Chapter 7

PHARMACOKINETICS AND BIODISTRIBUTION AFTER INTRA-ARTICULAR INJECTION

7.1 Introduction

No in-vitro testing can ensure optimum performance of the dosage form in the body. Therefore, a reasonable measure of performance of dosage form can be obtained by actual in vivo testing either in animal models or human volunteer. The in-vivo studies in animals provide an idea as to how the product will behave in human beings. The aim of the present work was to enhance the retention of the anti-arthritic drug in the inflamed synovium and thus prolonging its duration of anti-inflammatory effect. Two different approaches have been used to perform the in-vivo study after intra-articular injection. The first approach is the use of radiolabelling of celecoxib and its formulations and performing the pharmacokinetic studies in rabbits and biodistribution studies in rats which is discussed in the present chapter. The second approach is the use of γ-scintigraphy for the assessment of arthritis in rats. The assessment of arthritis in rats was done using ^{99m}Tc-labelled glutathione as a marker for arthritis which is discussed in chapter 8. The biodistribution and pharmacokinetic studies in arthritic rats after intravenous injection of celecoxib and its albumin microspheres as well as solid lipid nanoparticles was also done which is discussed in chapter 9.

In this chapter, the pharmacokinetic and biodistribution studies after the intra-articular injection of celecoxib and its formulations have been discussed.

7.2 Experimental

7.2.1 Materials

Complete Freund's adjuvant was purchased from Bangalore Genei limited.

7.2.2 Apparatus

Rat cages, glass syringe (1 ml capacity) with 26 gauge needle, Varnier calipers.

7.2.3 Selection of animals

Healthy New-Zealand albino rabbits of either sex, weighing about 3.0 kg were chosen for the blood kinetic study of the celecoxib and its microspheres. No diet restrictions were enforced prior to studies. Three rabbits were taken in each group.

Male Sprague-Dawley rats weighing 300-350 gms were chosen for biodistribution studies. No diet restriction was enforced prior to studies. Three rats were chosen for each group.

7.2.4 Adjuvant induced arthritis

Monoarticular arthritis was induced in the left knee joints of the white New-Zealand rabbits and Male Sprague-Dawley rats. In rabbits, arthritis was induced by injecting 0.5 ml of the complete Freund's adjuvant in the left knee joints (Vogel, 1997). In rats, arthritis was induced in left knee joints by injecting 0.1 ml of the Complete Freund's adjuvant through the supra-patella ligament using 27 gauge needle. The development of arthritis was monitored regularly by measuring changes in the knee joint diameter using varnier calipers, the mean of three readings being taken with the joint flexed at 90°C. Four days after the induction of the arthritis, the diameter of the arthritic joint in case of rabbits was 33.5±0.4 mm while that of the control joint was 25.4±0.3mm. In case of rats, four days after the induction of arthritis, the diameter of the arthritic joint was 19.4□0.2 mm while that of the control joint was 12.2□0.3mm.

7.2.5 Blood kinetic studies

The clearance of the ^{99m}Tc-celecoxib and its formulations (CS-chitosan microspheres, AMS-Albumin microspheres, SLN-Solid lipid nanoparticles and GMS-Gelatin microspheres) into the systemic circulation after intra-articular administration was studied in arthritic rabbits weighing 3-3.5 kg. The labelled preparation (500 µL, 500 µCi) was injected intra-articularly into the left knee joints of each rabbit of known

weight. Blood was withdrawn at different time intervals from the marginal ear vein. The radioactivity was measured in a well-type gamma ray counter (Gamma ray spectrometer, Type GRS23C; Electronics Corporation of India Ltd, Mumbai). The blood was weighed and the radioactivity present in the whole blood was calculated by keeping 7.3% of the body weight as the total weight of the blood (Wu et al, 1981).

7.2.6 Extra articular distribution of celecoxib and its formulations

All animal experiments were approved by the Animal Ethics Committee of the institution. Biodistribution of the ^{99m}Tc-labelled celecoxib and its radiolabelled formulations was studied in arthritic Sprague-Dawley rats, 300-350 g. Four days after the induction of the arthritis, 200 μL (200 μCi) of the radiolabelled preparation was injected intra-articularly into the left knee joint of each rat. Groups of three rats per time point were used in the study. The rats were sacrificed at different time intervals and blood was obtained by cardiac puncture. Subsequently tissues (heart, lung, liver, kidney, spleen, intestine, stomach) were removed. The knee joints, both the left and the right were exposed and cut into fragments. All the tissues were washed with normal saline, blotted dry, weighed and their radioactivity was measured in a well-type gamma scintillation counter. To correct for physical decay and to calculate radiopharmaceutical uptake in each organ as a fraction of the injected dose, samples of the injectate, containing 1% of the injected dose, were counted simultaneously at each time point.

7.2.7 Data analysis

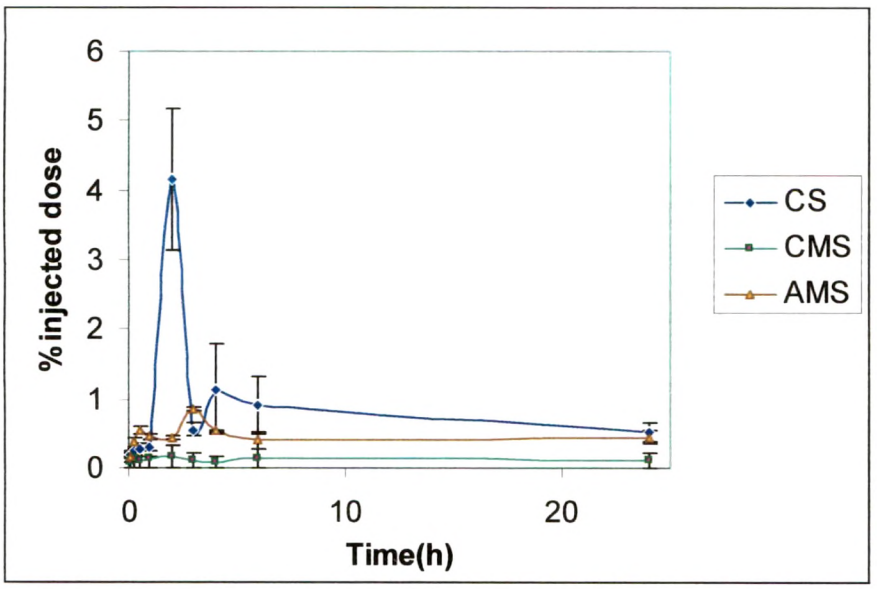
The results are expressed as mean±s.d. and were analysed using a Kruskal-Wallis multiple comparison test followed by post-hoc Dunn's test at the significance level of $P<0.05$ and 0.005.

Table 7.1: Blood kinetic studies of ^{99m}Tc-labelled CS, CMS and AMS after intra-articular injection

Time (h)	% injected dose		
	CS (Celecoxib)	CMS (chitosan microspheres)	AMS (Albumin microspheres)
0.08	0.198±0.04	0.235±0.08	0.152±0.061
0.25	0.241±0.05	0.217±0.14	0.377±0.063
0.5	0.263±0.10	0.226±0.12	0.562±0.054
1	0.289±0.13	0.342±0.13	0.458±0.039
2	4.162±1.02	0.418±0.17	0.443±0.037
3	0.564±0.09	0.36±0.11	0.850±0.026
4	1.14±0.65	0.346±0.08	0.537±0.024
6	0.921±0.41	0.218±0.14	0.406±0.123
24	0.532±0.14	0.172±0.12	0.449±0.099

All the values are expressed as mean±S.D(n=3)

Figure 7.1: Blood kinetics of ^{99m}Tc-labelled CS, CMS and AMS in rabbits after intra-articular injection



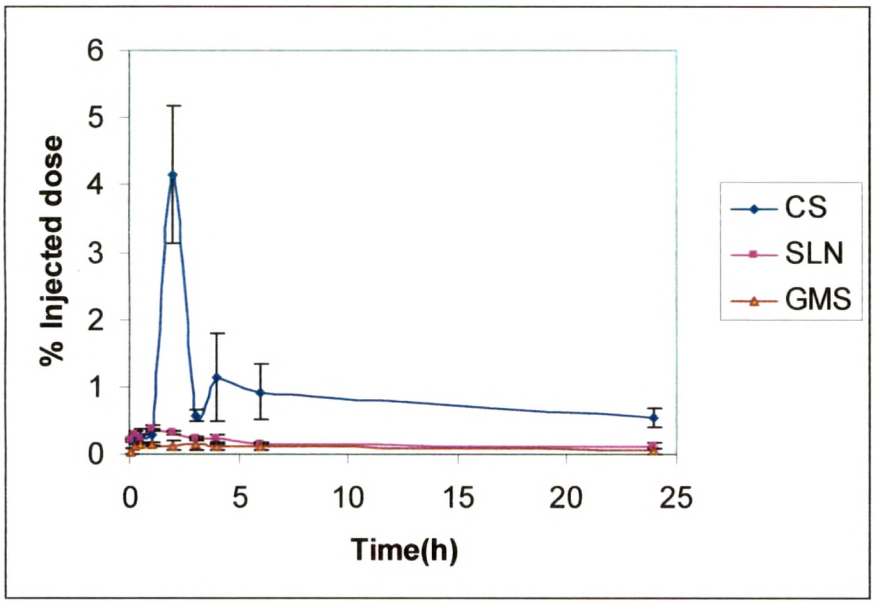
The rabbits were administered intra-articularly with 500µl (500µCi) of the ^{99m}Tc-labelled complexes and the radioactivity in the blood was measured at different time intervals. Each value is a mean of triplicate results. The error bars in the graph represents the standard deviation values.

Table 7.2: Blood kinetic studies of ^{99m}Tc-labelled SLN and GMS after intra-articular Injection

Time (h)	% injected dose	
	SLN (Solid lipid nanoparticles)	GMS (Gelatin microspheres)
0.08	0.205±0.018	0.041±0.040
0.25	0.283±0.050	0.105±0.029
0.5	0.226±0.068	0.153±0.016
1	0.361±0.018	0.142±0.034
2	0.307±0.023	0.127±0.067
3	0.235±0.027	0.143±0.074
4	0.230±0.056	0.102±0.034
6	0.148±0.005	0.119±0.059
24	0.124±0.038	0.048±0.042

All the values are expressed as mean±S.D(n=3)

Figure 7.2: Blood kinetics of ^{99m}Tc-labelled SLN and GMS in rabbits after intra-articular injection



The rabbits were administered intra-articularly with 500µl (500µCi) of the ^{99m}Tc-labelled complexes and the radioactivity in the blood was measured at different time intervals. Each value is a mean of triplicate results. The error bars in the graph represents the standard deviation values.

Table 7.3: Bio-distribution of ^{99m}Tc-labelled celecoxib in Sprague-Dawley rats after intra-articular administration

Organ/tissue	Percent injected dose/whole organ or tissue(± SD)	
	4 h	24 h
Blood	0.294±0.037	1.092±0.069
Heart	0.006±0.002	0.004±0.001
Liver	4.40±0.225	4.58±0.141
Spleen	0.185±0.010	0.145±0.015
Kidney	0.101±0.010	0.076±0.007
Lung	0.192±0.018	0.125±0.017
Intestine	0.023±0.007	0.025±0.012
Stomach	0.207±0.032	0.110±0.026
Non-inflamed joint	0.070±0.008	0.024±0.014
Inflamed joint	0.615±0.030	0.507±0.055

Each value is expressed as mean±S.D (n=3)

Table 7.4: Bio-distribution of ^{99m}Tc-labelled celecoxib in Sprague-Dawley rats after intra-articular administration

Organ/tissue	Percent injected dose/gram of organ or tissue(± SD)	
	4 h	24 h
Blood	0.014±0.008	0.052±0.005
Heart	0.005±0.001	0.002±0.001
Liver	0.440±0.0075	0.418±0.066
Spleen	0.324±0.050	0.255±0.050
Kidney	0.048±0.014	0.030±0.010
Lung	0.130±0.010	0.101±0.027
Intestine	0.002±0.001	0.002±0.001
Stomach	0.026±0.006	0.013±0.006
Non-inflamed joint	0.007±0.002	0.002±0.001
Inflamed joint	0.041±0.0095	0.035±0.004

Each value is expressed as mean±S.D (n=3)

Table 7.5: Bio-distribution of ^{99m}Tc-labelled chitosan microspheres in Sprague-Dawley rats after intra-articular administration

Organ/tissue	Percent injected dose/whole organ or tissue(± SD)	
	4 h	24 h
Blood	0.168±0.011	0.021±0.010
Heart	0.0008±0.001	0.0076±0.002
Liver	0.078±0.031	0.0036±0.002
Spleen	0.0017±0.001	0.0136±0.003
Kidney	0.107±0.030	0.032±0.017
Lung	0.125±0.035	0.134±0.027
Intestine	0.0134±0.002	0.029±0.016
Stomach	0.213±0.017	0.157±0.014
Non-inflamed joint	0.069±0.006	0.0226±0.010
Inflamed joint	9.024±0.644	5.274±0.182

Each value is expressed as mean±S.D (n=3)

Table 7.6: Biodistribution of Tc-labelled chitosan microspheres in Sprague-Dawley rats after intra-articular injection

Organ/tissue	Percent injected dose/gm organ or tissue(± SD)	
	4 h	24 h
Blood	0.008±0.006	0.001±0.0004
Heart	0.001±0.0006	0.007±0.0042
Liver	0.008±0.003	0.0003±0.0001
Spleen	0.002±0.005	0.016±0.0073
Kidney	0.041±0.013	0.013±0.0053
Lung	0.101±0.040	0.158±0.0326
Intestine	0.001±0.0008	0.003±0.0011
Stomach	0.022±0.007	0.015±0.0050
Non-inflamed joint	0.006±0.002	0.002±0.001
Inflamed joint	0.601±0.0743	0.327±0.045

Each value is expressed as mean±S.D(n=3)

Table 7.7: Bio-distribution of ^{99m}Tc-labelled albumin microspheres in Sprague-Dawley rats after intra-articular administration

Organ/tissue	Percent injected dose/whole organ or tissue(± SEM)	
	4 h	24 h
Blood	0.0309±0.0067	0.03±0.0135
Heart	0.0005±0.0004	0.0007±0.0004
Liver	0.0041±0.0039	0.026±0.0084
Spleen	0.0005±0.0004	0.0038±0.0018
Kidney	0.0174±0.0018	0.028±0.0197
Lung	0.0009±0.0006	0.002±0.0009
Intestine	0.0006±0.0004	0.0046±0.0032
Stomach	0.009±0.0067	0.0018±0.0017
Non-inflamed joint	0.0036±0.0026	0.0021±0.0028
Inflamed joint	12.28±1.395	5.52±0.248

Each value is expressed as mean±S.D (n=3)

Table 7.8: Bio-distribution of ^{99m}Tc-labelled albumin microspheres in Sprague-Dawley rats after intra-articular administration

Organ/tissue	Percent injected dose/gram of organ or tissue(± SEM)	
	4 h	24 h
Blood	0.0012±0.0004	0.0014±0.0010
Heart	0.0004±0.0003	0.0007±0.0004
Liver	0.0005±0.0004	0.0002±0.0001
Spleen	0.0006±0.0004	0.0055±0.0016
Kidney	0.0065±0.0028	0.013±0.0122
Lung	0.0004±0.0003	0.0012±0.0005
Intestine	0.00015±0.0001	0.0012±0.0007
Stomach	0.0007±0.0004	0.0006±0.0004
Non-inflamed joint	0.0003±0.0003	0.0002±0.0002
Inflamed joint	0.741±0.1951	0.410±0.2718

Each value is expressed as mean±S.D(n=3)

Table 7.9: Bio-distribution of ^{99m}Tc-labelled solid lipid nanoparticles in Sprague-Dawley rats after intra-articular administration

Organ/tissue	Percent injected dose/whole organ or tissue(± SEM)	
	4 h	24 h
Blood	0.032±0.0226	0.019±0.0080
Heart	0.0015±0.0006	0.0006±0.0003
Liver	0.036±0.0211	0.014±0.0108
Spleen	0.003±0.0020	0.0007±0.0003
Kidney	0.031±0.0145	0.016±0.0078
Lung	0.154±0.0312	0.0009±0.0004
Intestine	0.013±0.0040	0.0004±0.0003
Stomach	0.001±0.0009	0.0003±0.0002
Non-inflamed joint	0.0008±0.0007	0.0038±0.0021
Inflamed joint	10.136±0.776	7.27±0.9513

Each value is expressed as mean±S.D(n=3)

Table 7.10: Bio-distribution of ^{99m}Tc-labelled solid lipid nanoparticles in Sprague-Dawley rats after intra-articular administration

Organ/tissue	Percent injected dose/gram of organ or tissue(± SEM)	
	4 h	24 h
Blood	0.0012±0.0007	0.0008±0.0006
Heart	0.0014±0.0010	0.00061±0.0005
Liver	0.003±0.0014	0.0013±0.0010
Spleen	0.005±0.0017	0.0005±0.0004
Kidney	0.015±0.0114	0.0072±0.0015
Lung	0.086±0.0195	0.0006±0.0004
Intestine	0.0016±0.0010	0.0001±0.0001
Stomach	0.0003±0.0002	0.0005±0.0002
Non-inflamed joint	0.0003±0.0002	0.0003±0.0003
Inflamed joint	0.675±0.0813	0.494±0.0604

Each value is expressed as mean±S.D(n=3)

Table 7.11: Bio-distribution of ^{99m}Tc-labelled gelatin microspheres in Sprague-Dawley rats after intra-articular administration

Organ/tissue	Percent injected dose/whole organ or tissue(± SEM)	
	4 h	24 h
Blood	0.113±0.0270	0.030±0.0263
Heart	0.002±0.0010	0.0012±0.0008
Liver	0.032±0.0252	0.026±0.0093
Spleen	0.0027±0.0012	0.0038±0.0026
Kidney	0.973±0.0795	0.683±0.0745
Lung	0.027±0.0085	0.015±0.0129
Intestine	0.009±0.0053	0.013±0.0104
Stomach	0.009±0.0020	0.0063±0.0010
Non-inflamed joint	0.014±0.0066	0.0061±0.0021
Inflamed joint	16.27±0.4467	11.15±0.527

Each value is expressed as mean±S.D (n=3)

Table 7.12: Bio-distribution of ^{99m}Tc-labelled gelatin microspheres in Sprague-Dawley rats after intra-articular administration

Organ/tissue	Percent injected dose/gram of organ or tissue(± SEM)	
	4 h	24 h
Blood	0.0054±0.0022	0.0014±0.0003
Heart	0.0018±0.0009	0.0013±0.0011
Liver	0.0033±0.0015	0.0032±0.0008
Spleen	0.0027±0.0011	0.0056±0.0017
Kidney	0.352±0.0473	0.306±0.1105
Lung	0.017±0.0032	0.010±0.0062
Intestine	0.0023±0.0012	0.0017±0.0008
Stomach	0.003±0.0009	0.0019±0.0008
Non-inflamed joint	0.0009±0.0006	0.00041±0.0002
Inflamed joint	1.029±0.2541	0.698±0.0766

Each value is expressed as mean±S.D(n=3)

Figure 7.3: Levels of celecoxib and its formulations in different organs 4 hours post intra-articular injection

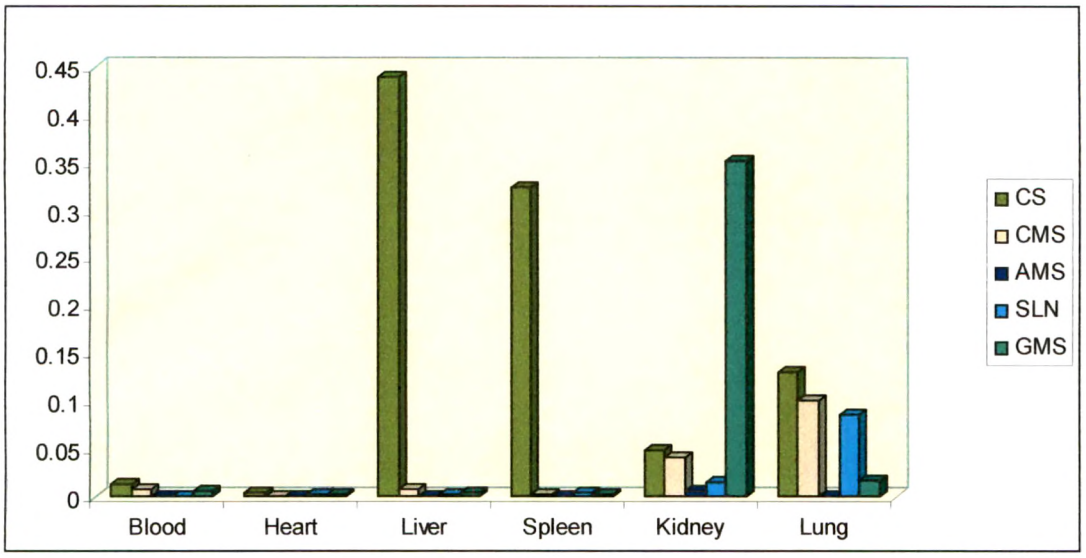


Figure 7.4: Levels of celecoxib and its formulations in inflamed as well as non-inflamed joints 4 hours post intra-articular injection

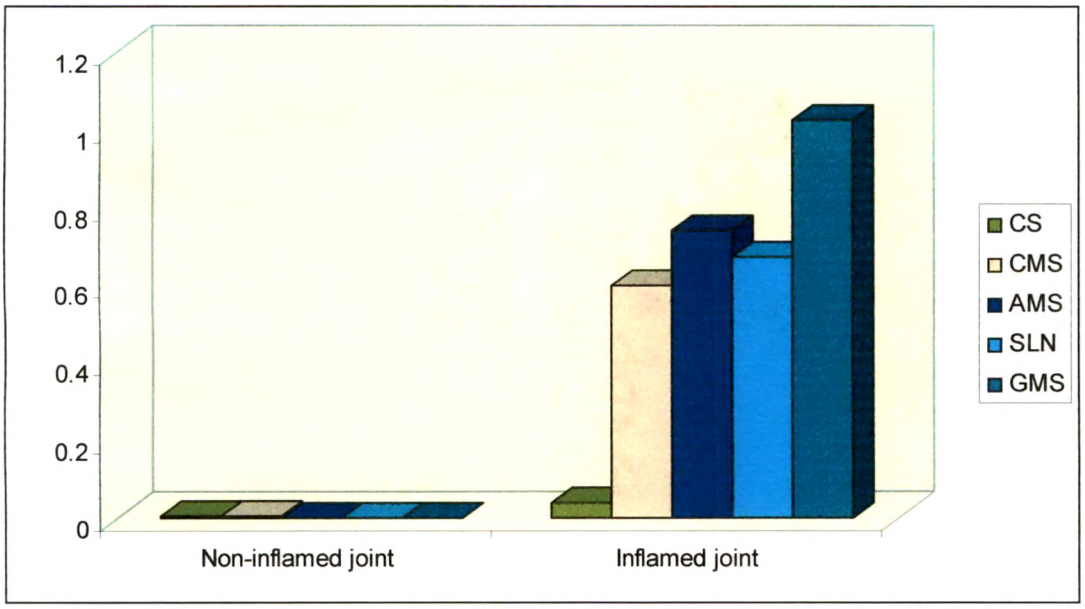


Figure 7.5: Levels of celecoxib and its formulations in different organs 24 hours post intra-articular injection

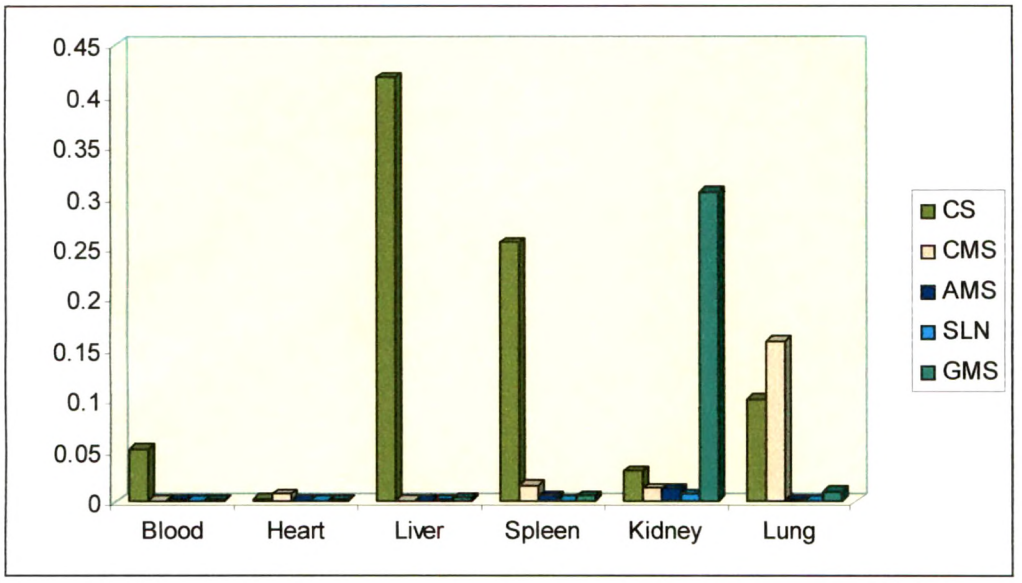
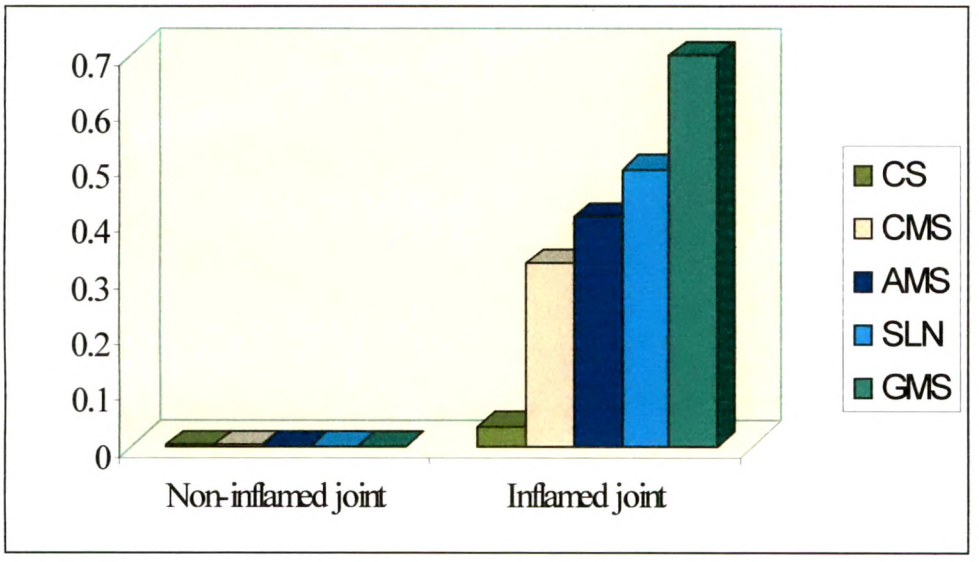


Figure 7.6:Levels of celecoxib and its formulations in inflamed as well as non-inflamed joints 24 hours post intra-articular injection



7.3 Results and discussion

The radioactivity present in the blood at various time intervals, after intra-articular injection of the celecoxib solution as well as the suspension of the formulations containing celecoxib is shown in tables 7.1 and 7.2 and figures 7.1 and 7.2.

Because of the rapid equilibration between the synovial fluid and plasma, clearance of the drug solution from the joint results in the release of appreciable levels of drug into the systemic circulation. Thus, the clearance of the celecoxib solution from the joint was much faster than the celecoxib loaded microspheres. Entrapment of the drug in the microspheres or solid lipid nanoparticles led to a delay in this clearance, as well as minimizing the exposure of the cartilage to significantly higher concentrations of the drug. Thus the peak blood concentration of the drug occurred 2 h after intra-articular injection of the drug solution. Similar results were obtained by previous workers (Bird et al, 1977, Wigginton et al 1980). The peak plasma concentration of the drug was detected 1–2 h after the intra-articular injection of methotrexate solution (Wigginton et al 1980). Two hours after intra-articular injection, the percent radioactivity present in the blood when chitosan microspheres were injected, was almost one-tenth of that of the celecoxib solution. Thus, there is 10-fold increase in the joint concentration of the drug when encapsulated in the microspheres, compared with the drug solution. The Mann–Whitney U-test indicated that a significant difference ($P<0.05$) was observed between the blood concentrations of celecoxib and microspheres, at different time points. In case of the albumin microspheres the radioactivity present in the blood after intra-articular injection is gradually increasing and the peak blood concentration occurs at 3 hours after intra-articular injection. The radioactivity present in the blood 3 hours post intra-articular injection was found to be 0.850% which is almost one-fifth of the peak blood concentration when celecoxib solution is injected. In case of solid lipid nanoparticles the

peak blood concentration was found to be 0.307% which is almost one-thirteenth of that of the celecoxib solution. The gelatin microspheres exhibited the lowest concentrations in the blood the peak blood concentration being only 0.142% which is almost one-thirtieth of the value obtained for celecoxib solution. The Kruskal-Wallis multiple comparison test reveals that there is a significant difference in the peak blood concentration values between the celecoxib solution and its formulations. A significant difference was observed between different formulations with respect to their clearance from joint into the blood. The difference in the peak plasma concentration between the celecoxib solution and gelatin microspheres was found to be highly significant. At 24 hours post intra-articular injection of the free drug, the percent radioactivity present in the blood was 10-15 folds higher than that obtained for the microspheres which clearly demonstrate the enhanced retention of the drug loaded microspheres/solid lipid nanoparticles in the joints.

Thus, from the results obtained, it can be inferred that entrapment of the drug in the microspheres or nano-particles leads to a reduction in its clearance from the joints into the systemic circulation. Thus the systemic side effects associated with the use of celecoxib are expected to reduce.

The bio-distribution of ^{99m}Tc–labelled celecoxib and its formulations (loaded with celecoxib) 4 h and 24 h after intra-articular injection is shown in Tables 7.3 to 7.12. The percent radioactivity is given per gram of the tissue as well as per whole organ or tissue. Blood was obtained by cardiac puncture, weighed and radioactivity present in the whole blood was calculated by keeping 7.3% of the body weight as total blood weight. Various organs or tissues, like lungs, liver, kidney, spleen, stomach and the inflamed, as well as non-inflamed, joints, were isolated and the radioactivity was determined.

In the case of ^{99m}Tc-celecoxib, the radioactivity present in the whole organ/tissue 4 h post injection was found as follows: blood 0.294%, liver 4.4%, spleen 0.185%, lungs 0.192%, kidney 0.101% and inflamed joint 0.615%.

As shown in table 7.5 and 7.6, with ^{99m}Tc-chitosan microspheres, the major amount of the injected activity remained at the arthritic joint, 9% of the injected dose (the weight of the inflamed joint was around 15 g) being recovered 4 h post intra-articular injection and 5.2% of the injected dose being recovered after 24 h. Thus, a 15-fold increase in percent radioactivity in the inflamed joint was observed 4 h ($P<0.005$) post intra-articular injection and an almost 10-fold increase 24 h ($P<0.005$) after the injection. The radioactivity present in the liver was almost 500 times higher for the drug solution, compared with the microspheres ($P<0.005$), while the radioactivity present in the spleen following microsphere administration was almost 160 times higher than when drug solution was injected ($P<0.005$). However, no significant difference in the % radioactivity was observed in lungs following the intra-articular injection of chitosan microspheres and celecoxib solution. This indicates that a major portion of the drug after its clearance from the joint is accumulated in the lungs.

In case of ^{99m}Tc-Albumin microspheres, the % radioactivity present in the inflamed joint 4 hours post intra-articular injection was found to be 12.28% which is almost 20 folds higher than that obtained for the celecoxib solution. The % radioactivity present in the inflamed joint 24 hours post intra-articular injection was found to be 5.52% which is almost 10 folds higher than that of the celecoxib solution. 24 hours post intra-articular injection of the celecoxib solution, the % radioactivity present in the liver is 175 folds higher and in spleen is 40 folds higher than that obtained for albumin microspheres. The % radioactivity in the organs like kidney and lungs is also significantly lower than that obtained for the celecoxib solution.

As shown in Table 7.9, the % radioactivity present in the inflamed joint 4 hours post intra-articular injection of the solid lipid nanoparticles was found to be 10.13% which is almost 16 folds higher than that obtained for the celecoxib solution. 24 hours post intra-articular injection of the celecoxib solution, the % radioactivity present in the inflamed joint was found to be 7.27% which is 15 folds higher than that obtained for celecoxib solution. 24 hours post intra-articular injection of the celecoxib solution, the radioactivity present in the liver is almost 120 folds higher and in spleen, almost 60 folds higher than that obtained for the solid lipid nanoparticles. The % radioactivity present in kidney was also found to be significantly lower than that obtained for the celecoxib solution. However, no significant difference in the % radioactivity in the lungs was observed between the solid lipid nanoparticles and the celecoxib solution. This indicates that after its clearance from the joint, a major portion of the solid lipid nanoparticles accumulate in the lungs.

In case of gelatin microspheres, the % radioactivity present in the inflamed joint 4 hours post intra-articular injection was found to be 16.27% which is almost 27 folds higher than that obtained for the celecoxib solution. The % radioactivity in the inflamed joint 24 hours post intra-articular injection was found to be 11.15% which is 20 folds higher than that of the celecoxib solution. The % radioactivity present in the liver post intra-articular injection of the celecoxib solution was found to be almost 130 folds higher than that obtained for gelatin microspheres and in spleen it was found to be 70 folds higher than that obtained for the gelatin microspheres. The radioactivity present in the lung is also significantly lower than that obtained for the celecoxib solution. However, the radioactivity present in the kidney in case of gelatin microspheres is significantly higher than that obtained for celecoxib solution. This indicates that the microspheres after being cleared from the joint are predominantly found to accumulate in the kidney.

All these results indicate that there is a rapid clearance of the drug solution from the joint and its extra-articular distribution. However, all the formulations are retained in the joint cavity for a longer duration of time and to a larger extent than the drug solution. The radioactivity present in the inflamed joint is significantly higher ($p<0.05$) in case of the formulations than the celecoxib solution. All the formulations shows enhanced retention in the joint compared to celecoxib which is rapidly lost in the systemic circulation. Out of all the formulations, gelatin microspheres were found to have highest retention in the joint cavity after intra-articular injection. This may be because of the larger particles size of the gelatin microspheres (20μm) compared to the other formulations. The higher particle size of the microspheres does not allow the clearance of the particles from the joint into the systemic circulation. The chitosan microspheres and the solid lipid nanoparticles exhibit the distribution to the lungs after the intra-articular injection. However, the majority of the activity remains in the inflamed joint. The gelatin microspheres are distributed to the kidney following intra-articular injection, even though the majority of the activity is retained in the inflamed joint. In case of albumin microspheres, the radioactivity present in all the organs, except the inflamed joint is significantly lower ($p<0.05$) than that obtained for the celecoxib solution.

Thus this study indicates that the highest retention is observed with gelatin microspheres and the lowest extra-articular distribution is observed with albumin microspheres. However, all the formulations exhibited significantly lower ($p<0.05$) extra-articular distribution compared to the celecoxib solution. Incorporation of the drugs in the biodegradable polymers resulted in greater reduction in distribution of drug to the organs such as liver and spleen Hence the potential side effects of celecoxib are expected to be reduced. The enhanced retention of the solid lipid

nanoparticles, chitosan microspheres and albumin microspheres in joints following intra-articular injection is believed to be due to the uptake of the particles by the macrophages of the inflamed synovium. For intra-articular injection, the particle size of the microsphere is also very important. The particle size should be small enough so that upon exposure to the inflamed synovium, the particles are phagocytosed by the macrophages. According to the work done by previous workers, particles with a size range of 0.0025 to 10μm are readily phagocytosed (Benacereff et al, 1975). The particle size of the chitosan microspheres, albumin microspheres and the solid lipid nanoparticles is below 10μm. Hence these particles are susceptible to be taken up by the macrophages of the inflamed synovium. According to the previous reports (Horisawa et al, 2002) the nanoparticles were found to be more suitable for intra-articular administration than the microparticles. But our studies indicated that the particles with a larger particle size are retained to a greater extent in the inflamed synovium than the particles with a lower diameter. The larger particles act as a depot in the inflamed synovium and thus reduce the clearance of the particles from the joint. Due to the higher particle size, the microspheres are not able to escape into the systemic circulation and hence are retained in the joint cavity.

The pharmacokinetic and the bio-distribution studies clearly indicates that celecoxib loaded in the microspheres or nanoparticles exhibits enhanced retention in the joint with a low uptake in other organs signifying a reduction in toxicity of the drug and prolonging its duration of action. The biodistribution of the drug and the formulations for a period longer than 24 hours would give a clear picture as to how long the formulations are retained in the joint cavity but the short half life of ^{99m}Tc does not allow the long term study of the retention behaviour of the microspheres in the joint.

7.4 References

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