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The in vivo degradation, absorption and excretion of PCL-based implant

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Abstract

The in vivo degradation of poly (ε-caprolactone)(PCL) was observed for 3 years in rats. The distribution, absorption and excretion of PCL were traced in rats by radioactive labeling. The results showed that PCL capsules with initial molecular weight (Mw) of 66 000 remained intact in shape during 2-year implantation. It broke into low molecular weight (Mw = 8000) pieces at the end of 30 months. The Mw of PCL deceased with time and followed a linear relationship between log Mw and time. Tritium-labeled PCL (Mw 3000) was subcutaneous implanted in rats to investigate its absorption and excretion. The radioactive tracer was first detected in plasma 15 days after implantation. At the same time radioactive excreta was recovered from feces and urine. An accumulative 92% of the implanted radioactive tracer was excreted from feces and urine by 135 days after implantation. In the mean while, the plasma radioactivity dropped to the background level. Radioactivity in the organs was all close to the background level confirming that the material did not cumulate in body tissue and could be completely excreted.

Keywords: Degradation; Absorption; In vivo test; Poly (ε-caprolactone); Drug delivery

1. Introduction

Nearly 10 million women have used subdermal contraceptive implants including Norplant®, Jadelle® and Implanon® over the past 3 decades. They are as effective in preventing pregnancy as permanent sterilization and have no serious health consequences [1,2]. However, these implants are all made of non-biodegradable polymers that have to be taken out surgically after completion of drug release. The retrieval operation is much more traumatic than original insertion that has been proven great burden on patients. For the last two decades biodegradable polymers have been extensively studied as control-releasing matrix in order to eliminate the device-retrieval surgery [3–5]. Poly (ε-caprolactone) (PCL) is one of the most potential candidates for this purpose due to its availability, biodegradability, non-toxicity and biocompatibility to many drugs [3]. The fact that PCL degrades much slower than other known biodegradable polymers makes it very suitable for making long-term drug delivery devices.

Our group developed a 2-year contraceptive implant made of PCL/Pluronic F68 (F68) compounds filed with levonorgestrel (LNG) powder. F68 is a FDA approved excipient under the trade name of Poloxamer. It is both water and organic solvent soluble. It has been used in pharmaceutical formulations primarily as emulsifier [6]. In this study, Pluronic F68 was incorporated into PCL matrices as a drug releasing enhancer. Recently the PCL/F68/LNG implant was approved by SFDA to conduct phase II human clinical trial in China. Previous studies [7] on in vitro and in vivo drug release behavior have demonstrated that PCL/F68/LNG implant achieved long-term, sustained LNG release for 2 years. The objectives of this study are to investigate the in vivo degradation of the novel PCL-based implant and the excretion of tritium-labeled low molecular weight PCL.

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2. Materials and methods

2.1. Materials

ε-Caprolactone monomer was Aldrich product with 99.9% purity. The monomer was further purified by vacuum distillation over CaH_2. Pluronic F68 with molecular weight (Mw) around 8300 containing about 80% poly (ethyl oxide) (PEO) segment and 20% of poly(propyl oxide)(PPO) segment, was purchased from BASF, Germany. The molecular formula of F68 was shown in Fig. 1. The F68 was incorporated into PCL matrix in 10% of weight ratio as a molecular distribution, which would leach out in aqueous medium to leave micro porous structure in the PCL matrix [7]. Tritium labeled PCL (^{3}H-PCL) was provided by the Chinese Institute of Atomic Energy (Beijing, China) as a chloroform–methanol solution with specific radioactivity of 0.93 mCi/ml. The labeling was did on low Mw PCL (Mw 3000) and its radiochemical purity was ⩾95%. All other reagents were AR grade and purchased from Tianjin No.1 Chemical Reagent Factory.

2.2. Synthesis of PCL/F68 compound

PCL/F68 was synthesized by bulk polymerization as described previously [7]. Briefly, the terminal hydroxyl groups in F68 molecules were capped with acetyl so that it became inactive and would not participate in the polymerization reaction of ε-caprolactone. The acetyl-capped F68 was dissolved in ε-caprolactone monomer before polymerization so that F68 was incorporated in PCL matrixes as a molecular dispersion instead of forming a copolymer. The polymerization was carried out at 140 °C under high vacuum for 24 h with 0.04% stannous octoate as catalyst. The end-capping reaction of F68 and the polymerization of PCL were shown in Fig. 1.

2.3. Preparation of PCL/F68 capsule for implantation

The PCL/F68 compound was melting extruded into a tube with 2.5/2.3 mm of OD/ID in diameter on an extruder (SJ-400/20, Shanghai Light Industry Machinery Co. LTD). The head temperature was 120 °C and the tube was cooled in thermostatic running water of 25 °C. The tube was further dried in vacuum before being cut into segments of certain length. The tubing segment was melting-sealed at both ends to form the capsule. For in vivo degradation studies, capsules with 2.3 cm in length were prepared for rat implantation.

2.4. Animals

Female Wistar rats (180–200 g) were purchased from Tianjin Medical Laboratory Animals Center (Tianjin, China). The Administrative Committee on Animal Research in the Institute of Biomedical Engineering, Chinese Academy of Medical Science approved all the protocols for animal experiments. Also, all the animal experiments were performed in compliance with the Guiding Principles for the Care and Use of Laboratory Animals, Peking Union Medical College, China.

2.5. In vivo degradation of PCL implants

Total 72 female Wistar rats were anesthetized by ether inhalation. A small incision of 2 mm long was made on the dorsal skin of each rat. One 2.3 cm length of PCL capsule (initial Mw 66 000) was subcutaneous inserted through the incision using a special trocar. The animals were given access to food and water ad libitum. Every random picked 6 rats were killed at predetermined time interval. The implants were retrieved from the implantation site, cleaned with distilled water and vacuum dried to constant weight. For Mw measurement, precisely weighed pieces of the dried capsule were dissolved in chloroform and the inherent viscosity was measured using a Ubbelohde-type viscometer at 30±0.1 °C according to the procedure described in Ref. [8]. The inherent viscosities [η] were defined by the following equation [8]:

$$[\eta] = [2(\eta_{sp} - \ln \eta_r)]^{1/2}/C.$$

The average molecular weight Mw was then obtained using the Mark–Houwink equation [9]:

$$[\eta] = 1.298 \times 10^{-4} \times Mw^{0.828} \quad \text{(Chloroform, 30 °C)}.$$

2.6. Adsorption and excretion of low molecular weight PCL

Adult female Wistar rats (185±3 g in weight) were used in this experiment. Rat's dorsal hair was shaved and the newly exposed skin was cleaned using PBS. The ^{3}H-PCL was reconstituted in glycol ether to get a radioactivity of 650 μCi/ml in solution. Each rats was subjected a subcutaneous injection of 0.1 ml of the ^{3}H-PCL solutions (a dose of 65 mCi per rat). Total 11 rats accepted the ^{3}H-PCL injections. These rats were then housed separately in metabolic cages in order to collect urine and feces samples.

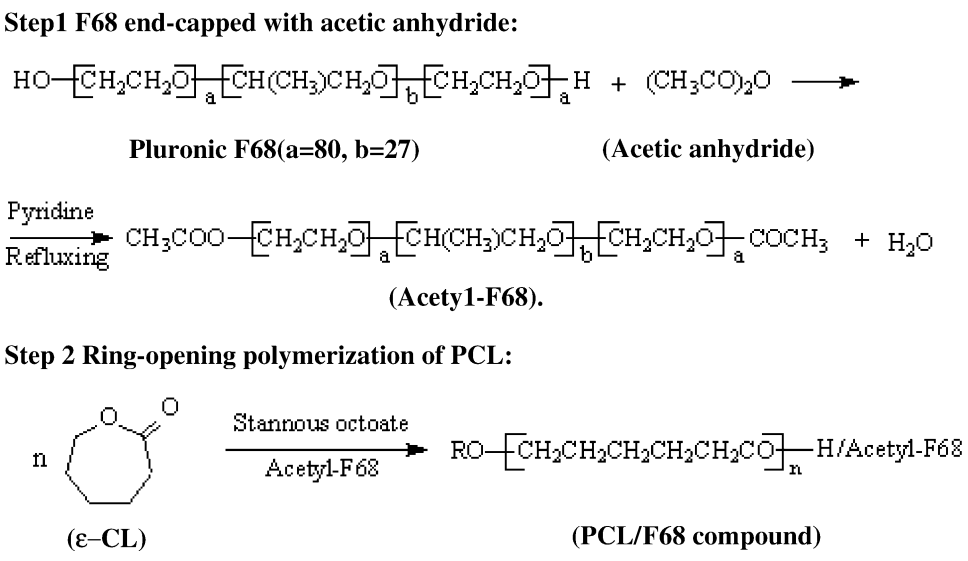

Fig. 1. The end-capping reaction of F68 and the polymerization of PCL.

2.6.1. Collection and treatment of urine and feces samples

The whole urine and feces samples of the rats were collected every day. The urine and feces samples were treated according to the procedure described in Ref. [10]. Briefly, the collected feces were pooled for each predetermined time interval and were dried by infrared ray. The dried feces samples were ground and blended thoroughly. For each sample, an aliquot of 10 mg the blended feces were added into distilled water containing 200 μl each of formic acid and H_2O_2, followed by adding two drops of octanoic acid. The mixture was heat digested at 80 °C for 1 h. The digestate was allowed to cool to room temperature. Toluene–ethanol scintillation solution was then added to the digestate to determine its radioactivity. The urine samples were also pooled for each 10-day time interval and mixed thoroughly. Scintillation solution was then directly added to the sample to determine its radioactivity.

2.6.2. Treatment of plasma samples [10]

Blood sample was collected periodically from each rat by capillary puncture of the orbital venous plexus and immediately centrifuged to get plasma. An aliquot of 10 μl plasma was digested in a mixed solvent containing 100 μl formic acid, 100 μl H_2O_2 and one drop of octanoic acid at 80 °C for 1 h. Toluene-based scintillation solution was then added to the digestate to determine its radioactivity.

2.6.3. Treatment of tissue and organ sample [10]

Five animals were sacrificed at 60 days, and the other six were sacrificed at 165 days after implantation. Such organs as heart, liver, spleen, kidney, stomach, duodenum, brain, ovary and uterus were harvested from the sacrificed animals. An aliquot of 10 mg wet tissue was weighed and homogenized in a mixed solvent containing 100 μl formic acid, 100 μl H_2O_2 and one drop of octanoic acid. The mixture then transferred into a scintillation vial and digested at 80 °C for 1 h. Toluene–ethanol scintillation solution was then added to the digestate to determine its radioactivity.

2.6.4. Radioactivity measurement

Five milliliter of scintillation solution was added into each sample. The radioactivity in each vial was determined on liquid scintillation spectrometer (LS-9800, Beckman. Instruments, Fullerton, California, USA). Quenching was automatically corrected using the "H-number" method, and then DPM (disintegrations per minute) results could be obtained. Data were expressed as mean ± SD.

3. Results

3.1. Molecular weight change of PCL implant in rats

The PCL capsules collected from rats at each time interval were evaluated by measuring molecular weight (Mw) change. As illustrated in Fig. 2, the PCL matrix gradually declined with time after the PCL capsules were implanted in rats. The results demonstrated a linear relationship between the logarithm of Mw and time, which was in accordance with the mechanism of random hydrolytic chain scission of the ester linkages [3]. The Mw of PCL dropped from original 66 000 to about 24 000 after 480 days implantation, and to about 15 000 Da at the end of 24 months implantation. In the mean while, photographs taken from the implantation site showed that the PCL capsules maintained its integrity during the 24 months implantation as illustrated in Fig. 3. A thin layer of connective tissues surrounding the capsules was observed after 480 days implantation. The Mw of PCL further dropped to 8000 Da by the time of 30 months. At this point, the implants became fragile and completely lost

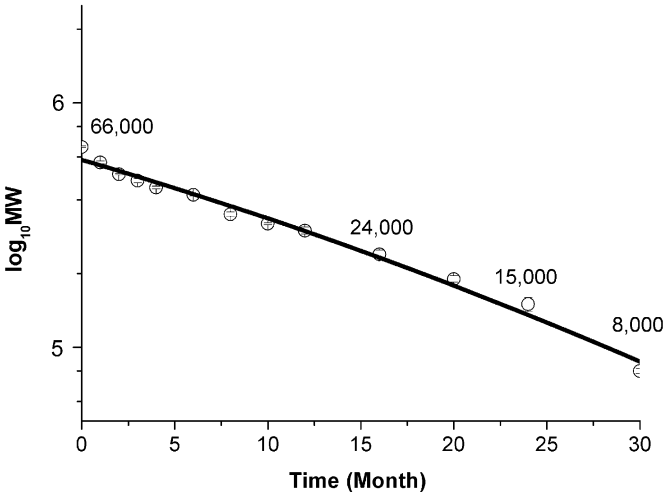

Fig. 2. Decrease in Mw of PCL/F68 capsules with time after implantated in rats. The results indicated a linear relationship between the logarithm of Mw and time. Data are shown as mean ± SD ($n = 6$).

mechanical strength. The implants were found as small pieces at the implantation site and could not be reclaimed at the end of 36 months implantation.

3.2. Adsorption, distribution and excretion of ^{3}H-labeled PCL in rats

3.2.1. The relationship between plasma radioactivity and time

The results were shown in Fig. 4. The plasma radioactivity measured at day 15 after implantation was 811 ± 108 DPM, which was significantly higher than the background level (110 DPM). The radioactivity reached peak value of 2253 ± 210 DPM at day 45, and decreased distinctly afterwards. The radioactivity level dropped to the background level (110 DPM) at 165 days after implantation of the low Mw ^{3}H-labeled PCL.

3.2.2. Distribution of radioactivity in organs

The results are shown in Fig. 5. As seen from these results, the radioactivity in all organs was close to the background level. Statistical assay demonstrated that ^{3}H-labeled PCL in all organs had no statistically significant difference after 60 days implantation ($P > 0.05$, mean ± SD, $n = 5$) and 165 days implantation ($P > 0.05$, mean ± SD, $n = 6$) with respect to background level (110 DPM). These results confirmed that the material did not cumulate in organs and tissues [11].

3.2.3. Urinary and fecal excretion of radioactivity

All rats were housed in individual metabolic cage after implantation of ^{3}H-labeled PCL. The urine and feces samples were collected every day for up to 135 days. Radioactivity in the collected samples was assayed at predetermined time intervals. The results are shown in Fig. 6. As seen from these results, first excretion of

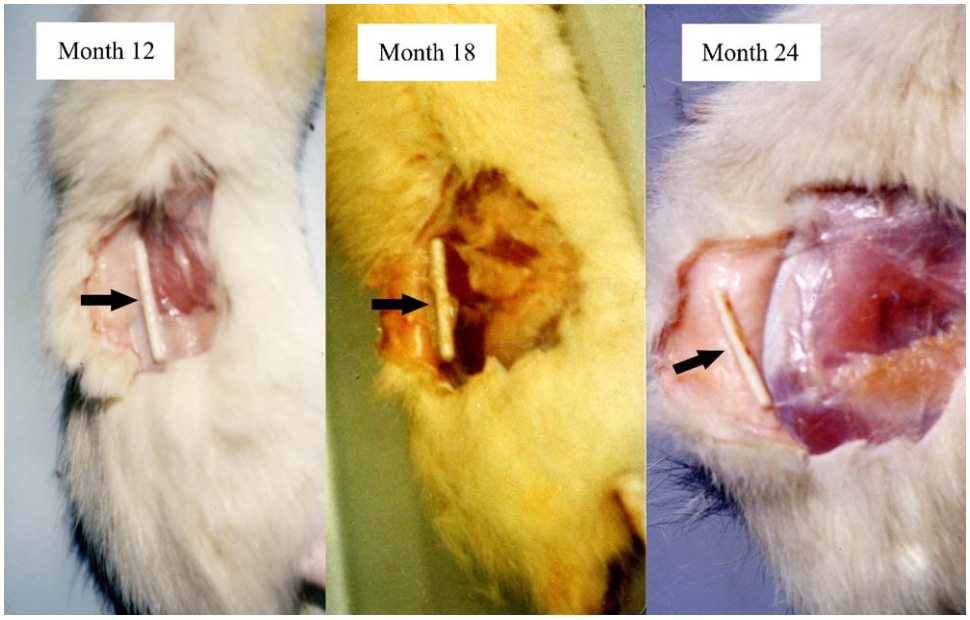

Fig. 3. Images of the PCL/F68 capsules in rats. The photographs were taken when the capsules were withdrawn from rats at different time points, showing the open site of the implantation and the appearance of the capsules. Black arrows indicated the PCL/F68 capsules.

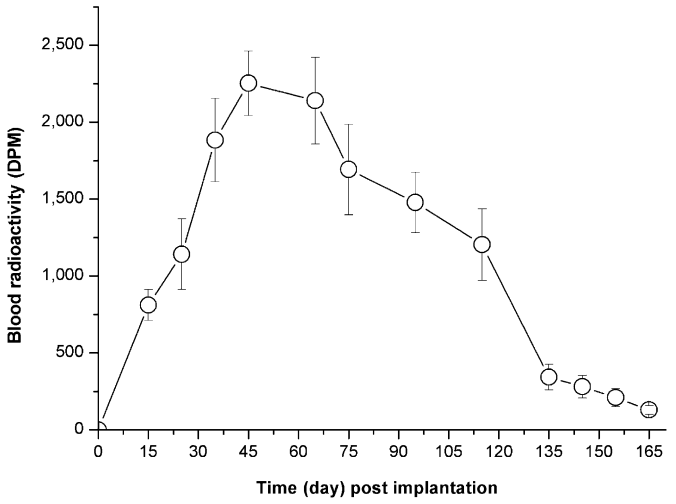

Fig. 4. The rat plasma radioactivity after subcutaneous implantation of ^{3}H-labeled PCL. Data are expressed as mean $\pm$ SD ($n = 11$).

radioactive tracer in urine and feces was detected at day 15 after implantation. About 56.4% of the total administered radioactivity was collected from urine and feces at day 65 representing a rapid excretion of the ^{3}H-labeled PCL with Mw 3000. The cumulative excretion of total radioactive tracer from urine and feces was 91.7% by the end of 135 days implantation.

4. Discussion

Pitt group at the Research Triangle Institute [3] revealed that the in vivo degradation of PCL showed a two stages pattern. The first stage involves a decrease in molecular weight without mass loss and deformation. The second degradation stage begins when the molecular weight dropped to 5000. At that point the material broke into pieces and mass loss occurs. They therefore predicted that the material would then gradually be absorbed and excreted by the body. They also found that in the first stage the degradation rate of PCL is essentially identical to the in vitro hydrolysis at 40 °C and obeyed first-order kinetics. They therefore concluded that the mechanism of PCL degradation is attributed to random hydrolytic chain scission of the ester linkages, which causes a decrease in molecular weight. Ali et al. [12] studied the mechanism of PCL degradation in vitro by mean of GPC, DSC, SEM. They hypothesized that the HO· radical is likely to be a significant cause of PCL degradation in implantable devices. Chen et al. [13] studied the in vitro degradation behavior of the PCL microparticles and compared it with that of PCL film in pH7.4 PBS at 37 $\pm$ 1 °C. They found that the shape of PCL had no obvious effect on its degradation rate. It suggested that homogeneous degradation dominated the process. Recently, a number of investigators focused primarily on the thermal degradation of poly(ε-caprolactone) [14–16]. Persenaire et al. [14] proposed a two-stage thermal degradation mechanism of PCL. They found in the first stage there was a statistical rupture of the polyester chains via ester pyrolysis reaction. The second stage lead to the formation of ε-aprolactone (cyclic monomer) as result of an unzipping depolymerization process. It was very important that they identified the produced gases during the thermal degradation as H_2O, CO_2, and 5-hexenoic acids. Sivalingam et al. [15] investigated the thermal degradation in bulk and solution. They found that the polymer degraded by random chain scission

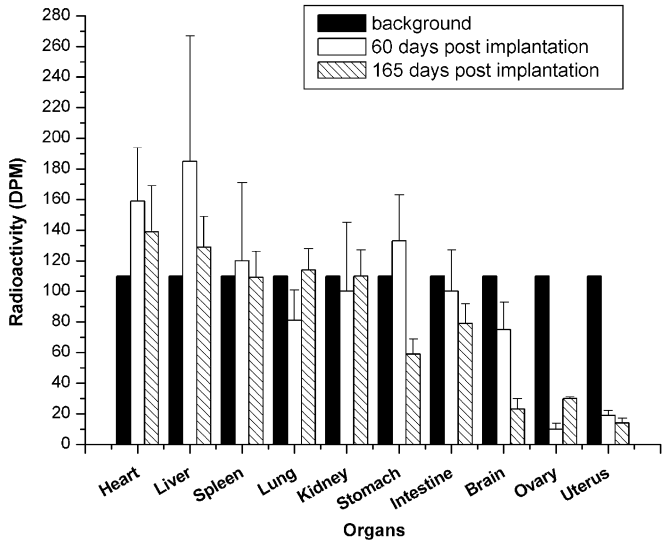

Fig. 5. The body distribution of radioactivity after implantation of ^{3}H-labeled PCL in rats. The results demonstrated that ^{3}H-labeled PCL in all organs had no statistically significant difference after 60 days implantation ($P>0.05$, mean$\pm$SD, $n=5$) and 165 days implantation ($P>0.05$, mean$\pm$SD, $n=6$) with respect to background level (110 DPM).

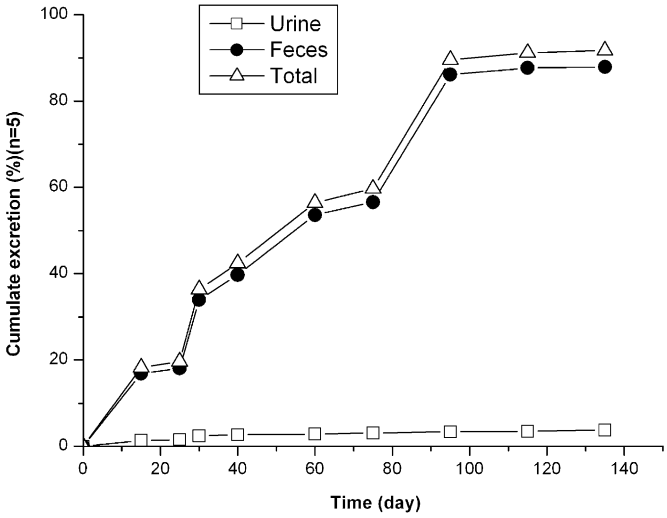

Fig. 6. Radioactive excretion after subcutaneous implantation of ^{3}H-labeled PCL in rats.

and specific chain end scission in solution and bulk, respectively.

Our results also demonstrated a perfect linear relationship between the logarithm Mw and time, which was in accordance with first-order rate law for ester hydrolysis. When the molecular weight decreased to approximately 24 000 at 480 days post-implantation, the PCL capsules were intact and maintained enough strength. When the molecular weight decreased to approximately 15 000 at 720 days post-implantation, the PCL capsules remained intact but started to lose strength. When the molecular weight decreased to 8000, the capsules lost their strength completely and broke into pieces. These results were in good agreement with Pitt's findings. It is hypothesized that in order to achieve 2-year controlled release of contraceptive medicine, the PCL polymer matrix should be so designed that the capsules maintain intact shape in vivo for at least 2 years. Therefore, PCL with Mw greater than 66 000 Da was used to construct the PCL/F68/LNG contraceptive implant.

Pitt et al. [3,17] studied in vivo adsorption of low molecular weight PCL at cellular level. They found that the PCL pieces was ingested and digested ultimately by phagocyte and giant cell. They conclude that the degradation of PCL in the second stage mainly involves intracellular phagocytosis. In this study, we observed the body distribution and excretion of ^{3}H-labeled low Mw PCL (molecular weight approximately 3000) in rats. Follow-up analysis of organ distribution and total urinary and fecal excretion of the radioactive tracer was performed. Plasma radioactivity was monitored simultaneously. The results showed that plasma radioactivity reached peak concentrations at 45 days after implantation. It decreased distinctly afterwards and disappeared completely at day 165. The excretion studies showed that when radioactive tracer occurred in the blood, it was detected in urine and feces simultaneously indicating that the excretion of ^{3}H-PCL was very rapid. Approximately 56.4% of the total administered radioactive tracer was excreted within 65 days. The cumulative excretion of total radioactivity into urine and feces was 91.7% at day 135, indicating that the excretion of ^{3}H-PCL was almost completed at this time point. Metabolites of drugs and other substances in vivo were mainly excreted via renal, biliary, mammary gland or gastrointestinal route [18]. In Fig. 6, we can see that almost all of cumulate excretion of metabolized PCL was found in feces. From the result we would infer that the metabolites of PCL were mainly excreted via biliary or gastrointestinal route. In the meanwhile, serum radioactive level decreased distinctly along with radioactive excretion in urine and feces. These results demonstrated that PCL could excrete from the body immediately after it was adsorbed and metabolized by the body. Body distribution studies showed that very low levels of radioactivity were detected in several organs after 60 days implantation of ^{3}H-PCL, for example, 185$\pm$51 DPM in liver, 159$\pm$45 DPM in heart. The levels of radioactivity were almost close to or below the background values (110 DPM) in other organs. The radioactivity levels in all organs were ultimately close to or below the background values by the end of 165 days indicating that the material did not cumulate in the body.

5. Conclusion

We reported the first long-term in vivo degradation, absorption and excretion studies ever done on PCL. The degradation of PCL can be split into two stages. The first stage involves a decrease in molecular weight without deformation. The PCL capsules with an initial molecular weight of 66 000 remained intact in vivo for 2 years.

Afterwards the PCL capsules gradually lost strength and broke into pieces. In the second stage, low molecular weight PCL pieces metabolized by unknown process and ultimately excreted from the body through urine and feces. The material did not cumulate in the any body organs.

Acknowledgments

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