Development and Evaluation of Nanoparticulate Drug Delivery Systems for Anticancer Drugs

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Abstract

The main aim of this study was to develop nanoparticulate drug delivery systems for chlorambucil (CHL) and asulacrine (ASL). CHL is a DNA alkylating agent. In an attempt to alter the pharmacokinetics (short half-life, rapid clearance and high volume of distribution) and improve the anticancer activity, CHL was incorporated into a lipid nanosphere (LN) formulation. The LN were composed of soybean oil as the internal oil phase, egg lecithin as the primary emulsifier, cholesterol as the phospholipid layer stabilizer, and water as the external phase. PEG-modified (long circulating) and DOTAP-modified (cationic) LN were prepared to evade the reticulo-endothelial system and enhance cellular delivery, respectively. LN prepared by ultra-sonicater and microfluidizer had an average particle size below 200 nm, with a CHL encapsulation efficiency of over 97%. Zeta potential of the LN ranged from -30.6 to +41.5 mV. The LN exhibited good physical stability over six months at 4°C and 25°C. *In vitro* evaluation on SKOV-3 cells showed that the cytotoxicity and the pro-apoptotic activity of CHL were significantly enhanced when given in the LN formulation compared to the CHL solution ($P<0.05$). An altered pharmacokinetics with increased plasma AUC and elimination half-life, and reduced clearance were observed after intravenous administration of CHL incorporated LN compared to a CHL solution ($P<0.01$). A marked reduction in the tissue distribution of CHL was also noted when it was given in LN. In addition, CHL incorporated in LN showed greater anticancer activity compared with the CHL solution in colon-38 tumour-bearing mice ($P<0.01$). These results suggest that LN could be an effective parenteral carrier for CHL delivery.

ASL is an inhibitor of topoisomerase II. In an attempt to improve its efficacy by altering the pharmacokinetic profile, ASL was formulated as a nanocrystal suspension (NS). The NS was consisting of ASL nanoparticles produced by high pressure homogenization and stabilized by stabilizers. The lyophilized NS exhibited good stability over three months at 4°C and 25°C. The dissolution and solubility of ASL were enhanced in NS form compared to un-milled ASL ($P<0.05$). Initial peak plasma concentration and AUC were remarkably reduced in the plasma after intravenous administration of NS compared to the ASL solution ($P< 0.01$). This suggests reduced systemic exposure to the drug.

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List of Abbreviations

ANOVA	Analysis of the variance
ASL	Asulacrine
ATCC	American Type Culture Collections
AUC	Area under the concentration-time
BCS	Biopharmaceutics Classification system
BCNU	1,3-bis(2-chloroethyl)-1-nitrosourea (carmustin)
CFC	Chlorofluorocarbon
CH	Cholesterol
CHL	Chlorambucil
Cl	Clearance
CLL	Chronic lymphocytic leukaemia
C-38	Colon-38 adenocarcinoma
DDS	Drug delivery systems
DIC	Differential interference contrast
DiD oil	1,1'-dioctadecyl-3,3,3'3'-tetramethylindodicarbocyanine perchlorate
DLS	Dynamic light scattering
DMA	Dimethylacetamide
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
DOTAP	1,2-dioleoyl-3-trimethylammonium-propane
DSC	Differential scanning calorimetry
DSPE	Distearyl phosphatidyl ethanolamine
EL	Egg lecithin
EPR	Enhanced permeation and retention
FDA	Food and Drug Administration
GRAS	Generally regarded as safe
HPMC	Hydroxypropyl methyl cellulose
IC	Inhibitory concentration
IV	Intravenous

IS	Internal standard
LHRH	Luteinising hormone relasing hormone
LN	Lipid nanospheres
MPS	Mononuclear phagocytic system
MRT	Mean residence time
MTT	3-[4, 5-dimethyl thiazolyl]-2, 5-diphenyl tetrazolium bromide
MW	Molecular weight
MCT	Medium chain triglycerides
NMP	N-methyl 2- pyrrolidone
NCA	Non-compartmental analysis
NS	Nanocrystal suspension
PBS	Phosphate buffered saline
PCS	Photon correlation spectroscopy
PC	Phosphatidylcholine
PDI	Polydisperisty index
PEG	Poly(ethylene glycol)
$PEG_{2000}DSPE$	1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (ammonium salt)
PE	Phosphatidylethanolamine
PG	Propylene glycol
PMNS	Polymorphonuclear neutrophils
PS	Phosphatidylserine
PXRD	Powder X-ray diffraction
QC	Quality control
RPM	Revolution per minute
RI	Refractive index
RP-HPLC	Reversed phase – High performance chromatography
RSD	Relative standard deviation
SD	Standard deviation
SEM	Scanning electron microscopy
SKOV-3	Human ovarian adenocarcinoma cells
SO	Soybean oil

SMANCS	Styrene maleic anhydride - neo carzinostain
TPP	Target product profile
TEM	Transmission electron microscopy
TUNEL	Transferase dUTP nick end labelling
UV	Ultra-violet spectrophotometry
WFI	Water for injection

List of Research Output

Journal Publications

1. Ganta S, Paxton JW, Baguley BC, Garg S. Development and validation of bioanalytical method for the determination of asulacrine in plasma by liquid chromatography. J Pharm Biomed Anal. 2008;46(2):386-390.

2. Ganta S, Devalapally H, Baguley BC, Garg S, Amiji M. Microfluidic preparation of chlorambucil nanoemulsion formulations and evaluation of cytotoxicity and pro-apoptotic activity in tumor cells. J Biomed Nanotechnol. 2008;4(2):165-173.

3. Ganta S, Paxton JW, Baguley BC, Garg S. Pharmacokinetics and pharmacodynamics of chlorambucil delivered in parenteral emulsion. Int J Pharm. 2008; 360: 115-121.

4. Ganta S, Paxton JW, Baguley BC, Garg S. Formulation and pharmacokinetic evaluation of an asulacrine nanocrystalline suspension for intravenous delivery. Int J Pharm. (In press).

5. Ganta S, Paxton JW, Baguley BC, Garg, S. Pharmacokinetics and biodistribution analysis of chlorambucil containing long-circulating nanoemulsion. Pharm Res. (communicated).

Book Chapters

1. Ganta S, Sharma P, Garg S. Permeability Assessment. In: S.C. Gad (ed.), Preclinical Development Handbook: ADME and Biopharmaceutical Properties. New Jersey, USA: John Wiley and Sons Inc. 2008; 227-248.

2. Sharma P, Ganta S, Garg S. Scale Up and Post Approval Changes (SUPAC) regulations. In: S.C. Gad (ed.). Pharmaceutical Manufacturing Handbook: Regulations and Quality. New Jersey, USA: John Wiley and Sons Inc. 2008; 67-95.

Conference Abstracts

1. Ganta S, Sharma P, Baguley BC, Paxton JW, Garg S. Comparative pharmacokinetics and anticancer activity of chlorambucil delivered in nanoemulsions. Annual meeting & exposition of the AAPS 2008, Atlanta, GA, USA.

2. Ganta S, Sharma P, Baguley BC, Garg S. Preparation and characterization of long circulating nanoemulsion for passive tumor targeting. 34th CRS Annual meeting & exposition of the Controlled Release Society 2007, Long Beach, California, USA.

3. Ganta S, Sharma, P, Baguley BC, Garg S. Pre-formulation studies to evaluate the chlorambucil properties for developing a lipid nanosphere formulation. NZ bio conference 2007, Auckland, NZ.

4. Ganta S, Sharma P, Garg S. Selection of parenteral dosage form composition through drug-excipient compatibility testing. APSA Annual conference 2006, Adelaide, Australia.