Prevention of Contrast Medium Induced Nephropathy by Liposome Encapsulation

Gi-Da Lee, Jyn-Wen Chai, Li-Che Hu, Pei-Hsuan Lu, and Kuo-Chih Liao

***Abstract*—The study is investigating prospectively the potentials of applying liposome as computed tomography (CT) contrast agent delivery vesicle for prevention of contrast medium induced nephropathy (CIN) incidence both in vitro and in vivo. From dynamic CT of nude mice, we found that partial encapsulation of CM in liposome increased the indication of biliary excretion up to 12 folds post CM administration, and reduced the concentration and duration of CM accumulation in kidney. From Madin–Darby canine kidney epithelial cell line (MDCK) viability studies, it showed that fully encapsulation of CM in liposome significantly improved the cell viability when exposed to clinical concentration of CM for 24 hours.**

***Index Terms*—Liposome, contrast medium, contrast medium induced nephropathy.**

I. Introduction

Contrast media (CM) induced nephropathy (CIN) has become the third leading cause of acute renal failure with the increase use for clinical diagnosis and intervention. It is associated with significant risk of morbidity and mortality [1]. Although the pathophysiology of CIN has been recognized with renal medulla ischemia and hypoxia resulting renal epithelial/tubular cells damage from toxic effect of CM, the incidence mechanism is still contentious [1]-[3]. The most popular hypothesis of CIN incidence is associated with the impact from hyper-osmolality of CM, however extensive clinical trial studies indicated that the role of osmolality is much lesser and its clinical correlated outcome exhibited in the limited reduction of CIN induced rate when administration with isotonic contrast agents [2]-[4]. Biophysical impacts other than osmolality, such as renal tubular viscosity (increased by CM) and renal interstitial pressure (increased by CM), or biochemical interaction of CM metabolites (such as gadolinium ion, releasing from chelating agent of magnetic resonance imaging CM, is a blocker of multiple calcium gated channels) with renal cells might play more important roles in CIN incidence [2]-[7].

Manuscript received April 1, 2013; revised July 11, 2013. This work was supported by grants from the Taichung Veterans General Hospital and National Chung Hsing University (TCVGH-NCHU 1017611) Taichung, Taiwan, Republic of China.

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Liposome is by far the most successful drug delivery vehicle in commercialization for clinical application [8]. Its long-lasting payload release characteristic has been utilized as anti-cancer, anti-infection and anti-HIV related Kaposi's sarcoma agents, vaccine (hepatitis, influenza), post surgical analgesia, menopause or age-related macular degeneration therapeutics. For chemotherapy, the liposome vesicle can accumulate higher concentration/percentage of drug in tumor (increase therapeutic index), and prevent the drug (toxic agent) exposed to the normal tissue and causing damage during transportation or been sabotaged before arriving target tumor site (reduce side effects). The liposome delivered anticancer agents, such as doxorubicin, have been proved to reduce side effects (cardio-toxicity, hear loss……) while exhibiting superior performance or preserving efficacy in clinical studies with maintaining extended period of above threshold value concentration for treatment [9]-[11]. Considerable efforts have been devoted for the lifetime and integrity of liposomes in the bloodstream for hours to days, increasing the successful rate of transportation to the target location [12], [13]. The enhanced permeability and retention effect (EPR effect) of liposome exhibits passively accumulation of macromolecule on tumor tissue due to the hyper-permeability from tumor neo-vasculature and the lack of lymphatic drainage [14], which contributes in the localization of delivered payload (drug or indicator) in high concentration (up to 177 folds in concentration than administration without liposome [15]).

The application of liposome in delivering CM is still under laboratory investigations for potentials, and a long road away from commercialization for clinical uses. Those studies focused on utilizing the characteristics of liposome for the following objectives:

1) Permeability of liposome through blood-brain barrier (BBB) can allow early prognosis from brain tumor imaging with CM before significant compromised BBB in the later stage of cancer development and metastasis [16], [17].
2) Surface modification of liposome with active targeting agents (antibody……etc.) can increase the tumor enhancement of encapsulated CM, such as overcoming the low relaxivity by higher accumulation for MRI [18], or with cell-targeted markers can aid visualization of pathological process [19].
3) High capacity of payload encapsulation and high flexibility of formula or surface modification allow CM included liposome to evolve as multi-model tools for both diagnosis and therapy [19]-[21].
4) The extending circulation lifetime of liposome in vivo allows incorporated CM for prolonged tumor enhancement or revelation of pathological index [21], [22].

Theoretically, delivery of CM with liposome has the potential to prevent the accumulation of CM in kidney by alternative route of biliary/fecal excretion, undesired exposure of CM with renal cells, and reduce amount of CM administrated for maintaining the same contrast effect. The application of liposome in preventing CIN incidence has not been systematically investigated to the authors' knowledge. The study is aiming to investigate prospectively the potentials of applying liposome as computed tomography (CT) contrast agent delivery vesicle for prevention of contrast medium induced nephropathy (CIN) incidence both in vitro and in vivo.

II. Materials and Methods

A. Dynamic CT of Nude Mice

NU/NU nude mice were administrated with iobitridol (Xenetix, Guerbert, France), or partially encapsulated iobitridol in liposome (dried lipid film, 10mg or 20mg, was rehydrated by iobitridol) intravenously as a bolus through the tail vein at the dosage of 1mg iodine / g. The long circulating liposome was composed of 1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine (DPPC), L-α-phosphatidylcholine (SoyPC), cholesterol (Chol), 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[amino (polyethylene glycol)-2000] in the molar ratio DPPC:SoyPC:Chol:DSPE-PEG2k = 54 :27:16:3 [15].

Dynamic CT imaging was acquired with Philips BR64 (Andover, MA, USA) at following the setting (0.5sec rotation time, 80kV, 80mAs, 0.6mm beam collimation, 1mm slice thickness) at different time points (0, 1, 3, 8, 10, 30, 60, 90, 120minutes……etc). Imaging data from the CT scans was transferred to a PACS workstation (Centricity, GE Medical Systems, Milwaukee, Wis, USA) for quantification evaluation. The signal intensity expressed in Hounsfield units (HU) was assessed for the contrasts achieved by CM with or without liposome.

B. Cell Viability of MDCK Cells

MDCK cells were seeded on 24-well plate to reach 70% confluence, and then treated with mixture of DMEM medium and iobitrodol or saline (same osmolarity as iobitridol) or column chromatography purified iobitridol encapsulated liposome (distributed in saline with the same osmolarity as iobitridol) in the volume ratio, 1:1, for 24 hours.

Cell viability was quantified by MTT (3-[4, 5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay. The yellow solution was reduced by succinate dehydrogenase located in the inner membrane of mitochondria and resulted in the formation of purple crystal, which was dissolved by dimethyl sulfoxide and quantified by absorbance at 570nm with spectrophotometer (Synergy Mx , BioTeK).

III. Results and Discussions

The clinically administrated CMs and their metabolites are primarily excreted through renal excretion due to their hydrophilic characteristics, and with much smaller portion cleared through biliary excretion. Generally, only drugs (and metabolite) with a molecular weight of > 300 g/mole and with both polar and lipophilic groups are more likely to be excreted in bile [23]. We found that the presence of liposome had influence on the distribution of CM excretion routes indicated by the signal increase in bladder (waste storage of renal excretion) and intestine (waste storage of biliary excretion) as shown in Fig. 1 and Fig. 2. The partial encapsulation of iobitridol in 10mg liposome increased the excretion through biliary route up to 12 folds (at 1 hour post administration) and accounted for majority of clearance (>50%) after 5 hour (Fig. 2).

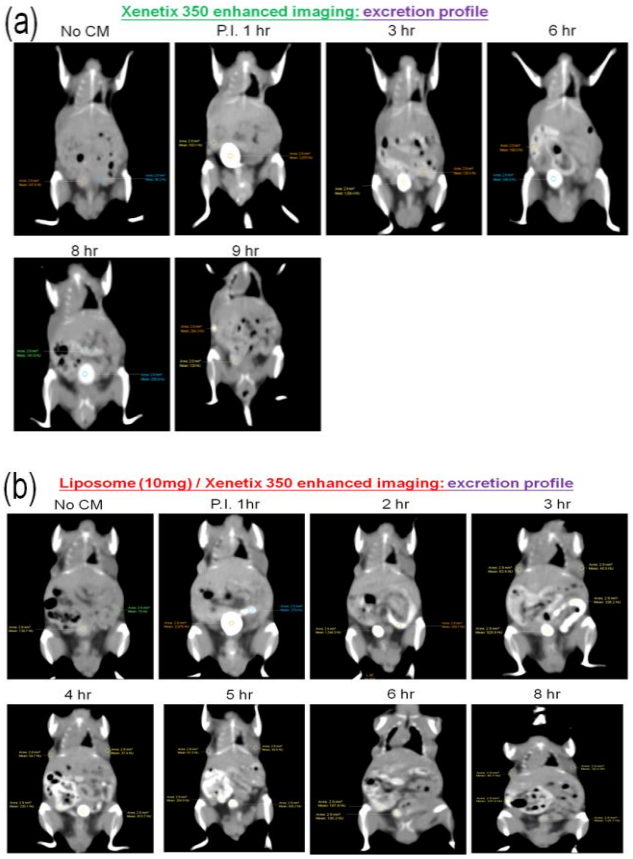


Fig. 1. Contribution of renal and biliary excretion in CM clearance: signal intensity at bladder (renal excretion storage) and intestine (biliary excretion storage) for CM contrasted images (a), and CM/liposome (10mg) contrasted images (b).

In order to investigating the potential dose dependent CIN risk elimination of liposome encapsulation, the accumulation dynamics of CM in kidney were observed under 3 different contrast enhanced conditions: iobitridol only, iobitridol partially encapsulated with 10mg liposome and iobitridol partially encapsulated with 20mg liposome (Fig. 3). We found that the presence of 10mg/ml liposome encapsulation reduced the CM signals by 50% at peak (1 hour post CM administration), and the higher the dosage of liposome, the quicker the clearance of CM in kidney. The group with 20mg/ml liposome showed no sign of CM presence in kidney after 3rd hour post CM injection, however the 10mg/ml liposome group had CM in kidney up to 5th hour, and pure CM group had CM in kidney lasting more than 7 hours.

In addition to altering the clearance route of CM and reducing CM accumulation in kidney, encapsulation by liposome can theoretically prevent the CM cytotoxicity due

to the elimination of direct contact / interaction between renal cells and CM, which is shielded by well-proven biocompatible liposome. It is indicated by exposing MDCK cells with medium/iobitridol, or medium/saline (same osmolarity as iobitridol), or medium/column chromatography purified iobitridol encapsulated liposome (distributed in saline with the same osmolarity as iobitridol). The iodine concentration was maintained as 150mg/l for both medium/iobitridol and medium/iobitridol/liposome groups to simulating the dilution effect of iobitridol in circulation. The liposome encapsulation had significantly restored the cell viability from 39.8% to 95.3% (Fig 4).

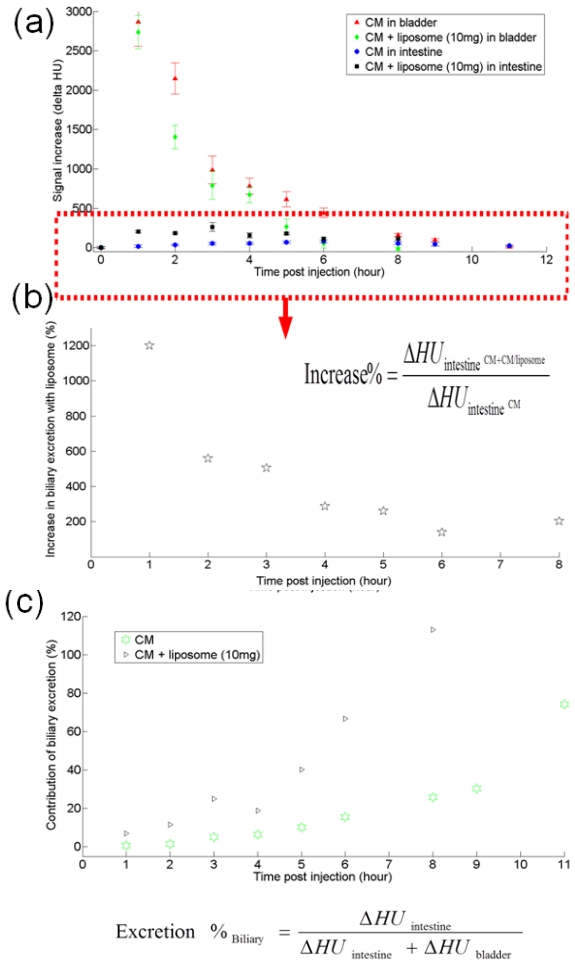
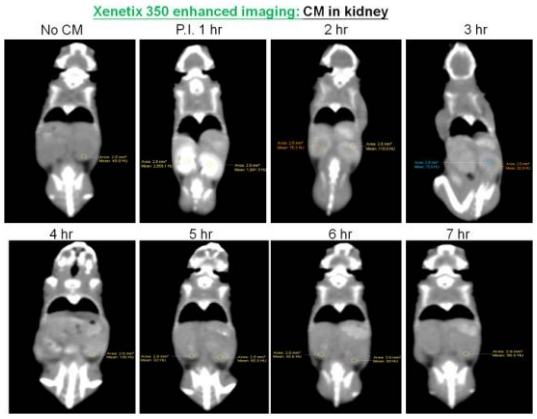
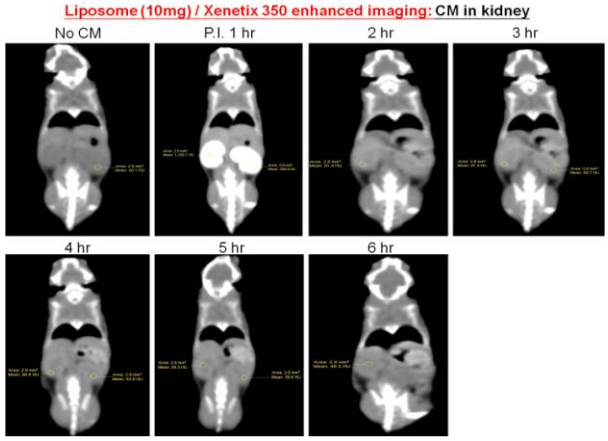


Fig. 2. Contribution of renal and biliary excretion in CM clearance: (a) Excretion dynamics indicated by signal at bladder (renal excretion storage) and intestine (biliary excretion storage). (b) Enhancement of biliary excretion by application of liposome (10mg). (c) Contribution of biliary excretion in the presence of liposome (10mg).





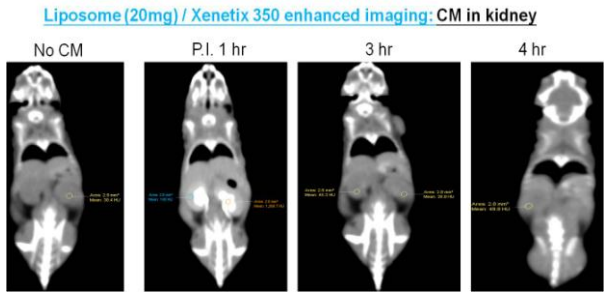


Fig. 3. Accumulation dynamics of CM in kidney under 3 different conditions:iobitridol only, partial encapsulation of iobitridol with 10mg liposome and partial encapsulation of iobitridol with 20mg liposome.

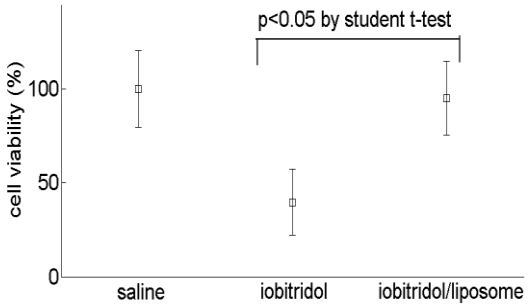


Fig. 4. CM cytotoxicity reduction by liposome encapsulation.

IV. Conclusion

The preliminary results in the study suggested that liposome encapsulation could reduce the risk of CIN by altering the clearance route to avoid accumulation of CM in kidney (in vivo dynamic CT of mice study) or prevent the direct cytotoxicity of CM on renal cell (MDCK cell viability study).

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