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RESEARCH ARTICLE

PREPARATION AND *In Vivo* STUDY OF DOXORUBICIN HCL LOADED CHITOSAN NANOPARTICLES PREPARED BY W/O EMULSION METHOD

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ABSTRACT

The present study was carried out to determine and compare the pharmacokinetics and tissue distribution of Doxorubicin Hydrochloride (Doxorubicin) delivered as solution or through Chitosan Nanoparticles after intravenous (i.v.) injection. Doxorubicin loaded Chitosan nanoparticles were prepared by ionic gelation method (IGM). Plasma, tissue distribution profiles were quantified in an animal model of cancer and were compared to treatment with IGNP (ionic gelation method nanoparticles) to treatment with drug solution (DS). An isocratic high-pressure liquid chromatography (HPLC) method was developed to quantify Doxorubicin Hydrochloride in Rats plasma,vital organs. The Nanoparticles prepared by IGM show significantly increased the half life ($T_{1/2}$) and mean residence time (MRT) of Doxorubicin in blood. The area under curve (AUC^{0-8} and $AUC^{0-\infty}$) was also higher for Doxorubicin delivered through Nanoparticles prepared by both the methods, while the clearance and elimination half life ($K_{1/2}$) significantly lower. The Doxorubicin delivered through Nanoparticles experienced enhance distribution to the organs of reticuloendothelial system it lowered the distribution of Doxorubicin to heart and resulted in significantly lower concentration than Doxorubicin solution at all the time points studied. This signifies the advantage of nanoparticles in increasing the elimination half-life and reduce the Doxorubicin-associated systemic toxicity and Doxorubicin-associated cardiotoxicity.

INTRODUCTION

Development of efficient drug delivery system has attracted great attention during the last two decades. The delivery of drug molecules through the carrier system is assumed to avoid their unwanted effects because of controlled biodistribution [i] A major limiting factor to the systemic use of particulate delivery systems is the rapid clearance of carrier from the blood circulation by reticuloendothelial system (RES). Various techniques such as suppression of RES [ii] and modification of surface characteristic of drug carriers by coating with PEG were attempted to reduce the RES uptake. The second approach has been shown to be highly effective in altering the biodistribution pattern of colloidal drug carriers.[iii] It is imperative to develop selective release system for antineoplastic agent which would spare normal cell and reduce toxicity, such selective delivery would allow the use of higher doses of the drug and put eventually lead to a certain efficacy against some resistant tumors. Doxorubicin hydrochloride is a cytotoxic anthracycline antibiotic widely used in the treatment of non-Hodgkin's lymphoma, acute lymphoblastic leukemia, breast carcinoma and several others types of cancers. The mechanism of cytotoxicity involves the specific intercalation of planar anthracycline nucleus of DH to the DNA double helix resulting in the prevention of further DNA replication.

Chitosan has its unique properties for organ targeting such as colon, liver, lung and kidney. Also it can be modified and made nano-sized for passive and active targeting for cancer.[iv] Chitosan nanoparticles investigated for cancer chemotherapeutics have drawn considerable attention of the researchers. [v] The high toxicity, indiscriminate distribution and poor solubility of traditional anticancer drugs were attempted to be overcome by chitosan based drug delivery system. The drug release profiles of various drugs are attributed to the considerations of both properties of drug and carrier.

EXPERIMENTAL

Materials

Doxorubicin HCL was supplied as a gift sample from Khandelwal Laboratory Ltd., Mumbai, India. Chitosan was gifted from Central Institute of Fisheries Technology (Cochin, India), Sodium tripolyphosphate (TPP) was purchased from National Chemicals, Vadodara. Span 20 and Hexane – LR was purchased from S.D. Fine chemicals Ltd., Mumbai. Acetic acid glacial was purchased from Allied chemicals, Vadodara.

Methods

Preparation of plain nanoparticles

The preparation had been done in two steps as follows:

Step – I: In 25 ml liquid paraffin 5% w/v span 20 was added and stirred under magnetic stirrer for 15-20 min. 1% w/v solution of chitosan was added drop wise into above solution under the Ultra Turrax at 9500 rpm for 5 min. Step II: 0.5 ml of 0.5%w/v solution of sodium TPP was added drop wise in it under the UT-at the speed of 9500 rpm for 5 min that is plain Nanoparticles.

Preparation of Drug loaded nanoparticles

Doxorubicin HCL (30% w/w Drug: Polymer) is dissolved in distilled water added into Step I, then the procedure for step II was followed.

Pharmacokinetic and biodistribution studies

All the animal studies were approved by the CPCSEA and local animal ethics committee. The studies were performed on female S.D. rats weighing about 200-250 gm. The rats were fasted overnight before experimentation and were accessed to water ad libitum. The aqueous Nanoparticulate dispersion of Doxorubicin hydrochloride prepared by IGM (ionic gelation method) equivalent to 5mg/kg [vi] (Gulyaev et al., 1999) Doxorubicin hydrochloride (Doxorubicin) in saline was administered to rats by intravenous injection. After predetermined time intervals, the rats were sacrificed and organs such as liver, lung, kidney, heart and spleen were isolated. For pharmacokinetic studies, blood was collected from the retro-orbital plexus of rat eye. Estimation of Doxorubicin in blood and tissues was performed by HPLC analysis (Shimadzu 10 AT VP Model, Shimadzu Corporation, Japan).

Statistical Analysis

All the data are reported as mean ± SD of all three experiments. Statistical comparison of the data was done by ANOVA as a significance level of $p<0.001$, and student's t-test at a significance level of $p<0.001$.

RESULTS AND DISCUSSION

Doxorubicin loaded Chitosan nanoparticles were synthesized by W/O emulsion techniques. Fig 1 and 2 represents the blood clearance profiles of Doxorubicin solutions (DS) and Nanoparticles prepared by W/O emulsion method. Doxorubicin loaded Nanoparticles experienced an initial rapid clearance of the blood followed by slow clearance after 2 h of injection. Nanoparticles show higher blood conc. throughout the study. The pharmacokinetic parameters determined by Wagner Nelson Method. The parameters after i.v. injection shown in Table 2. The Nanoparticles showed significantly increased the half life ($T_{1/2}$) and mean residence time (MRT) of Doxorubicin in blood. The area under curve (AUC^{0-8} and $AUC^{0-\infty}$) was also higher for Doxorubicin delivered through Nanoparticles, while the clearance and elimination half life ($K_{1/2}$) significantly lower. Fig 3 represents tissue conc. of Doxorubicin hydrochloride loaded Nanoparticles prepared by W/O emulsion method after i.v. administration in rats. The Doxorubicin delivered through Nanoparticles experienced enhance distribution to the organs of reticuloendothelial system. Nanoparticles show significantly lowered the distribution of Doxorubicin to heart and resulted in significantly lower concentration than Doxorubicin solution at all the time points studied.

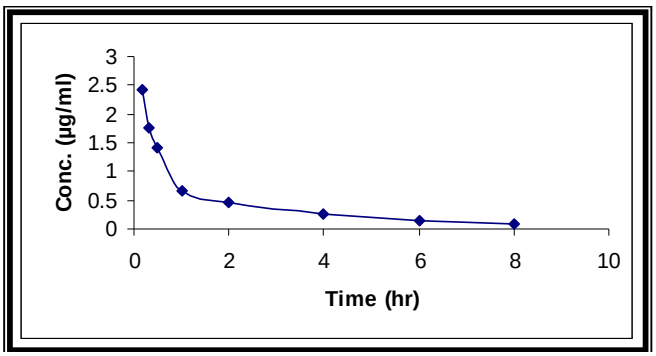

Figure 1. Blood Level of Doxorubicin Hydrochloride Solution (Ds)

Table 1. Formulation of Nanoparticles

Batch No.	Quantity of oil	Conc. Of span – 20	Conc. Of Chitosan	Conc. Of TPP
B1	25 ml	10%	2.0%	1.0%
B2	25 ml	7.5%	2.0%	1.0%
B3	25 ml	5 %	2.0%	1.0%
B4	25 ml	2.%	2.0 %	1.0%
B5	25 ml	1%	2.0%	1.0%
B6	25 ml	5%	1.5%	1.0%
B7	25 ml	5%	1.0%	1.0%
B8	25 ml	5%	1.0%	0.75%
B9	25 ml	5%	1.0%	0.5%
B10	25 ml	5%	1.0%	0.5%
B11	25 ml	5%	1.0%	0.25%

Table 2. Tissue Distribution of Doxorubicin after Intravenous Injection In Solution

Parameters	Doxorubicin solution	ENP
K_{el}, (elimination rate constant) h^{-1}	1.1163	0.5163
$T_{1/2,}$	0.620	1.34
Cl,clearance, ml/min	0.3802	0.1671
MRT h	2.3614	3.747
AUC^{0-8}	3.1981	6.519
$AUC^{0-\infty}$	3.2876	7.48

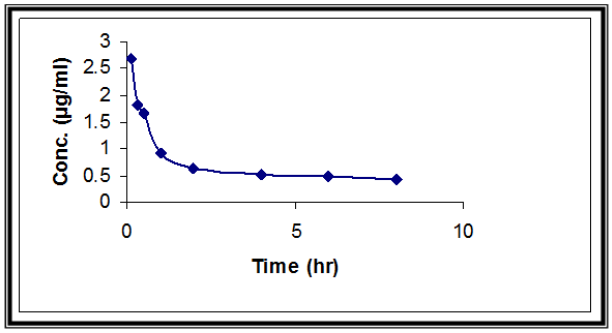

Figure 2: Blood Level of Doxorubicin Hydrochloride Loaded Chitosan Nanoparticles

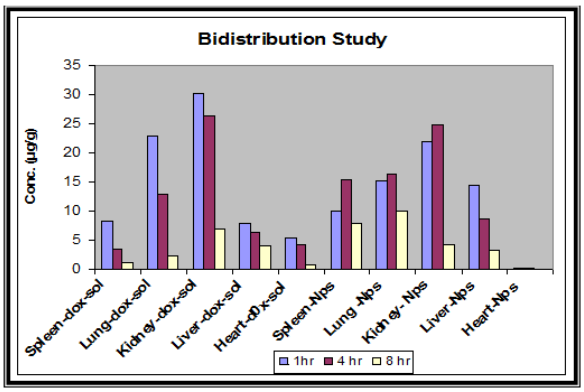

Figure 3: Biodistribution Study of Drug Solution and Nanoparticles in Spleen, Lung, Kidney, Liver and Heart Up To 8 Hrs

Conclusion

The experimental results revealed that the biodistribution of Doxorubicin is greatly altered when delivered through chitosan Nanoparticles. The Nanoparticles significantly enhanced the circulation half-life of Doxorubicin in blood.

A very important aspect is the significant reduction in distribution of Doxorubicin to heart by Nanoparticles prepared by W/O emulsion method, indicating their potential in reducing the cardiotoxicity associated with Doxorubicin therapy and also shows significant reduction in distribution of Doxorubicin. to kidney by nanoparticles prepared by W/O emulsion method compare to plain drug solution indicating their potential in reducing the Nephrotoxicity associated with Doxorubicin therapy.

REFERENCES

i Kreuter J. Nanoparticles. In: Kreuter J editor 1994. Colloidal drug delivery system, Marcel Dekker New York., 219-342.

ii Illum L, Davis SS. 1984. The organ uptake of intravenously administered colloidal particles can be altered using a non ionic surfactant (Poloxamer 338) FEBS Lett., 167: 79-82

iii Lue D, Manthey B, Kreuter J, Speiser P, Deluca PP. 1984. Distribution and elimination of coated polymethyl [2-14C) methacrylate nanoparticles after intravenous injection in rats. J Pharm Sci 73., 1433–1437.

iv Park JH, Saravanakumar G, Kim K, Kwon IC. 2010. Targeted delivery of low molecular drugs using chitosan and its derivatives. Adv. Drug Deliv. Rev., 62 (1): 28-41.

v Bisht S, Maitra A. 2009. Dextran-doxorubicin/chitosan nanoparticles for solid tumor therapy. Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol., 1 (4): 415-425.

vi Gulyaev AE, Gelperina SE, Skidan IN, Antropov AS, Kivman, GY, Kreuter J. 1999. significant transport of doxorubicin into the brain with polysorbate 80 coated nanoparticles,. Pharm. Res.,16 :1564-1569

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