Chapter - 5

SUMMARY AND CONCLUSIONS

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5.1 SUMMARY

The present day research in the field of cancer chemotherapy aims at selective toxicity of the anticancer agents to the tumour cells by means of site specific delivery of the carrier system. This is necessary because conventional cancer chemotherapeutic agents are highly potent, toxic, non-selective and are harmful to both the tumour as well as the host cells. Site specific drug delivery or drug targeting is achieved by associating the drug selectively to the desired site of action with little or no interaction with the non-target tissues.

Of the various approaches used to achieve drug targeting, Natural or Passive targeting involves exploiting physiological processes to provide selective delivery of the drug. For example, delivery of drugs to the organs of the reticuloendothelial system (RES) can be achieved by injecting the drug after incorporating it in particulate carriers. These particulate carriers can be easily directed either to the lungs or to the liver by controlling their particle size. Thus, intravenously administered particles greater than 7 μm in size are mechanically filtered in the capillary beds of the lungs whereas smaller particles are taken up by the macrophages of the liver.

This concept of natural targeting has been utilized in the present study in an attempt to achieve site specific delivery of the widely used anti-cancer agent, 5-fluorouracil (5-FU). This drug is effective in the treatment of neoplasms of the intestine, liver, breast, pancreas, prostate etc. but has the drawback of being non-selective and toxic.

Three different polymers of different characteristics were chosen as carriers for 5-fluorouracil. They include the slowly biodegradable **polyamides**, the non-biodegradable **polyacrylamide** and the rapidly biodegradable **polyalkylcyanoacrylate.** Microcapsules of these polymers were prepared by interfacial polycondensation and emulsion polymerisation and all variable parameters in the formulation procedures were optimized in each case. Thereafter, they were evaluated for particle size distribution, drug entrapment efficiency, in vitro drug leaching and in vitro drug release profiles. Infrared spectra of the microcapsules were used to characterize them.

In vivo organ distribution of 5-FU in the liver, lungs, kidneys, intestine and spleen from intravenously administered microcapsules was studied in healthy rats upto various time periods.

5-FU was estimated from various organs by an extraction method which was first tested for its applicability in the laboratory. According to this procedure, the homogenates obtained after centrifuging the homogenized organs were extracted with a solvent

system comprising of ethyl acetate : propanol (7:3) and the absorbance of the organic layer was read at 266 nm on a Hitachi-2000 Spectrophotometer against an appropriate blank.

5.1.1 Polyamide Microcapsules

Polyterephthalamide microcapsules were prepared by interfacial polycondensation reaction between a diacid, terephthaloyl chloride and various diamino acids viz. L-Arginine, L-Asparagine, L-Cystine, L-Citrulline, L-Glutamine, L-Lysine and L-Ornithine. Cross-linked haemoglobin microcapsules were similarly prepared by reacting haemoglobin with terephthaloyl chloride. Spherical microcapsules with a distinct boundary in the size range of 1 to 15 μm and having drug entrapment efficiency from 40 to 80%, were obtained. The infrared spectra of these microcapsules showed characteristic peaks of polyamides. The particle size, drug entrapment efficiency, in vitro drug leaching and in vitro drug release rates were found to be a function of the oil/water partition coefficient ($K_{o/w}$) of the amino acid moiety of the polymer. Thus, the microcapsules prepared from amino acids with high $K_{o/w}$ values had larger size and greater rates of drug release as compared to those prepared from readily water soluble amino acids having low $K_{o/w}$ values.

The results of the in vivo organ distribution studies showed a preferential distribution of 5-FU in the lungs followed by the liver and kidneys from all batches of polyterephthalamide microcapsules.

This was attributed to the size dependent distribution of intravenously administered particles. The polyterephthalamide microcapsules had a size range of 2 to 20 μm, of which the larger particles would have been mechanically filtered in the lungs, leading to high levels of drug therein. Peak drug levels were observed within 4 hrs. from almost all batches of these microcapsules. However, the extent of the total amount of the drug distributed from the microcapsules was different in each case and was again found to be a function of the partition coefficient ($K_{o/w}$) of the amino acids. Thus, maximum drug recovery was obtained from the Cy-Tc batch of microcapsules prepared from cystine which had the highest $K_{o/w}$ whereas minimum values were obtained from the Gl-Tc and Ci-Tc batches of microcapsules prepared from glutamine and citrulline respectively, which had lowest $K_{o/w}$ values.

Application of ANOVA and Dunnett's test revealed that there was a significant modification in the biodistribution pattern of 5-FU when administered in microencapsulated form. The total amount of drug accumulated per gram of organ (μg/gm) was significantly higher from almost all batches of polyamide microcapsules as compared to that from free 5-FU. Cy-Tc, As-Tc and Ly-Tc batches of microcapsules showed most significant increase in overall drug accumulation in the five organs studied ($P < 0.05$). Except for Ci-Tc batch, all other batches of polyterephthalamide microcapsules produced significantly higher levels in the lungs ($P < 0.05$) compared to free 5-FU solution. Higher drugs levels in the liver were observed from Cy-Tc, Ly-Tc

($P < 0.05$) and As-Tc ($P < 0.01$) batches while only Cy-Tc and Ci-Tc batches showed significantly higher drug distribution ($P < 0.05$) in the intestine and spleen respectively.

CROSS-LINKED HAEMOGLOBIN MICROCAPSULES

A significant feature in the organ distribution pattern of cross-linked haemoglobin microcapsules was a relatively higher drug accumulation in the liver and kidneys as compared to that into the lungs. In sharp contrast to the other polyamide microcapsules studied, administration of cross-linked haemoglobin microcapsules produced appreciable drug levels in the spleen, which on a weight basis (µg drug per gram of organ) were higher than those found in the liver, kidneys and intestine. Another noteworthy feature of the organ distribution pattern of 5-FU from cross-linked haemoglobin microcapsules was the maintenance of appreciable drug levels even upto 6 hours, accounting for almost 30% of the administered dose.

5.1.2 Polyacrylamide Microcapsules

Polyacrylamide microcapsules were prepared using an emulsion polymerisation procedure wherein N,N' methylene bisacrylamide was used to crosslink acrylamide in presence of ammonium peroxodisulphate as catalyst, tetra ethyl methylene diamine (temed) as initiator and Pluronic F 68 as the emulsifier. Spherical microcapsules containing a distinct boundary surrounding the core material

having a mean diameter of 2.65 ± 0.37 μm and drug entrapment efficiency of 60.33 ± 3.68% were obtained. The infrared spectrum of the microcapsules showed characteristic peaks which helped in identifying the polymer.

In vitro drug leaching and in vitro release rates of 5-FU from these microcapsules were very slow, due to the highly cross-linked nature of polyacrylamide.

In vivo organ distribution of 5-FU from intravenously injected polyacrylamide microcapsules was studied upto 48 hours in healthy rats. The results confirmed the slow rate of elimination of polyacrylamide. Peak drug levels were detected 24 hours post injection as opposed to 4 hours from polyamide microcapsules. However, the organ distribution pattern of 5-FU from these microcapsules was similar to the other microcapsules studied, confirming the size dependent biodistribution of intravenously administered particulate carriers. Thus, maximum drug levels were found in the lungs, followed by the liver and kidneys.

Application of Student's t test to the in vivo data generated by these experiments indicates that the total organ distribution of 5-FU on a weight basis (μg of drug per gram organ) was significantly higher ($P < 0.01$) as compared to that achieved by the free drug solution. A significant increase in the drug accumulation was observed in the lungs ($P < 0.001$) and kidneys ($P < 0.01$) from

polyacrylamide microcapsules whereas it was lower in the liver ($P < 0.1$), spleen ($0.001 < P < 0.02$) and intestine ($0.001 < P < 0.01$) as compared to that from free 5-FU.

5.1.3 Polyisobutylcyanoacrylate Microparticles

Spherical, solid polyisobutylcyanoacrylate (PiBCA) microparticles with mean diameter of 6.60 ± 3.24 µm were obtained using the emulsion polymerisation technique. The size distribution of these microparticles was broader as compared to the other types of microcapsules studied, owing to the instantaneous reactivity of the alkylcyanoacrylate monomer in undergoing polymerisation. The drug entrapment efficiency of these microparticles was 33.33 ± 4.52%.

In vivo organ distribution studies of 5-FU from PiBCA microparticles in healthy rats confirmed the rapidly biodegradable nature of polyalkylcyanoacrylates. Thus, peak drug levels in various organs were attained within ½ hour, were maintained upto 1 hour and fell rapidly within 1½ to 2 hours.

As observed in earlier studies with other microcapsules, the PiBCA microparticles also showed maximum drug accumulation in the lungs. But a noteworthy feature of the in vivo data generated after administration of these microcapsules was that appreciable drug levels were detected in the spleen upto 2 hours and on a weight basis (µg drug per gram organ), the drug levels in the spleen were much higher than those in the liver, kidneys and intestine.

Application of Student's t test to the data generated from the pharmacokinetic study of 5-FU from intravenously administered PiBCA microparticles showed that on a weight basis (µg/gm), there was a significantly higher ($P < 0.001$) total distribution of 5-FU from PiBCA microparticles as compared to that from free 5-FU solution. Significantly higher drug levels were observed in the lungs and spleen ($P < 0.001$) after administration of PiBCA microparticles. However, the drug distribution in the kidneys and intestine was significantly lower ($0.02 < P < 0.05$) while that in the liver remained unchanged following administration of 5-FU loaded PiBCA Microcapsules.

5.2 CONCLUSIONS

Three different types of polymeric materials were studied as carriers for achieving site specificity of the widely used anti-cancer agent, 5-fluorouracil. After optimisation of Formulation, conditions in each case, spherical microcapsules in the size range of 2 to 20 µm were obtained. The drug entrapment efficiency of these microcapsules ranged from 33% (PiBCA) to 80% (As-Tc batch of polyterephthalamide series). In the polyterephthalamide series, the various properties like particle size, drug entrapment efficiency, in vitro drug release and in vivo drug release pattern were influenced by the oil/water partition coefficient ($K_{o/w}$) of the amino acid moiety of the polyamide membrane. The low drug entrapment efficiency of the polyisobutylcyanoacrylate microparticles was due to

the fact that only 20 µl of the monomer was used to entrap 25 mg of 5-fluorouracil. Of all the three types of microcapsules prepared, polyacrylamide microcapsules had the lowest particle size, owing to the slow rate of polymerisation, whereas the polyisobutylcyano-acrylate microparticles had the biggest size because of the instantaneous reactivity of the monomer.

The photomicrographs showed that the polyterephthalamide and the polyacrylamide microcapsules were spherical with a distinct boundary surrounding the core material. The polyisobutylcyano-acrylate products were nearly spherical and non-capsular and hence are referred to as microparticles. The infrared spectra of the microcapsules showed characteristic peaks.

In vitro drug release rates were also minimum for polyacrylamide microparticles, probably due to the highly cross-linked nature of the polymer wall enveloping the drug while the permeability of 5-FU from polyamide microcapsules was observed to depend directly on the $K_{o/w}$ of the amino acids. Appreciable rate of drug release was also observed from cross-linked haemoglobin microcapsules.

The results of in vivo organ distribution studies with the various types of microcapsules highlighted the differences in their properties. Thus, the peak drug levels for the rapidly biodegradable polyalkylcyanoacrylates were obtained within ½ to 1 hour, those for the biodegradable polyamides within 2 to 4 hours

while those for the non-biodegradable polyacrylamide within 24 hours after injection.

Irrespective of the type of the polymer, the common feature in all the microcapsules studied was a preferential drug accumulation in the lungs as compared to the other organs. This shows that the biodistribution of intravenously administered particulate carriers is a function of their particle size.

It has been widely reported that after intravenous administration, particles greater than 7 μm are mechanically filtered by the capillary beds of the lungs and remain embedded therein, whereas particles smaller than 7 μm are opsonized by the macrophages of the other organs of the reticuloendothelial system (RES), including the liver, kidneys and spleen.

All types of microcapsules studied had particle size range of 2 to 20 μm, so that upon intravenous administration, the larger particles would have been retained by the lungs and only the smaller particles would have passed through and got distributed to the other organs of the RES. Thus, in most batches of microcapsules, significant drug levels were observed in the lungs, followed by the liver and kidneys ($0.001 < P < 0.05$). However, only a few batches of microcapsules (Ci-Tc, Hb-Tc, PiBCA) produced appreciable levels of 5-FU in the spleen and only Cy-Tc batch of microcapsules produced appreciable drug levels in the intestine.

The results obtained in the present study show the potential of carrier-mediated delivery systems in achieving site-specificity of 5-fluorouracil in the treatment of cancers of specific organs.

At present, although 5-fluorouracil is effective in the treatment of cancers of various organs including those of the liver, kidneys and intestine, its use is restricted due to its non-specificity, non-selectivity and pronounced toxicity. These limitations can be overcome by using carrier-mediated targeted delivery systems. The investigations carried out by us using various polymeric carriers for 5-fluorouracil show that such delivery systems are capable of distributing the drug mainly to the lungs, liver and kidneys.

The preferential organ distribution of the prepared batches of microcapsules is shown below :

Organ	Batches showing preferential distribution
Liver	Cy-Tc, Ly-Tc, As-Tc, Hb-Tc
Kidneys	Cy-Tc, Ly-Tc, Ci-Tc, Hb-Tc, Polyacrylamide
Intestine	Cy-Tc
Spleen	Ci-Tc, PiBCA
Lungs	Cy-Tc, Ly-Tc, As-Tc, Ar-Tc, Gl-Tc, Or-Tc, Hb-Tc, Polyacrylamide, PiBCA

The data is indicative of the possibility of using these target-specific microcapsules for delivering 5-FU in the treatment of cancers of liver, kidneys and intestine.

The preferential delivery of 5-fluorouracil to the lungs by almost all the types of microcapsules studied, opens up new vistas for their use as potential carriers of 5-fluorouracil in the treatment of lung cancer.

However, there is scope for further detailed investigations with respect to stability, clinical trials, toxicity and scale up procedures before such targetted drug delivery systems can prove themselves viable in more effective and safer antineoplastic therapy.