CHAPTER 9

In Vivo Studies

9.1 INTRODUCTION

To ensure the optimum performance and to obtain a reasonable measure of performance of the dosage form in the body, in vivo testing was done using animal models. The study provides an idea of behavior of the formulation in the human beings. The aim of the present work was to design a stable localized controlled delivery system with reduced dose and improved retention time to reduce the toxicity and resistance against various periodontal infectious diseases. However, specificity towards the infection site is required to reduce any adverse effects of the antibiotics to normal cells. In this chapter the pharmacokinetic and gamma scintigraphy study of plain minocycline hydrochloride/ clindamycin phosphate and their periodontal formulations were discussed.

9.2 EXPERIMENTAL

9.2.1 Selection of animals

Healthy New Zealand albino rabbits, of either sex, weighing about 2.5-3.0 kg were chosen for blood kinetic study of minocycline hydrochloride/ clindamycin phosphate and their periodontal formulations. No diet restriction was enforced prior to studies. Three rabbits were taken for the study in each group.

Sprague Dolly rats of either sex, of 2-3 months old were chosen for gamma scintigraphy study of minocycline hydrochloride/ clindamycin phosphate and their periodontal formulations. Turpentine oil was used to create periodontitis in the dental cavity of S.D. rats. No diet restriction was enforced prior to studies. Three rats were taken for the study in each group.

9.2.2 Implantation of periodontitis

In order to evaluate the activity of radio isotope labeled minocycline hydrochloride/ clindamycin phosphate and their periodontal formulations, an abscess model was developed artificially (Paola et.al, 2004) in Sprague Dolly rats of either sex (2-3 months old, weighing about 200-300gm).

The rats were divided into separate groups each consisting of three rats. To induce periodontitis artificially, a piece of 2/0 braided silk sterile thread was placed around the cervix of lower molar teeth of each rat (Gyorfi et. al, 1994) and then they were allowed to eat commercial laboratory food and drink tap water ad libitum. The formulations were injected locally into the inflamed periodontal cavities regularly for 8 days. The improvement in the healing property i.e. the decrease in the diameter of inflammation was recorded by gamma scintigraphy imaging on zero day (control), 1 day, 4 day and 8 day. The results are expressed as mean ± S.D. and were analyzed using kruskal- Wallis multiple comparison test.

9.2.3 Blood kinetic studies

Healthy New Zealand albino rabbits, of either sex, were administered with the radio labeled preparations of minocycline hydrochloride/ clindamycin phosphate and their periodontal formulations (500µl; 500µCi) by locally injecting into the periodontal cavity of each rabbit of known weight. At different time intervals blood samples (0.5 ml) were withdrawn from the marginal dorsal ear vein of the rabbit and the radioactivity was measured in the well type gamma ray counter (Gamma ray spectrometer, Type GRS23C; Electronics corporation of India Ltd., Mumbai) calibrated for ^{99m} Tc Energy. An amount equal to 7% of the body weight was considered to represent amount of whole blood in the body (Wu et. al., 1981) and data were expressed as the percentage of dose administered at each time interval.

9.2.4 Gamma scintigraphic imaging

The gamma scintigraphy study of minocycline hydrochloride/ clindamycin phosphate and their periodontal formulations were carried out using the abscess bearing Sprague-Dawley rats (three in each group) weighing about 250-400gm after administration of ^{99m} Tc labeled complex formulations locally into the periodontium.. No prior diet restrictions were enforced. The rat was fixed on a board and imaging was performed using a Single Photon Emission Computerized Tomography (SPECT., Lc 75-005, Diacam, Siemens, USA) gamma camera. The gamma imaging photograph of radio labeled minocycline

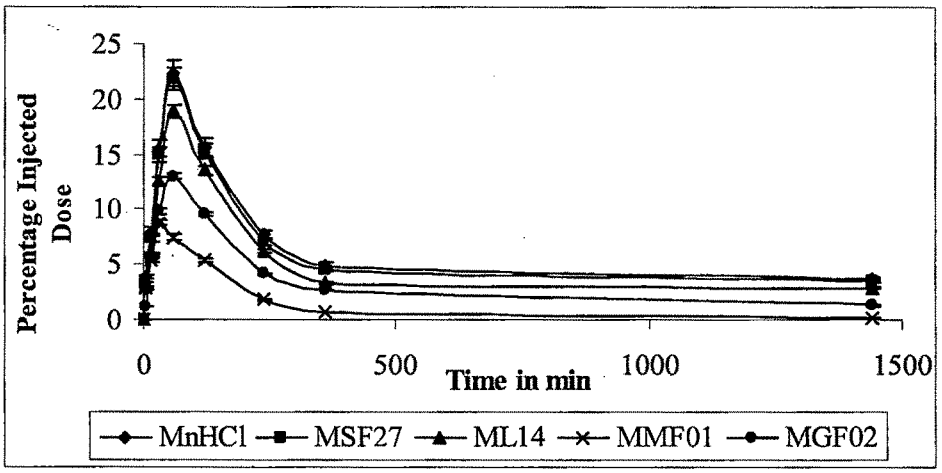
hydrochloride/ clindamycin phosphate and their periodontal formulations showing the extent of healing property is shown in figure no. 9.03.

The results are expressed as mean ± S.D. and were analyzed using kruskal- Wallis multiple comparison test.

Table no. 9.01: Blood kinetic study of ^{99m} Tc labeled minocycline hydrochloride and their periodontal formulations

Time in min	Concentration in mcg/ ml (± S.D.)				
	MnHCl	MSF27	ML14	MMF01	MGF02
5	3.89 ± 0.11	3.16±0.15	2.95± 0.18	2.8±0.21	1.19± 0.29
15	7.93± 0.15	7.42± 0.25	5.86± 0.34	5.41± 0.32	5.68± 0.38
30	15.51± 0.21	14.98± 0.31	12.59± 0.13	8.79± 0.26	9.83± 0.62
60	22.26± 0.23	21.8± 0.26	18.94± 0.28	7.43± 0.19	12.98± 0.24
120	15.63± 0.18	15.21± 0.16	13.54± 0.47	5.41± 0.25	9.54± 0.38
240	7.68± 0.26	7.1± 0.25	6.22± 0.51	1.8± 0.21	4.15± 0.17
360	4.91± 0.11	4.58± 0.37	3.28± 0.18	0.718± 0.11	2.68± 0.11
1440	3.74± 0.17	3.55± 0.21	2.86± 0.32	0.191± 0.54	1.26± 0.16

Figure no. 9.01: Blood kinetic of ^{99m} Tc labeled minocycline hydrochloride and their periodontal formulations in rabbits

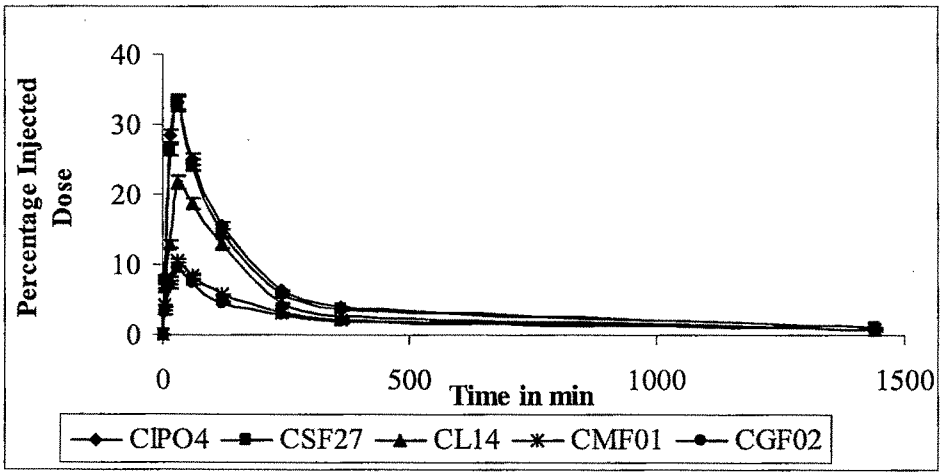


n=3

Table no. 9.02: Blood kinetic study of ^{99m} Tc labeled clindamycin phosphate and their periodontal formulations

Time in min	Concentration in mcg/ml (± S.D.)				
	$ClPO_4$	CSF27	CL14	CMF01	CGF54
5	8.11±0.41	7.67 ± 0.35	6.85 ± 0.25	4.17 ±0.21	3.16±0.29
15	28.31±0.37	26.32 ± 0.38	12.89 ±0.38	7.97 ± 0.38	6.83±0.31
30	33.15±0.32	32.95 ± 0.72	21.71± 0.92	10.48 ± 0.30	9.37±0.19
60	24.90±0.43	24.10 ± 0.37	18.67± 0.34	8.54 ± 0.14	7.48±0.27
120	15.61±0.35	14.50 ± 0.24	13.01± 0.25	5.72 ± 0.79	4.59±0.35
240	6.19±0.51	5.67 ± 0.06	4.39 ± 0.29	3.20 ± 0.36	2.81±0.22
360	3.91±0.29	3.66 ± 0.93	2.52 ± 0.83	2.08 ± 0.49	1.76±0.31
1440	1.06±0.17	0.97 ± 0.15	0.76± 0.42	0.74 ± 0.54	0.68±0.26

Figure no. 9.02: Blood kinetic of ^{99m} Tc labeled clindamycin phosphate and their periodontal formulations in rabbits

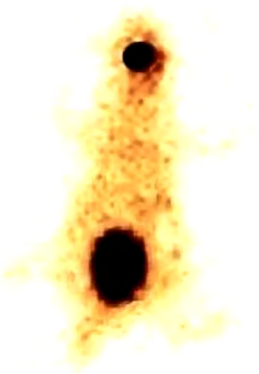


n=3

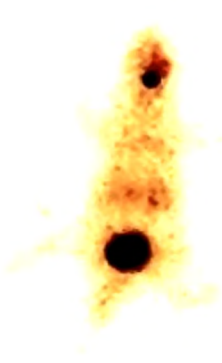
Table no. 9.03: Pharmacokinetic parameters for the in vivo studies of ^{99m}Tc labeled minocycline hydrochloride/ clindamycin phosphate loaded periodontal formulations in rabbits

Formulation	Dose (mcg/ml)	Cmax (mcg/ml)	Tmax (min)	Clearance (ml/min)	Elimination rate (min^{-1})	$T_{1/2}$ (min)	V_d (ml)	$\int_{0-24}$ AUC (min/mcg/ml)	$\int_{0-\infty}$ AUC (min/mcg/ml)
MnHCl	100	22.26	30	0.402	0.001	680.1	394.31	8772.9	12443.2
MSF27	100	21.8	30	0.425	0.001	673.3	412.73	8320.4	11769.8
ML14	100	18.94	30	0.535	0.0011	640.3	494.06	6708.4	9351.5
MMF01	100	8.79	30	2.50	0.0016	429.2	1567.2	1857.6	1975.9
MGF02	100	12.98	30	0.80	0.0008	790.4	955.12	4530.2	5970.7
$ClPO_4$	100	33.15	30	0.6158	0.0013	506.93	449.92	7347.2	8124.71
CSF27	100	32.95	30	0.6562	0.0014	503.66	476.29	6917.6	7624.92
CL14	100	21.71	30	0.8724	0.0013	523.84	658.10	5161.2	5738.97
CMF01	100	10.48	30	1.2655	0.0011	620.73	1129.20	3297.4	3964.60
CGF54	100	9.37	30	1.4458	0.0011	652.94	1352.90	2830.0	3474.58

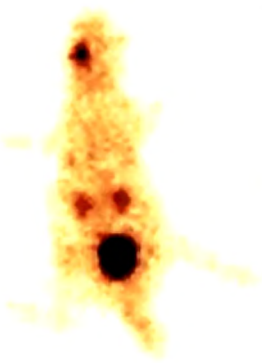
Figure no. 9.03: Gamma scintigraphy image of ^{99m}Tc-minocycline hydrochloride/ clindamycin phosphate injected rat bearing the periodontium in the oral cavity



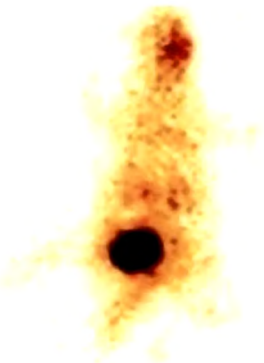
Periodontal Pocket Zero Day



MGF27 (1day)



MGF27 (4 day)



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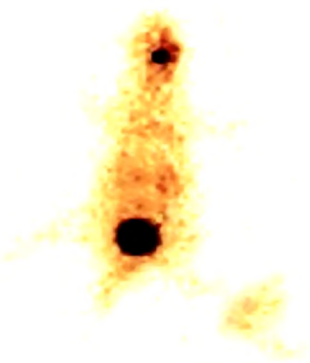

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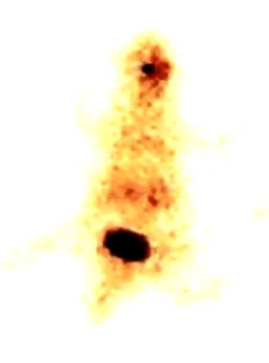

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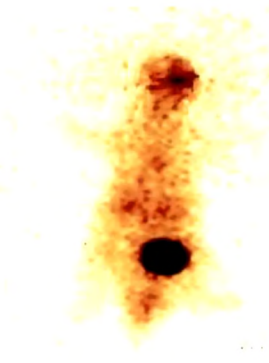

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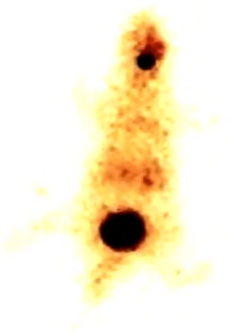
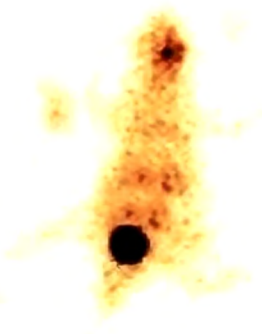


MMF01 (1day)



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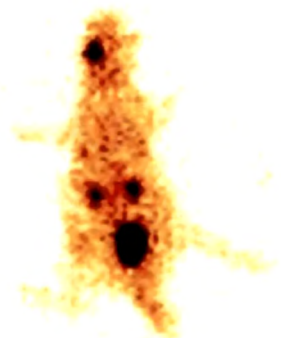
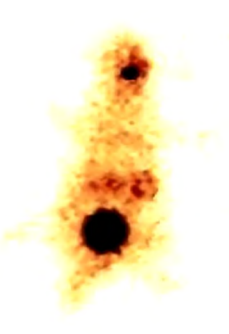


MMF01 (8 day)

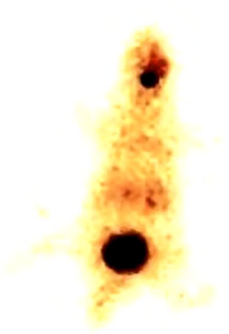
CMF01 (1day)



CMF01 (4 day)

CMF01 (8 day)

ML14 (1day)



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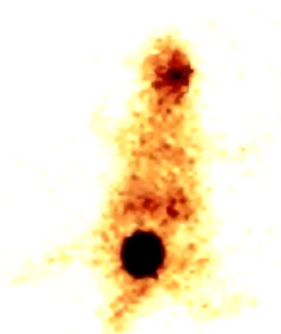
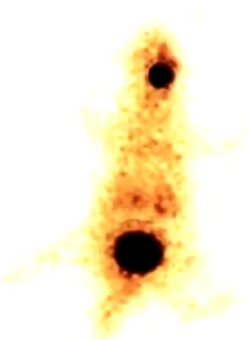
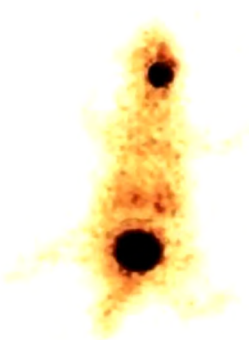


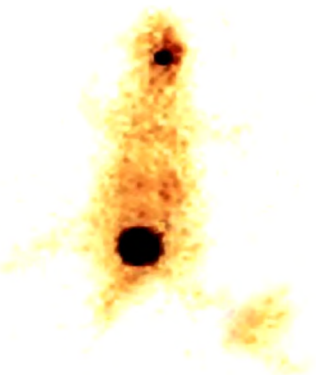
ML14 (8 day)



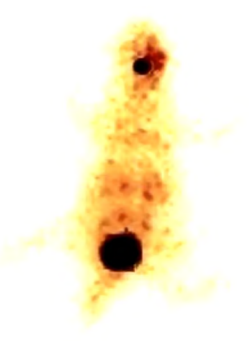
CL14 (1day)



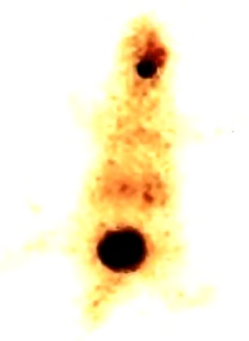
CL14 (4 day)



CL14 (8 day)

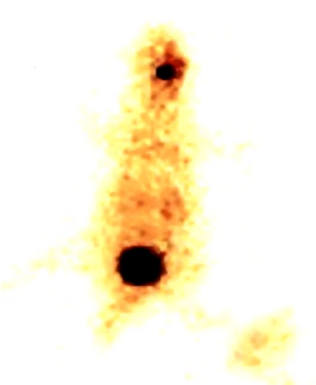


MSF27 (1 day)



MSF27 (4 day)

MSF27 (8 day)



CSF27 (1day)

CSF27 (4 day)

CSF27 (8 day)

9.3 RESULTS AND DISCUSION

Locally administered periodontal mucoadhesive thermoreversible gel, strip, liposomal gel and microspheres loaded with minocycline hydrochloride/ clindamycin phosphate were prepared with an aim to improve the localized action of the antibiotic at the targeted site of action to thereby decreasing the resistance of drug, which would also lead to significant reduced side effects.

In order to ascertain the achievement of these objectives, in vivo blood pharmacokinetic study was performed by administration of an appropriate volume of the respective formulations labeled with technetium-99m.The ^{99m}Tc labeled minocycline hydrochloride/ clindamycin phosphate and their periodontal formulations were injected into the ear vein of rabbits and measurement of the blood labels at predetermined time intervals was performed. The data so obtained was treated to analysis using WinNonline-5 software to get an idea of the blood kinetic profile of minocycline hydrochloride/ clindamycin phosphate and their periodontal formulations in rabbits. The table no. 9.01 and 9.02 tabulate the raw data obtained from the experiment and the calculated value from the analysis. Table no. 9.03 gives the pharmacokinetic parameters describing the in vivo behavior of the prepared periodontal formulations generated by data analysis. Figure 9.02 and 9.03 shows the comparison of the blood kinetics pattern of minocycline hydrochloride/ clindamycin phosphate and their periodontal formulations, respectively. An appropriate volume of the radio labeled complexes was locally injected into the oral cavity of the rats bearing artificial abscissa in the periodontium individually in regular intervals. The gamma scintigraphy imaging was performed using a Single Photon Emission Computerized Tomography (SPECT., LC 75-005, Diacam, Siemens USA) gamma camera and are shown in figure 9.03

9.4 CONCLUSION

The rapid elimination of free drugs and the conventional periodontal formulations containing the drug proved their inability to remain in the systemic circulation for a longer period of time and also showed a very less amount of release of free drug into the circulation thereby increasing the accumulation of drug at the periodontium. These studies are preliminary investigations showing the potential of the agents used for periodontal infectious diseases. Hence minocycline hydrochloride/ clindamycin phosphate and their periodontal formulations may be used as locally administrable delivery systems with improved drug action with lesser side effects and reduced drug resistivity.

9.5 REFERENCES

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Györfi A, Fazekas A, Suba Z, Ender F, Rosivall L. Neurogenic component in ligature-induced periodontitis in the rat. J Clin Periodontol. 1994 Oct;21(9):601-5.

Wu M.S., Robbins J.C., Ponpipom M.M., Shen T.V. Modified in vivo behavior of liposomes containing synthetic glycolipids. Biochim. Biophys Acta. 1981; 674: 19-29.