BIODEGRADATION, DRUG RELEASE MECHANISM AND PHYSIOLOGICAL ACTIVITY OF A PROTEIN'S SUSTAINED RELEASE SYSTEM

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I.INTRODUCTION

The classical superovulatory treatment consists in 8 intramuscular injections of pituitary extracts (porcine Follicle Stimulating Hormone : pFSH; porcine Luteinizing Hormone: pLH) every 12 hours during 4 consecutive days. This schedule is justified by the weak half-life of pFSH ($t1/2 = \pm 5$ h. : Demoustier M., 1988(a)).

The studied sustained release system released the pituitary hormone on 4 days and induced a optimal pFSH plasma concentration near to 0,3 ng/ml (Demoustier, 1988 (a)). This system was biocompatible and moreover, biodegradable. The polylactide 50 (PLA50) was chosen as polymer. The system, in the shape of a rod of 3,4 mm diameter, was subcutaneously injected with a trocar in the ear of the cow. The implant 80/20 (%,w/w : PLA50/pituitary extracts) gave in cows, the wished plasma profile (Demoustier, 1988 (b)).

This communication relates

- the study of the impact of different quantities of pituitary extracts on the biodegradability of the polymer PLA50,
- the explanation of the drug release mechanism of pituitary extracts from the sustained release system, and
- the proof that the sustained release system has not only the same quantitative physiological response than the repetitive intramuscular injections, but also that it has a more reproducible responsiveness of the ovaries.

II. MATERIALS AND METHODS

Estimation of degradation of PLA50

The kinetic of degradation was estimated by the rate of decrease in the weight-average molecular weight (Mw) of PLA50 evaluated by gel permeation chromatography (GPC). The eluent was tetrahydrofurane (Carlo Erba) and the columns were µstyragels (Waters, Millipore Corporation; porosity : 104 Å, 103 Å and 5 102 Å). The used standard solutions were polystyrene (Polymer Laboratories LTD) of molecular weight (Mw) equal to 520000, 200000, 120000, 52000, 22000, 9200, 5050 and 2150. A differential refractometer R-401 (Waters Millipore) was used as detector. Before molecular weight determination, the samples and the standards were dissolved in tetrahydrofurane and filtered on aerodisc (Gelman; 45 µm).

In vivo degradation of PLA50

In the degradation experiments, implants of PLA50 containing different quantities of pituitary extracts (40%, 20% and 0%) were subcutaneously introduced in the back of male rats. At time $t = 0, 1, 3, 7, 14, 21, 49, 63$ and 84 days, implants were recovered and stored under hight vacuum in presence of silicagel until molecular weight determination.

Determination of physiological activity

The physiological activity of continuous infusion and repetitive injections of pFSH was evaluated by the test described by Steelman and Pohley (1953). These authors demonstrated on immature female rats (20 to 21 days old), a linear relationship between the increase in administered FSH dosage and the augmentation of the ovarian weight. The Follicle Stimulating Hormone standard solutions containing a pFSH dosage comprised between 0,0 and 17 µg/ml were prepared. Every pFSH dosage was tested on a group of 10 female rats.

The ovaries' responses induced by repetitive injections and by continuous infusion of pFSH were compared for a same total pFSH dosage (2,5 µg) distributed over 3 days. In the case of repetitive injections, the pFSH was administered in 6 subcutaneous injections of 0,2 ml each. In the other hand, implant was introduced in the back muscle of the female rat.

Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM : Hitashi S-570) observations were carried out on implants frozen in liquid nitrogen and fractured.

Firstly, implants 100/0 and 80/20 were placed for 45 minutes in phosphate buffer (pH = 7,4) and the cross section directly in contact with the buffer was observed by SEM. Secondly, implants 100/0, 80/20 and 50/50 were incubated in phosphate buffer (pH = 7,4) and at time $t = 0, 3, 6$ and 24 hours, implants were recovered, dried under high vacuum : implants were then frozen and fractured before SEM observations.

III. RESULTS

Impact of the drug on the biodegradation of PLA50

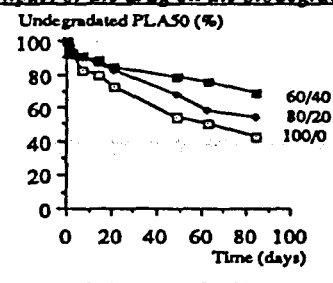

Figure 1 : influence of different quantities of pituitary extracts (0%, 20% and 40% (w/w)) on the degradation kinetic of PLA50 (n = 3).

The impact of the drug concentration on the biodegradability of PLA50 showed that the more the quantity of pituitary extracts incorporated increased, the more the kinetic of degradation slowed down. Indeed, after 63 days of degradation test, the PLA50 of the system without any pituitary extracts (100/0) lost 50% of its molecular weight against 42% and 34% for the systems respectively containing 20% (system 80/20) and 40% (60/40) of pituitary extracts (figure 1).

These results confirmed the macroscopic observations : the cross-section of the implant 100/0 revealed a yellowish transparent inner part surrounded by a whitish outer layer. After 63 days, the inner part of the system 100/0 was completely degraded and it only subsisted an empty shell. In contrast, the systems 80/20 and 60/40 presented at the same time a homogeneous full cross-section.

Drug release mechanism

Contrary to the cross-section of implants 80/20 before any treatment (SEM photographies : figure 2a), the cross-section directly in contact with phosphate buffer (figure 2b) clearly showed gaps in the polymer due to the leaving of the pituitary extracts which dissolved in the aquous solution. This SEM observation proved that the implant was a matrix system with 2 phases : the polymer and the pituitary extracts.

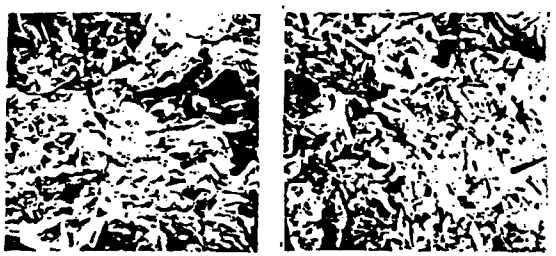

Figure 2 : SEM appearance of cross-sections of implant 80/20 before (a) and after (b) an incubation of 45 minutes in phosphate buffer. In the last case, the cross-section was directly in contact with buffer. Magnification : x 350

It's important to notice that the system of PLA50 alone (without any pituitary extract) was not porous, the cross-section was full homogeneous.

The cross-sections of fractured implants 80/20 and 50/50 incubated in phosphate buffer revealed an outer porous layer whose surface became larger with increasing release time. These observations prove that the release front progressively moves from the surface into the center of the system.

The SEM photographies also demonstrated that porous density was directly proportional to the quantity of pituitary extracts present in the system.

Physiological activity of the sustained release system

Thought there was no significant difference between the physiological responses (average ovaries' weight) induced by the two different types of pFSH administration, the reproductiveness was better for the sustained release system than for the repeated injections (table I)

	pFSH dosage		
	0,0 (µg)	2,5 (µg)	
	untreated	repeated injections	implant
average weight (mg)	26,6	62,1	63,4
SE (σ_m)	0,33	1,18	0,6

Table I : comparison of average weight of ovaries' rats, treated by repeated injections or by continuous administration of pFSH (number of ovaries, n = 20).

IV. DISCUSSION

The incorporation of pituitary extracts in PLA50 slowed down the degradation kinetic of polymer. This observation can be explained if one rememberes that degradation of polylactides is easier at extreme pH values. However the pituitary extracts, composed of proteins (isoelectric point (pI) near to 5) buffers the system at a pH value between the extreme pH values, so that the degradation kinetic of polymer is slowed down.

From SEM photographies, it seems that the matricial device is two phases, and that the drug is released by channels. Moreover the more the incorporated pituitary extracts quantities increases, the more the kinetic of liberation is quick (Demoustier, 1988(b)). This observation could be explained by the following facts : the release is achieved by the channels and the number of these channels is directly proportional to the pituitary extracts quantity incorporated in the system. We also can affirm that this release is exclusively carried out by diffusion through channels and not by diffusion through the polymeric chains : indeed diffusion tests achieved on PLA50 films in diffusion cells have been negative due to the too high molecular weight of the glycoproteins (PM ± 32000).

In the fundamental physiology point of view, it's interesting to observe that the 2 types of pFSH administration (continuous and repetitive) induces on rats, ovarian responses which are statistically identical : further these experiments will be directly carried out on cows.

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