System (Nalge Nunc, USA). After 24 h, when the cells were attached to the surface of the slides as a monolayer, they were incubated with DOX-nanoparticle (powder form) at the concentration of 0.625 μg/mL for 24 h. Then the nanoparticles were rinsed out using PBS. The cells were fixed by adding 0.5 mL of 4% solution of paraformaldehyde in PBS for 10 min. This solution was then removed and 20 μL of 1:9 PBS–glycerol containing 1 μL of 1 mg/mL solution of 4′-6-diamidino-2-phenylindole (DAPI) in PBS was added as mounting medium. Control samples were prepared by incubating cells with 100 μL of 0.625 μg/mL solution of free DOX (doxorubicin injection) in PBS under the same conditions.

2.10. Confocal laser scanning microscopy (CLSM)

For imaging the cells and examining the morphology and size of the carrier particles, a Zeiss LSM 510 confocal microscope (Oberkochen, Germany) was used. This model can collect 12 bit images using four detectors for fluorescent signals and a transmission detector for bright field images. It has four lasers with multiple laser lines for excitation of fluorophores. The LSM 510 Software, version 2.01 was used to control the microscope and to analyze the data.

2.11. Measurement of the intensity of fluorescence in CLSM images

The cell uptake of doxorubicin nanoparticle and control solution was determined and the intensity of fluorescence in CLSM images was measured using Metamorph software version 6.2.6 (Universal Imaging Corp., USA). Average gray value was used as a criterion to compare the intensity in different images.

2.12. Statistical analysis

ANOVA and a paired *t*-test at a *P*-value of 0.05 were used for statistical analysis.

3. Results

The drug loading study showed that approximately 85% of DOX was adsorbed on the poly(butylcyanoacrylate) nanoparticles which was in accordance with the other published data (Gulyaev et al., 1999; Gelperina et al., 2002). The loading amount of DOX in the carrier particles after spray-freeze drying was calculated as 1.39 μg DOX/mg powder. The mean particle sizes of DOX-loaded nanoparticles after re-dissolving of the spray-freeze-dried powders were 173 ± 43 nm. The spray-freeze dried powder particles had a spherical shape, among which some

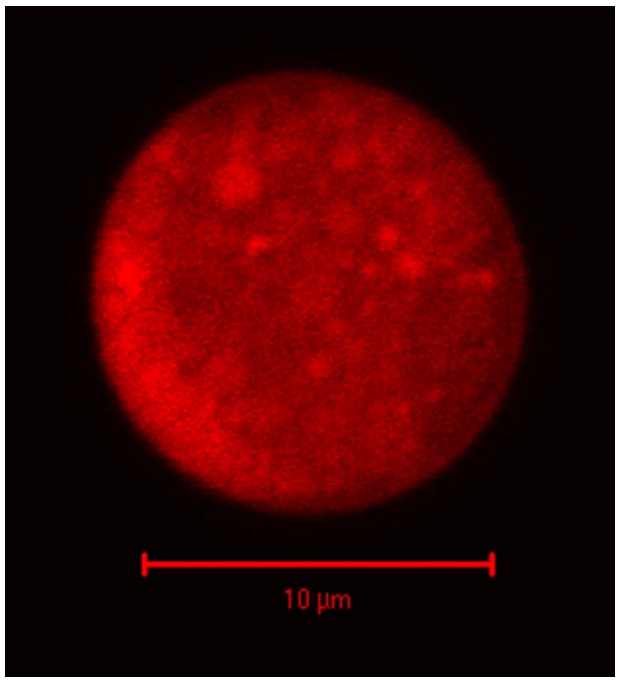

Fig. 1. CLSM image of the DOX-loaded nanoparticles incorporated into a lactose carrier particle via spray-freeze drying method.

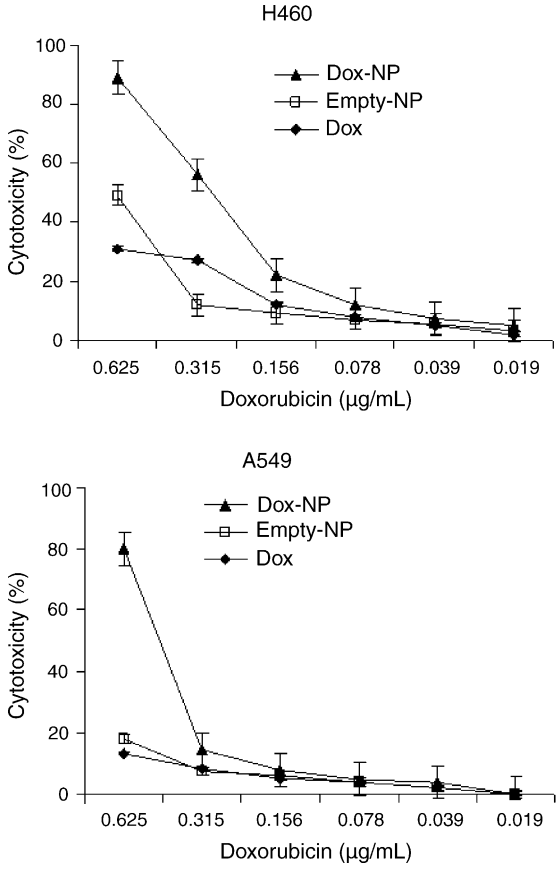

Fig. 2. Comparison of cell toxicity of free doxorubicin (DOX), carrier particles containing DOX-loaded nanoparticles (DOX-NP), or blank nanoparticles (Empty-NP) in H460 (A) and A549 (B) lung cancer cells. Each data point represents the average result of three wells and two independent experiments (the empty nanoparticles had the same concentration based on dry weight as doxorubicin nanoparticles).

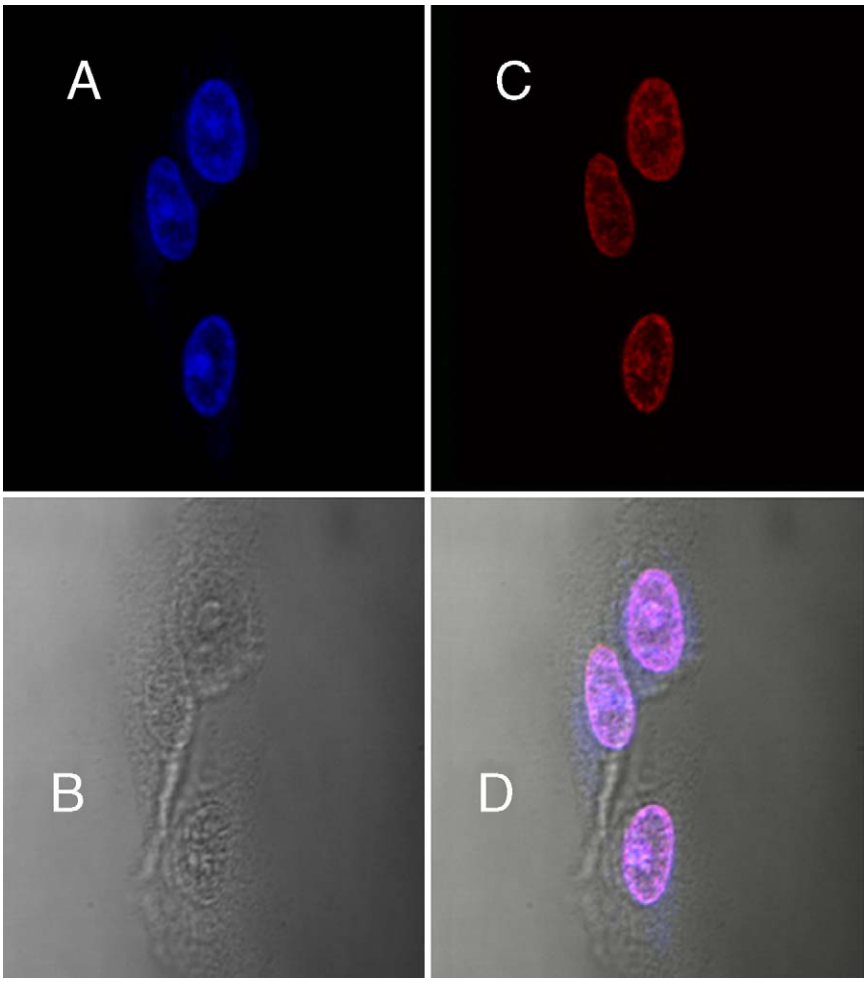

Fig. 3. CLSM image of the cellular uptake of DOX delivered via DOX-loaded nanoparticles in A549 lung cancer cells. (A) DAPI staining, (B) bright field, (C) DOX detection and (D) overlay of (A)–(C).

carrier particles were sponge like and others had a continuous matrix. Fig. 1 shows a CLSM image of a carrier particle loaded with nanoparticles. It can be seen that DOX, a red fluorescent anti-cancer drug, has been successfully incorporated into lactose carrier particles and DOX-loaded nanoparticles clustered in different spots within the lactose carriers. The mean geometric carrier particle size of 10 ± 4 μm was determined from more than 50 particles. However, the aerodynamic particle sizes of these carrier particles loaded with blank and doxorubicin nanoparticles were 3.45 ± 0.11 ($n = 3$) and 3.41 ± 0.22 ($n = 6$), respectively.

The results of the XTT test in H460 and A549 lung cancer cells are shown in Fig. 2A and B. In H460 cells free DOX showed a gradually increase in cytotoxicity from the lowest concentration to 0.156 μg/mL. Then a more rapid increase in cytotoxicity with further increases in DOX concentration was observed. Some of that increase can be attributed to the rapid increase in cytotoxicity for blank nanoparticles at these levels.

However, a significant increase in cytotoxicity was observed at the highest concentration only ($P < 0.05$). DOX-nanoparticles showed a profound increase in cytotoxicity at concentrations higher than 0.156 μg/mL.

In A549 cells, a similar trend in cytotoxicity was observed. Free DOX and blank nanoparticles gradually increased their cytotoxicity with increasing concentrations. DOX-nanoparticles showed a sharp increase in cytotoxicity at the highest tested concentration. Compared to H460 cells, A549 cells showed less sensitivity to the treatment with free DOX. The DOX-nanoparticles showed in both cell lines a higher cytotoxicity at the highest tested concentration compared to the blank nanoparticles and the free DOX.

Figs. 3 and 4 show the uptake of DOX loaded nanoparticles and DOX solution in A549 cells, respectively. Confocal laser scanning microscopy showed that DOX as free drug or bond to nanoparticles was accumulated as red staining in the cell nucleus of both cell lines (pictures for H460 cells not shown).

4. Discussion

In the present study DOX-loaded nanoparticles were incorporated as model drug delivery system into inhalable carrier particles. In order to avoid chemical decomposition and a loss of drug activity, spray freeze-drying technology (Maa et al., 1999) rather than a conventional heat spray-drying technology

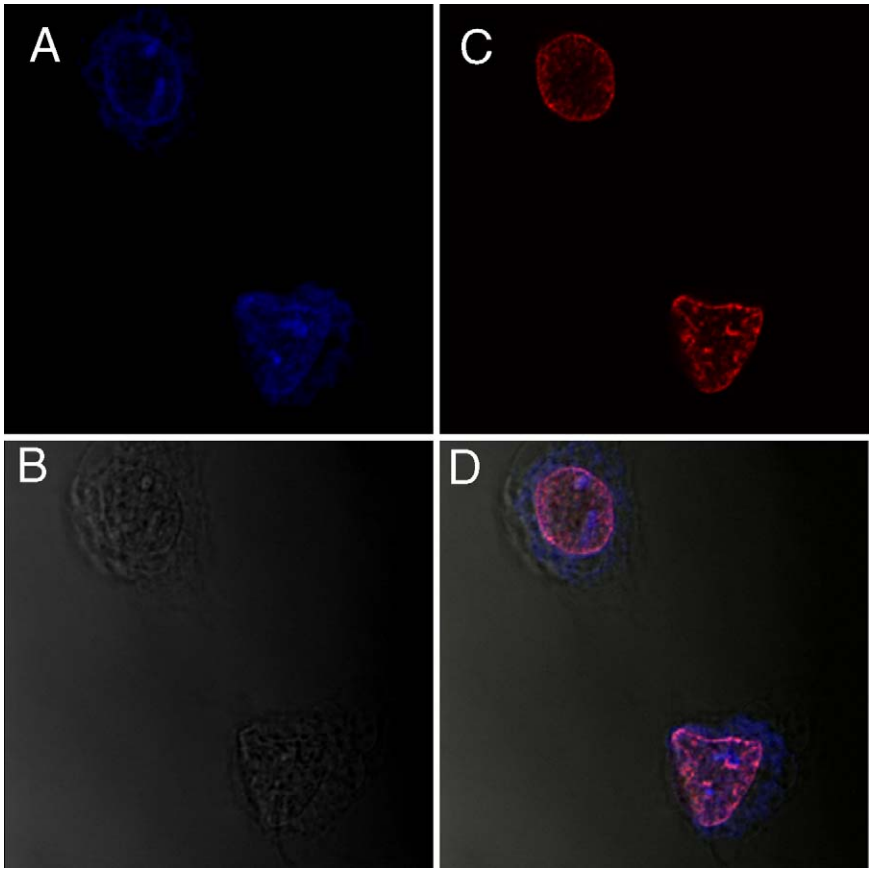

Fig. 4. CLSM image of the cellular uptake of DOX control solution in A549 lung cancer cells. (A) DAPI staining, (B) bright field, (C) DOX detection and (D) overlay of (A)–(C).

was used to obtain the carrier microparticles. The spray freeze-drying into liquid nitrogen and storage of the particles at 4 °C can prevent a possible Millard reaction or decrease the reaction rate (Qiu et al., 2005) between the amino group of DOX and the reducing sugar lactose. During 6 months storage of powder at both room temperature and refrigerator (2 °C) in well-closed containers, we did not observe any instability. The size of the carrier particles can be modified due to the spray-freeze drying conditions. In this study the shape and size of the particles were similar to carrier particles made by conventional spray drying techniques (Sham et al., 2004). Our study showed that the nanoparticles had a tendency to cluster within the carrier matrix as reported for carrier particles made by spray drying (Sham et al., 2004).

The nanoparticles were coated using polysorbate 80 prior to the incorporation into the carriers. The coating decreased the tendency of the nanoparticles to agglomerate as indicated by the particle size differences between freshly synthesized nanoparticles and re-dispersed spray freeze-dried particles. The effect of the coating of nanoparticles by surfactants has been shown in several studies. Coating of nanoparticles by polysorbate 80 can enhance the drug delivery through the blood brain barrier (Alyautdin et al., 1995, 1997, 1998; Schroeder et al., 1998; Gulyaev et al., 1999) and alter the body distribution after an intravenous injection (Araujo et al., 1999). Recent nanotoxicological investigations have shown that surfactant coatings can improve the translocation of nanoparticles across the alveolar capillary barrier (Kato et al., 2003). Therefore, coated nanoparticles were used in this study.

One of the aims of this study was to determine whether the spray freeze-dried DOX loaded nanoparticles maintained their cytotoxic effect. We tested free DOX, carrier particles containing DOX-loaded nanoparticles or blank nanoparticles to differentiate between drug related and dosage form related cytotoxicity. This was done by using two different human non-small cell lung carcinoma cell lines, H460 and A549. Our results confirmed that A549 cells are less sensitive to doxorubicin compared to H460 (Kraus-Berthier et al., 2000). The lower sensitivity to DOX treatment showed by A549 was ascribed to drug sequestration by lung resistance-related protein (LRP) inside the cytoplasmic compartments (Meschini et al., 2002). Our study showed that carrier particles containing blank nanoparticles exhibit some degree of cytotoxicity. The sharp increase in cytotoxicity at the highest tested concentration might be due to the presence of polysorbate 80. The cytotoxic effect of polysorbate 80 on different cell lines has been shown previously (Bacskay et al., 2005).

However, the results of other researchers showed that entrapment of paclitaxel in cetyl alcohol/polysorbate nanoparticles significantly increases its toxicity toward human glioblastoma cell line (U-118) (Koziara et al., 2004). Inhibition of P-glycoprotein

is believed to be the involved mechanism (Nerurkar et al., 1996; Wang et al., 2004).

Nanoparticulate drug delivery has shown to be a promising way to overcome multi drug resistance phenomenon for DOX (Vauthier et al., 2003). Resistant cells treated with DOX-loaded poly(alkyl cyanoacrylate) nanoparticles showed higher sensitivity to the drug relative to the free drug (Treupel et al., 1991; Cuvier et al., 1992; Nemati et al., 1996; Colin de Verdiere et al., 1997).

In our study we observed a steep increase in cytotoxicity in both cell lines when carrier formulations containing DOX-nanoparticles were used. This indicates that DOX-loaded nanoparticles are more potent than free drug. A possible explanation for the activity enhancement of DOX-loaded nanoparticles is that nanoparticles are more readily internalized by an endocytosis mechanism compared to a passive diffusion mechanism of DOX into cells (Yoo et al., 2000). On the other hand, the observed increase might be a combined effect of nanoparticles and polysorbate 80. The confocal study supports these findings (Fig. 3). The results of measuring fluorescence intensity in different pictures based on average gray value showed that the red fluorescent was higher when nanoparticles were used.

In conclusion, drug-loaded nanoparticles carried by dry powders showed a concentration related increased cytotoxicity in vitro. This study supports the approach of local lung cancer treatment using nanoparticles as a drug delivery vector. The development of inhalable nanoparticles loaded with bioactive molecules is a new delivery platform which can allow targeting of lung specific diseases in the future.

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References

Alyautdin, R.N., Gothier, D., Petrov, V.E., Kharkevich, D.A., Kreuter, J., 1995. Analgesic activity of the hexapeptide dalargin adsorbed on the surface of polysorbate 80-coated poly(butylcyanoacrylate) nanoparticles. Eur. J. Pharm. Biopharm. 41, 44–48.

Alyautdin, R.N., Petrov, V.E., Langer, K., Berthold, A., Kharkevich, D.A., Kreuter, J., 1997. Delivery of loperamide across the blood–brain barrier with polysorbate 80 coated polybutylcyanoacrylate nanoparticles. Pharm. Res. 14, 325–328.

Alyautdin, R.N., Tezikov, E.B., Ramge, P., Kharkevich, D.A., Begley, D.A., Kreuter, J., 1998. Significant entry of tubocurarine into the brain of rats by adsorption to polysorbate 80-coated polybutylcyanoacrylate nanoparticles. J. Microencapsul. 15, 67–74.

Araujo, L., Löbenberg, R., Kreuter, J., 1999. Influence of the surfactant concentration on the body distribution of nanoparticles. J. Drug Target. 6, 373–385.

Bacskay, I., Kocsan, R., Fenyvesi, F., Feher, P., Sipos, T., Varadi, J., Vecsernyes, M., 2005. Cytotoxicity of different types of surfactants on HeLa cells. Eur. J. Pharm. Sci. 25, S46–S47.

Colin de Verdiere, A., Dubernet, C., Nemati, F., Soma, E., Appel, M., Ferte, J., Bernard, S., Puisieux, F., Couvreur, P., 1997. Reversion of multidrug resistance with polyalkylcyanoacrylate nanoparticles: towards a mechanism of action. Br. J. Cancer 76, 198–205.

Cuvier, C., Roblot-Treupel, L., Millot, J.M., Lizard, G., Chevillard, S., Mnfait, M., Couvreur, P., Poupon, M.F., 1992. Doxorubicin-loaded nanospheres bypass tumor cell multidrug resistance. Biochem. Pharmacol. 44, 509–517.

Finlay, W.H., 2001. The Mechanics of Inhaled Pharmaceutical Aerosols: An Introduction. Academic Press, San Diego.

Finlay, W.H., Wang, Z.L., 2003. Device and method for deagglomeration of powder for inhalation. US Patent Pending (Ref. 002555-0013).

Gelperina, S.E., Khalansky, A.S., Skidan, I.N., Skidan, I.N., Smirnova, Z.S., Bobruskin, A.I., Severin, S.E., Turowski, B., Zanella, F.E., Kreuter, 2002. Toxicological studies of DOX bound to polysorbate 80-coated poly(butylcyanoacrylate) nanoparticles in healthy rats and rats with intracranial glioblastoma. Toxicol. Lett. 126, 131–141.

Gulyaev, A.E., Gelperina, S.E., Skidan, I.N., Antropov, A.S., Kivman, G.Y., Kreuter, J., 1999. Significant transport of doxorubicin into the brain with polysorbate 80-coated nanoparticles. Pharm. Res. 16, 1564–1569.

Jemal, A., Clegg, L.X., Ward, E., Ries, L.A.G., Wu, X., Jamison, P.M., Wingo, P.A., Howe, H.L., Anderson, R.N., Edwards, B.K., 2004. Annual report to the nation on the status of cancer, 1975–2001, with a special feature regarding survival. Cancer 101, 3–27.

Kato, T., Yashiro, T., Murata, Y., Hebert, D.C., Oshikawa, K., Bando, M., 2003. Evidence that exogenous substances can be phagocytized by alveolar epithelial cells and transported into blood capillaries. Cell Tissue Res. 311, 47–51.

Koziara, J.M., Lockman, P.R., Allen, D.D., Mumper, R.J., 2004. Paclitaxel nanoparticles for the potential treatment of brain tumors. J. Contr. Release 99, 259–269.

Kraus-Berthier, L., Jan, M., Guilbaud, N., Naze, M., Pierre, A., Atassi, G., 2000. Histology and sensitivity of anticancer drugs to two human non-small cell lung carcinomas implanted in the pleural cavity of nude mice. Clin. Cancer Res. 6, 297–304.

Maa, Y., Nguyen, P., Sweeney, T., Shire, S.J., Hsu, C.C., 1999. Protein inhalation powder: spray drying vs spray freeze drying. Pharm. Res. 16, 249–254.

Meschini, S., Marra, M., Calcabrini, A., Monti, E., Gariboldi, M., Dolfini, E., Arancia, G., 2002. Role of the lung resistance-related protein (LRP) in the drug sensitivity of cultured tumor cells. Toxicol. In Vitro 16, 389–398.

Nemati, F., Dubernet, C., Fessi, H., Colin de Verdiere, A., Poupon, M.F., Puisieux, F., Couvreur, P., 1996. Reversion of multidrug resistance using nanoparticles in vitro: influence of the nature of the polymer. Int. J. Pharm. 138, 237–246.

Nerurkar, M.M., Burton, P.S., Borchardt, R.T., 1996. The use of surfactants to enhance the permeability of peptides through Caco-2 cells by inhibition of an apically polarized efflux system. Pharm. Res. 13, 528–534.

Qiu, Z., Stowell, J.G., Morris, K.R., Byrn, S.R., Pinal, R., 2005. Kinetic study of the Maillard reaction between metoclopramide hydrochloride and lactose. Int. J. Pharm. 303, 20–30.

Sham, J.O.H., Zhang, Y., Finlay, W.H., Roa, W., Löbenberg, R., 2004. Formulation and characterization of spray-dried powders containing nanoparticles for aerosol delivery to the lung. Int. J. Pharm. 269, 457–467.

Sharma, S., White, D., Imondi, A.R., Placke, M.E., Vail, D.M., Kris, M.G., 2001. Development of inhalational agents for oncologic use. J. Clin. Oncol. 19, 1839–1847.

Schroeder, U., Sommerfeld, P., Ulrich, S., Sabel, B.A., 1998. Nanoparticle technology for delivery of drugs across the blood–brain barrier. J. Pharm. Sci. 87, 1305–1307.

Sweeney, L.G., Wang, Z., Loebenberg, R., Wong, J.P., Lange, C.F., Finlay, W.H., 2005. Spray-freeze-dried liposomal ciprofloxacin powder for inhaled aerosol drug delivery. Int. J. Pharm. 305, 180–185.

Treupel, L., Poupon, M.F., Couvreur, P., Puisieux, F., 1991. Vectorisation of doxorubicin in nanospheres and reversion of pleiotropic resistance to tumor cells. C.R. Acad. Sci. III 313, 1–174.

Vauthier, C., Dubernet, C., Chauvierre, C., Brigger, I., Couvreur, P., 2003. Drug delivery to resistant tumors: the potential of poly(alkyl cyanoacrylate) nanoparticles. J. Contr. Release 93, 151–160.

Wang, S.W., Monagle, J., McNulty, C., Putnam, D., Chen, H., 2004. Determination of P-glycoprotein inhibition by excipients and their combinations using an integrated high-throughput process. J. Pharm. Sci. 93, 2755–2767.

Wang, Z., Chen, H.T., Roa, W., Finlay, W.H., 2003. Farnesol for aerosol inhalation: nebulization and activity against human lung cancer cells. J. Pharm. Pharm. Sci. 6, 95–100.

Yoo, H.S., Lee, K.H., Oh, J.E., Park, T.G., 2000. In vitro and in vivo antitumor activities of nanoparticles based on doxorubicin–PLGA conjugates. J. Contr. Release 68, 419–431.