Chapter 9

PHARMACOKINETICS AND BIODISTRIBUTION AFTER INTRAVENOUS INJECTION

9.1 Introduction

Colloidal particles introduced into the circulation can concentrate in the inflammatory lesions (Alpar et al, 1989). For intravenous administration, the particle size of the microspheres should not be more than 5μm. The albumin microspheres and the solid lipid nanoparticles being in this size range were used for intravenous administration. The fate of albumin microspheres and that of the solid lipid nanoparticles after the intravenous injection in arthritic rats was studied by radiolabelling and biodistribution studies in rats. The pharmacokinetic studies were performed in rabbits.

9.2 Experimental

9.2.1 Materials

Complete Freund's adjuvant was purchased from Bangalore Genei limited

9.2.2 Apparatus

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

9.2.3 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 formulations. 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.

9.2.4 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. In rats, arthritis was

induced in left knee joints by injecting 0.1 ml of the Complete Freund's adjuvant (Vogel, 1997) 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.

9.2.5 Blood kinetic studies

The clearance of the ^{99m}Tc-celecoxib (CS), ^{99m}Tc-Albumin microspheres (AMS) and ^{99m}Tc-Solid lipid nanoparticles (SLN) into the systemic circulation was studied in arthritic rabbits weighing 3-3.5 Kg. 500µl of the labeled preparation (500µCi) was injected intra-venously through the left marginal ear vein of each rabbit of known weight. Blood was withdrawn at different time intervals from the marginal vein of the right ear. The radioactivity was measured in a well type gamma ray counter (Gamma ray spectrometer, Type GRS23C, Electronics Corporation of India Ltd, Mumbai, India) 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.

9.2.6 Biodistribution studies

All animal experiments conducted were approved by the Institutional Animal Ethics Committee. Biodistribution of the ^{99m}Tc-celecoxib, ^{99m}Tc-Albumin microspheres and ^{99m}Tc-Solid lipid nanoparticles was studied in Arthritic Sprague-Dawley rats weighing 300-350 gms. Four days after the induction of the arthritis, 250 µl (250 µCi) of the radiolabelled preparation was injected intra-venously via the tail vein to each rat. Groups of three rats per group per time point were used in the study. The rats were

sacrificed at 1 hour, 4 hours and 24 hours post intra-venous injections and blood was obtained by cardiac puncture. Subsequently, tissues (heart, lung, liver, kidney, spleen, intestine, stomach, lungs) were dissected out. The knee joints, both the left and the right were exposed and were cut into fragments. All the tissues were washed with normal saline, blotted dry, weighed and their radioactivity was measured in 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, aliquots of the injectate, containing 1% of the injected dose, were counted simultaneously at each time point.

9.2.7 Data analysis

The results are expressed as mean ± S.D and were analyzed 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. The data of the blood kinetic studies was analyzed using the quickcalc software, Plexus supporting systems, Ahmedabad.

Table 9.1: Blood kinetic studies of ^{99m}Tc-labelled Celecoxib (CS)

Time(h)	% radioactivity in blood (±SD)	
	Actual	Calculated
0.08	5.48±0.52	3.42
0.25	3.33±0.35	3.39
0.5	3.31±0.50	3.35
1	3.19±0.47	3.27
2	3.04±0.35	3.11
4	2.96±0.44	2.82
6	2.64±0.27	2.56
24	1.05±0.26	1.06

Table 9.2: Blood kinetic studies of celecoxib loaded albumin microspheres (AMS)

Time (h)	% radioactivity in blood	
	Actual	Calculated
0.08	15.67±1.21	13.24
0.25	13.82±0.39	13.19
0.5	13.65±0.39	13.12
1	12.84±0.40	12.98
2	12.45±0.22	12.70
4	11.92±0.12	12.16
6	11.78±0.86	11.65
24	7.91±0.41	7.89

Table 9.3: Blood kinetic studies of celecoxib loaded solid lipid nanoparticles (SLN)

Time (h)	% radioactivity in blood	
	Actual	Calculated
0.08	13.46±0.77	11.20
0.25	11.30±0.60	11.11
0.5	10.96±0.34	10.97
1	10.07±0.27	10.70
2	10.0±0.31	10.18
4	9.83±0.15	9.22
6	8.62±0.54	8.35
24	3.38±0.79	3.42

Figure 9.1: Blood clearance of celecoxib (CS) and its formulations (AMS and SLN)

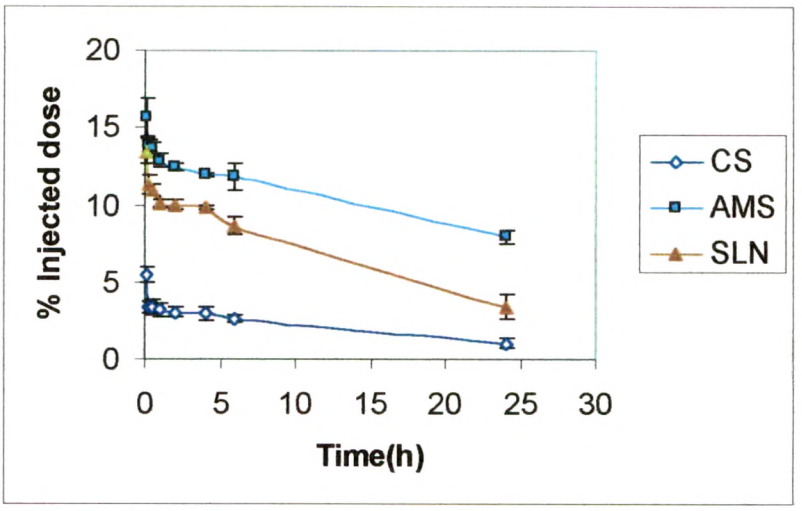


Table 9.4: Pharmacokinetic parameters of celecoxib and its formulations

Batch code	Elimination rate Kel(h^{-1})	Elimination half life(h)	Vd (L)	AUC
CS	0.0489	14.16	0.290	70.255
AMS	0.0216	32.028	0.075	613.20
SLN	0.0495	13.99	0.089	227.20

Table 9.5: Biodistribution of ^{99m}Tc-celecoxib (CS) in Sprague-Dawley rats after intra-venous administration

Organ/Tissue	Percent injected dose/ whole organ or tissue (±) SD		
	1 hour	4 hours	24 hours
Blood	0.861±0.136	0.543±0.060	0.085±0.028
Heart	0.030±0.019	0.133±0.014	0.106±0.088
Liver	33.442±4.002	27.181±3.638	17.11±3.300
Spleen	5.59±0.584	5.58±0.947	5.295±1.494
Kidney	7.104±1.521	3.99±0.900	3.266±0.806
Lung	0.98±0.159	1.33±0.242	1.28±0.139
Intestine	0.361±0.182	0.321±0.089	0.336±0.103
Stomach	0.430±0.024	0.409±0.172	0.331±0.084
Non-Inflamed joint	0.044±0.015	0.033±0.020	0.063±0.018
Inflamed joint	0.071±0.013	0.048±0.030	0.077±0.022

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

Table 9.6: Biodistribution of ^{99m}Tc-celecoxib (CS) in Sprague-Dawley rats after intra-venous administration

Organ/Tissue	Percent injected dose/ whole organ or tissue (±) SD		
	1 hour	4 hours	24 hours
Blood	0.041±0.005	0.024±0.007	0.0036±0.011
Heart	0.021±0.009	0.089±0.013	0.075±0.010
Liver	3.52±0.399	2.78±0.422	1.67±0.369
Spleen	6.29 ±0.265	6.81±0.716	6.23±1.533
Kidney	2.84±0.293	1.58±0.277	1.37±0.244
Lung	0.57±0.061	0.91±0.056	0.97±0.020
Intestine	0.038±0.010	0.034±0.015	0.041±0.012
Stomach	0.051±0.012	0.045±0.008	0.036±0.012
Non-Inflamed joint	0.0036±0.001	0.0027±0.001	0.0055±0.001
Inflamed joint	0.0046±0.001	0.0031±0.001	0.0048±0.001

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

Table 9.7: Biodistribution of ^{99m}Tc-Microspheres (AMS) in Sprague-Dawley rats after intra-venous injection

Organ/Tissue	Percent injected dose/whole organ or tissue (±) SD		
	1 hour	4 hours	24 hours
Blood	3.210±0.905	3.531±0.459	2.433±0.416
Heart	0.054±0.013	0.059±0.028	0.022±0.013
Liver	1.58±0.305	0.980±0.138	0.997±0.177
Spleen	0.138±0.045	0.094±0.028	0.121±0.035
Kidney	1.342±0.580	1.358±0.056	0.580±0.157
Lung	6.207±0.657	3.390±0.051	2.952±0.113
Intestine	2.44±0.412	0.119±0.021	0.151±0.018
Stomach	0.646±0.049	0.174±0.066	0.129±0.013
Non-Inflamed joint	0.073±0.013	0.0691±0.022	0.042±0.018
Inflamed joint	0.255±0.058	0.204±0.161	0.106±0.018

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

Table 9.8: Biodistribution of ^{99m}Tc-Microspheres (AMS) in Sprague-Dawley rats after intra-venous injection

Organ/Tissue	Percent injected dose per gram of organ or tissue (±) SD		
	1 hour	4 hours	24 hours
Blood	0.146±0.013	0.165±0.009	0.101±0.013
Heart	0.049±0.008	0.047±0.011	0.0208±0.008
Liver	0.169±0.016	0.106±0.011	0.115±0.022
Spleen	0.154±0.018	0.108±0.015	0.169±0.027
Kidney	0.562±0.036	0.541±0.033	0.273±0.044
Lung	2.506±0.223	2.29±0.275	1.97±0.265
Intestine	0.298±0.029	0.013±0.003	0.018±0.006
Stomach	0.071±0.018	0.019±0.006	0.015±0.011
Non-Inflamed joint	0.0055±0.001	0.005±0.003	0.0031±0.001
Inflamed joint	0.0162±0.005	0.0134±0.007	0.0075±0.001

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

Table 9.9: Biodistribution of ^{99m}Tc-solid lipid nanoparticles (SLN) in Sprague-Dawley rats after intra-venous injection

Organ/Tissue	Percent injected dose/whole organ or tissue (±) SD		
	1 hour	4 hours	24 hours
Blood	2.394±0.302	1.810±0.164	1.604±0.190
Heart	0.010±0.006	0.009±0.008	0.005±0.001
Liver	3.043±0.464	3.133±0.142	4.162±0.564
Spleen	0.110±0.049	0.092±0.006	0.121±0.018
Kidney	0.184±0.046	0.128±0.021	0.141±0.045
Lung	1.484±0.409	0.712±0.063	0.632±0.029
Intestine	0.034±0.008	0.048±0.014	0.031±0.011
Stomach	0.011±0.004	0.008±0.004	0.007±0.001
Non-Inflamed joint	0.019±0.009	0.003±0.002	0.004±0.002
Inflamed joint	0.010±0.004	0.004±0.001	0.004±0.001

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

Table 9.10: Biodistribution of ^{99m}Tc-Solid lipid nanoparticles (SLN) in Sprague-Dawley rats after intra-venous injection

Organ/Tissue	Percent injected dose per gram of organ or tissue (±) SEM		
	1 hour	4 hours	24 hours
Blood	0.114±0.012	0.086±0.016	0.072±0.011
Heart	0.013±0.003	0.0013±0.001	0.006±0.002
Liver	0.396±0.030	0.408±0.101	0.498±0.065
Spleen	0.167±0.022	0.146±0.038	0.178±0.015
Kidney	0.115±0.011	0.095±0.011	0.101±0.016
Lung	1.237±0.091	0.551±0.012	0.461±0.024
Intestine	0.004±0.001	0.008±0.004	0.004±0.001
Stomach	0.001±0.001	0.001±0.0009	0.0008±0.0004
Non-Inflamed joint	0.002±0.001	0.0003±0.0002	0.0004±0.0002
Inflamed joint	0.001±0.001	0.0004±0.0003	0.0003±0.0002

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

Figure 9.2: Levels of celecoxib and its formulations in different organs at 1 hour post intravenous injection

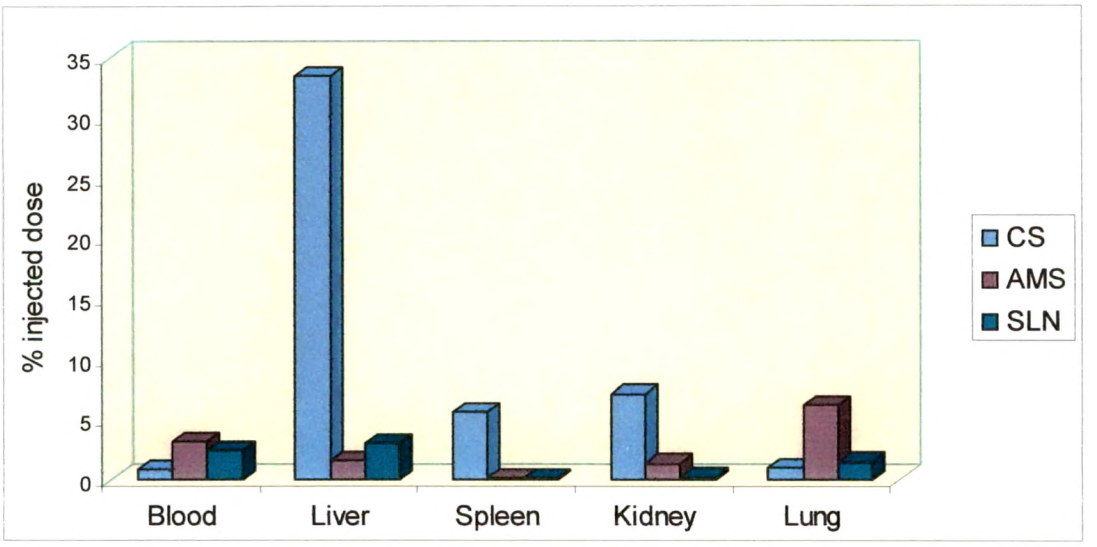


Figure 9.3: Levels of celecoxib and its formulations in inflamed and non-inflamed joint 1 hour post intra-venous injection

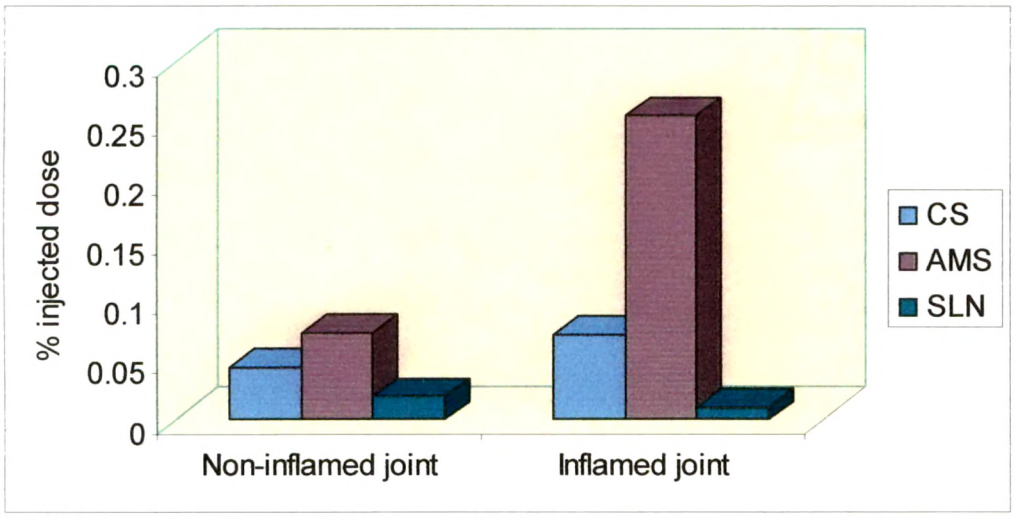


Figure 9.4: Levels of celecoxib and its formulations in different organs 4 hours post intra-venous injection

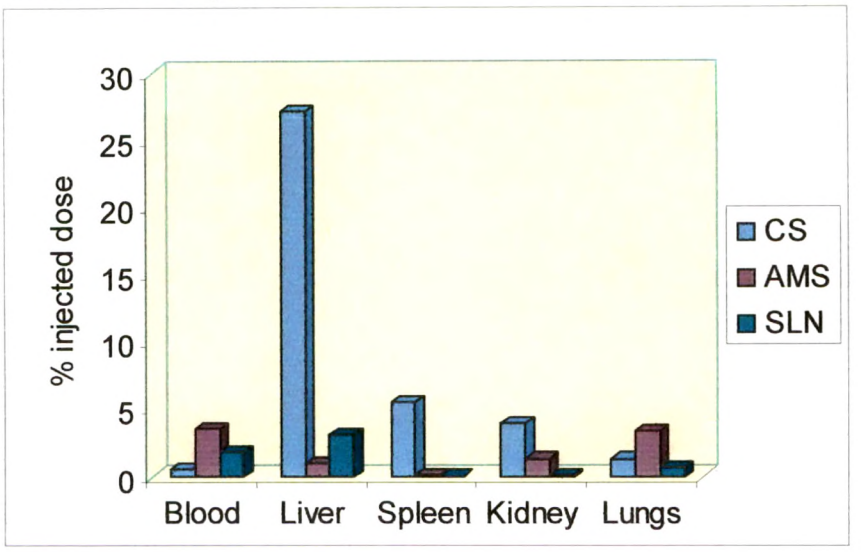


Figure 9.5: Levels of celecoxib and its formulations in inflamed and non-inflamed joint 4 hours post intra-venous injection

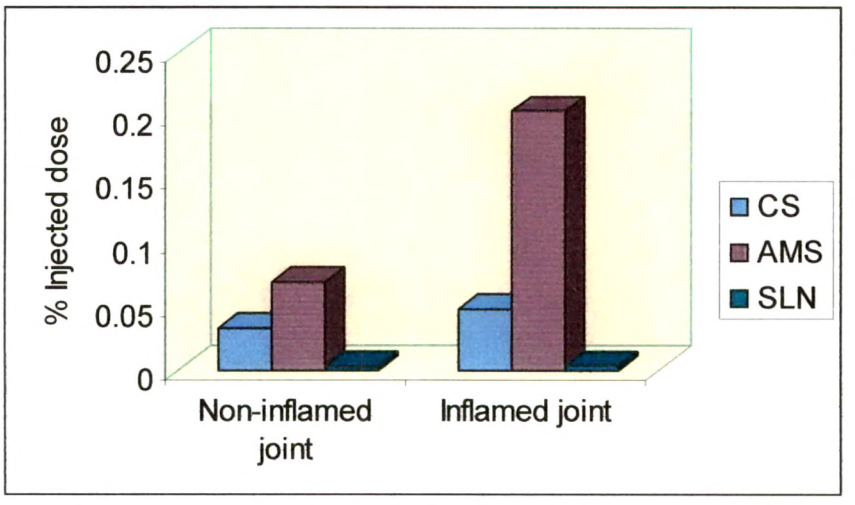


Figure 9.6: Levels of celecoxib and its formulations in different organs 24 hours post Intravenous injection

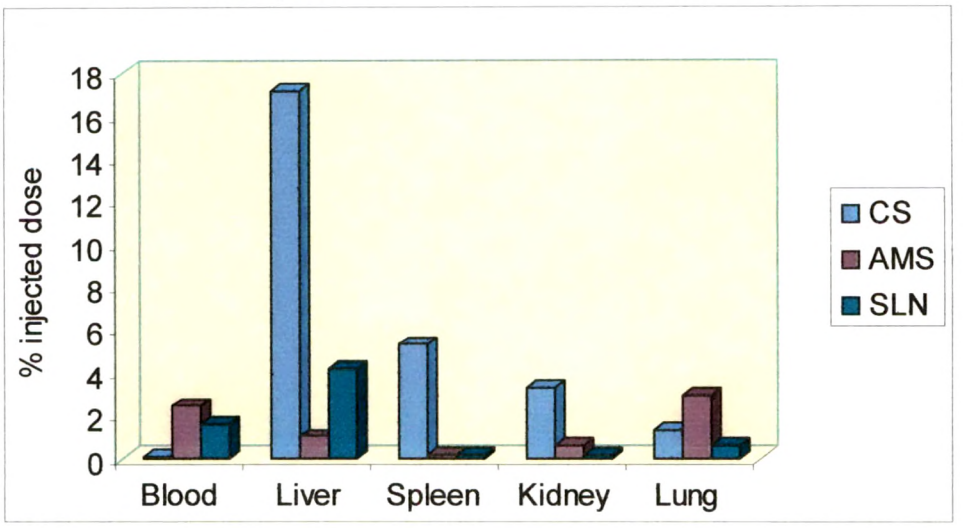


Figure 9.7: Levels of celecoxib and its formulations in inflamed and non-inflamed joint 24 hours post intravenous injection

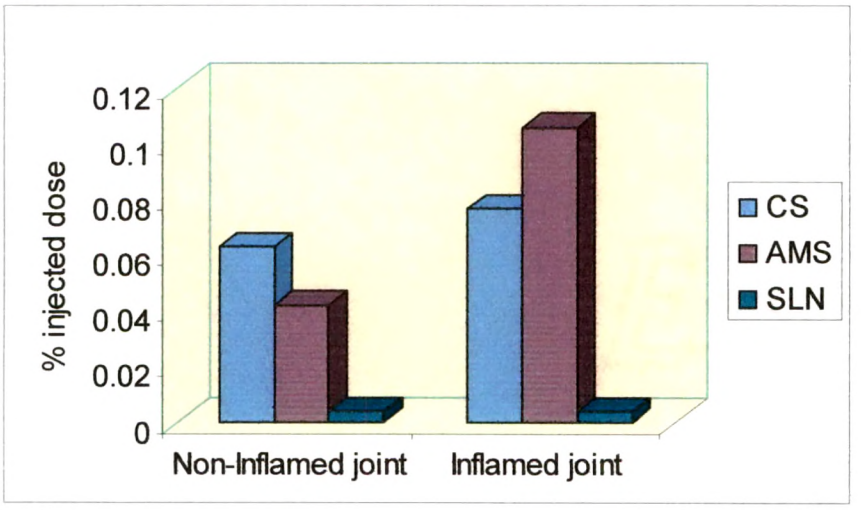
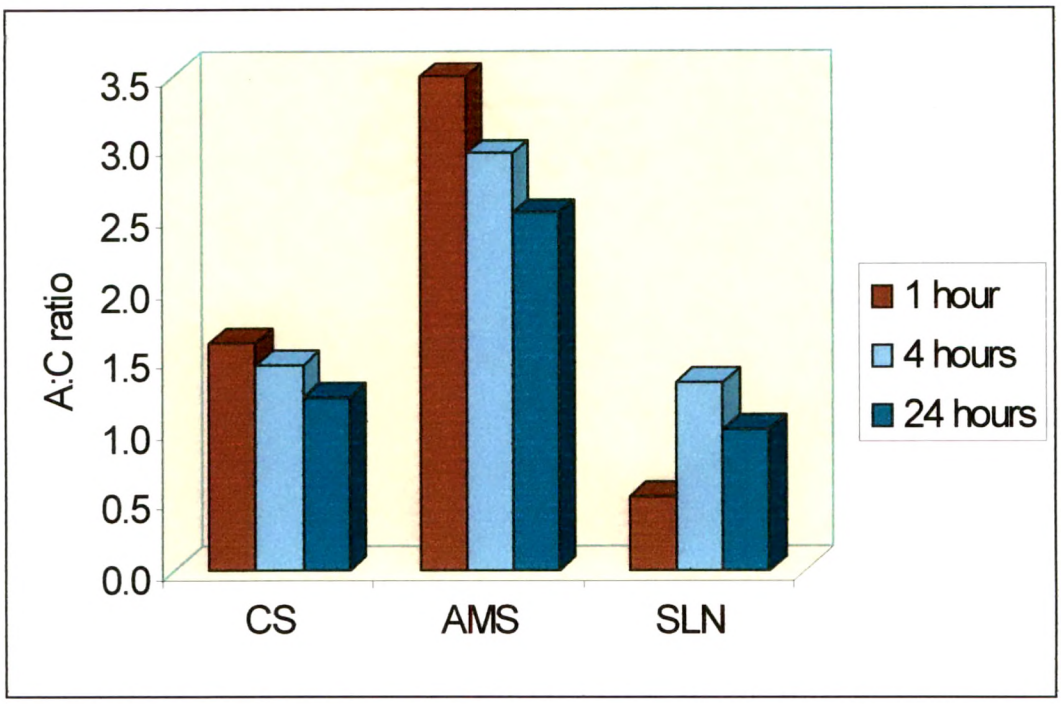


Figure 9.8: A:C (Inflamed:Non-inflamed joint) ratios of CS,AMS and SLN 1hour, 4 hours and 24 hours post intravenous injection



9.3 Results and Discussion

The pharmacokinetic studies of the celecoxib and its formulations (Solid lipid nanoparticles and Albumin microspheres) were carried out in rabbits. Rabbits were administered with the radiolabelled celecoxib and the formulations intra-venously and the radioactivity in the blood was determined at various time intervals. The radioactivity present in the blood at various time intervals, after intra-venous injection of Celecoxib (CS) is shown in table 9.1. As shown in figure 9.1, the drug is very rapidly cleared from the blood, only 5% radioactivity being detected 5 minutes post intra-venous injection, while only about 1% of the injected dose is detected 24 hour post intra-venous injection. On the other hand, in case of albumin microspheres, 15% of the injected dose is detected at 5 minutes post-injection and around 8% of the injected dose is detected 24 hours post injection, indicating an 8 fold increase in the concentration of celecoxib in blood when entrapped in albumin microspheres. In case of solid lipid nanoparticles, 13% of the

injected dose is present in the blood 5 minutes post intravenous injection which is 2.5 folds higher than that of the ^{99m}Tc-CS. Around 3% of the injected dose is detected 24 hours post intra-venous injection which is around 3 folds higher than that of ^{99m}Tc-CS. As shown in figure 9.1, at all time points, the % injected dose in the blood is significantly higher ($p<0.05$) for ^{99m}Tc-AMS and ^{99m}Tc-SLN as compared to ^{99m}Tc-CS. Table 9.4 depicts the pharmacokinetic parameters of celecoxib and its formulations.

The circulation half lives of the drugs have been reported to dramatically increase when the drug is conjugated with albumin (Breton et al, 1995). Increasing the circulation half life of the formulation by reducing its uptake by the reticuloendothelial system has been shown to improve the targeting efficiency of the formulation to the arthritic paws (Srinath et al, 2000). Solid lipid nanoparticles also exhibits prolonged circulation in the blood as reported by the previous workers (Zara et al, 2002). This property of the solid lipid nanoparticles is used for targeting the drug to the tumors. Since the synovium of the rheumatoid arthritic joint shares various features observed in the tumors like the leaky endothelium, long circulating formulations are expected to target the drug to the inflamed synovium. Thus, we tried to develop the formulations of celecoxib with prolonged circulation half life. The results obtained after the blood clearance studies indicated that the albumin microspheres and solid lipid nanoparticles exhibits prolonged circulation in blood compared to celecoxib and thus are expected to target the drug to the inflamed joint of the rat. The volume of distribution value for celecoxib is significantly higher than that of its albumin microspheres as well as solid lipid nanoparticles indicating an extensive distribution of celecoxib. The elimination half life of celecoxib is significantly lower ($p<0.05$) than that of albumin microspheres indicating a prolonged circulation of albumin microspheres.

Biodistribution

The bio-distribution of ^{99m}Tc-CS after 1 hour, 4hours and 24 hours is shown in Table 9.5 and 9.6. Various organs/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-CS, the radioactivities present in the whole organ/tissue at 1 hour post injection were found as follows: blood (0.861%), liver (33.44%), spleen (5.59%), lungs (0.98%), kidney (7.1%). Thus, celecoxib is extensively distributed in the organs like liver, kidney and spleen. High concentration of celecoxib is present in these organs even 24 hours post intra-venous injection.

In case of ^{99m}Tc-AMS, major amount of the injected activity accumulated in lungs, 6.2% being present per whole organ, 1 hour post injection. The radioactivities present in the whole organ/tissue at 1 hour post injection of ^{99m}Tc-AMS were found as follows: blood (3.21%), liver (1.58%), spleen (0.138%) and kidney (1.34%). The radioactivity present in these organs post intravenous injection of ^{99m}Tc-CS was significantly higher ($p<0.05$) than that after the injection ^{99m}Tc-AMS. The radioactivity present in the blood 1 hour post intra-venous injection of ^{99m}Tc-AMS is four folds higher than that of ^{99m}Tc-CS, which is in concurrence with the blood kinetic studies. In case of ^{99m}Tc-CS, a negligible activity was detected in the inflamed joint and there was no significant difference ($p>0.1$) in the radioactivity in the inflamed or non-inflamed joint. While in case of ^{99m}Tc-AMS, the radioactivity present in the inflamed joint was 2.5 folds higher than the non-inflamed joint. Around 0.25% of the injected dose was able to reach the inflamed tissue in case of ^{99m}Tc-AMS, which was significantly higher ($p<0.05$) than the ^{99m}Tc-CS. The radioactivity present in the inflamed joint in case of ^{99m}Tc-AMS group was significantly higher ($p<0.05$) than in ^{99m}Tc-CS group. Thus, it can be inferred that celecoxib loaded albumin microspheres are able to reach the inflamed tissue of the arthritic joint. The

endothelium at the site of inflammation is more permeable and leaky leading to preferential accumulation of the long circulating microspheres in these regions. Albumin microspheres have a prolonged presence in the circulation and consequently have ample time to escape from the circulation to the inflamed region through the leaky endothelium. Albumin is not highly immunogenic (Lee et al, 1981) which probably accounts for the fact that the particles were able to remain in the circulation long enough to enter into the inflamed tissues of the joint. Intra-venous administration of the drugs coupled with albumin has been reported to improve the targeting efficiency of the drug to arthritic regions (Wunder et al, 2003). The higher particle size of the microspheres does not allow the redistribution of the microspheres to the other organs. Thus, there is no significant difference ($p<0.05$) between the radioactivity present in the inflamed joint 4 hours and 24 hours post injection.

A major amount of the injected radioactivity was present in blood and lungs. As reported by earlier workers, a particle of diameter 3-12μm becomes entrapped within the capillary networks of the lungs, liver and spleen (Tomilson and Burger, 1987). The particle size of the microspheres was around 5μm. Thus, a major amount accumulated in the lungs. Celecoxib is also reported to have a beneficial effect on lung carcinoma (Komaki et al, 2004, Cuzzocrea et al, 2002, Cerchietti et al, 2004, Diperna et al, 2003, Peluffo et al, 2004). The high concentrations of the celecoxib loaded microspheres detected in the lungs for prolonged period of time after intra-venous injection indicate it's potential use as targeted drug delivery system for lung carcinoma.

Negligible amount of free label (^{99m}Tc) is observed over the course of the experiment, which is evidenced by less activity in stomach. The free ^{99m}Tc tends to accumulate in the stomach. The negligible amount of radioactivity present in the stomach indicates the stability of the radiolabelled complexes in-vivo.

The bio-distribution of ^{99m}Tc-SLN after 1 hour, 4hours and 24 hours is shown in Table 9.9 and 9.10. Various organs/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-SLN, the radioactivities present in the whole organ/tissue at 1 hour post injection were found as follows: blood (2.394%), liver (3.043%), spleen (0.110%), lungs (1.484%), kidney (0.184%). Thus, the radioactivities present in the organs like liver, spleen and kidney post intravenous injection of ^{99m}Tc-SLN is significantly less($p<0.005$) as compared to ^{99m}Tc-CS. Thus the distribution of celecoxib in these organs can be dramatically reduced by entrapping celecoxib in solid lipid nanoparticles. However, a negligible amount of radioactivity was detected in the inflamed joint and there was no significant difference in the radioactivity present in the inflamed as well as non-inflamed joint indicating that solid lipid nanoparticles are not able to reach the inflamed tissue of the arthritic joint. The fact that the amount accumulated in organs like liver, spleen and kidney is significantly lower($p<0.005$) than that of the celecoxib solution indicates that the solid lipid nanoparticles have the potential to reduce the hepatotoxic and nephrotoxic side effects associated with the use of celecoxib. In spite of having a prolonged circulation in blood, the solid lipid nanoparticles failed to get accumulated in the inflamed synovium, probably due to the smaller particle size. Small particles may manage to escape easily from the inflamed synovium and redistribute in other organs. Thus, it may be concluded that solid lipid nanoparticles have the potential of reducing the side effects of celecoxib but may need some other strategies to target them to the arthritic joints. The distribution of celecoxib loaded solid lipid nanoparticles in the organs like liver and spleen can further be reduced by preparing stealth solid lipid nanoparticles.

9.4 References

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