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COMMUNICATION

Administration of Human Chorionic Gonadotropin with a Controlled-Release Function to Immature Rats for Application in Male Infertility Therapy

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ABSTRACT

Biodegradable poly(DL-lactic acid) (PLA) devices with controlled release of human chorionic gonadotropin (hCG) were fabricated in a 2-mm cylinder form by the melt-pressing technique. The devices fabricated were inserted subcutaneously in the backs of immature rats. It was found that the serum level of testosterone (T) remained constant at approximately 0.7 ng/ml for an experimental period of 14 days, resulting in greater Leydig cell production of testosterone. This was also suggested from the results of immunohistochemical observation of the testis and weight changes of prostates, such as the ventral prostates and seminal vesicle.
Key Words: *Biodegradable device; Controlled release; Gonadotropin; Poly(DL-lactic acid); Testis.*

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INTRODUCTION

It is known that human chorionic gonadotropin (hCG) shows a luteinizing-hormone-like effect in stimulating testosterone (T) secretion by the testis (1,2). The administration of hCG, therefore, is expected to be used for spermatogenetic maintenance therapy in infertile patients with low serum gonadotropin levels (3). However, maintaining a durable pharmacological effect in vivo requires that the patient go to the hospital at short intervals to receive intramuscular injections two or three times a week. To overcome this disadvantage, we incorporated hCG into a biodegradable drug delivery system that releases hCG over a long period of time. The device used as the drug delivery system was a fine cylinder made by fusing a mixture of hCG and D-mannitol with biodegradable poly(DL-lactic acid) (PLA) under mild heat-pressure conditions (4,5). The device was inserted subcutaneously in the back of Wistar strain immature male rats. This is the first report to investigate the in vivo controlled-release function of hCG from a biodegradable device.

MATERIALS AND METHODS

The male anti-infertility agent hCG (obtained from Teikoku Hormone Mfg. Co., Ltd., Tokyo, Japan) was dissolved in distilled water containing D-mannitol at 25°C. This solution was then lyophilized as it contained 343 i.u. of hCG per 5 mg of the samples lyophilized.

The number-average molecular weight M_n of the biodegradable PLA was 1600, the same as that reported earlier (6,7). The 2-mm diameter cylindrical device was prepared by the melt-pressing technique (4,5) as follows. A blended powder of PLA (45 mg) and D-mannitol (5 mg) containing hCG (343 i.u.) was charged into a 2-mm interior diameter poly(tetrafluoroethylene) tube. Stainless piston rods were inserted from both sides of the tube, followed by the melt pressing under a pressure of 100 kg/cm^2 at 37°C. The device was solidified by slightly decreasing the temperature; the device was placed into a poly(ethylene) package, sealed in an atmosphere of dried air, and sterilized by irradiation at 15 kGy using γ-rays from a ^{60}Co source.

Wistar strain immature male rats weighing approximately 50 g were used. Under ether anesthesia, a fine cylindrical PLA device containing 343 i.u. of hCG was inserted subcutaneously in the backs of 42 rats (group 1). Subcutaneous injection with 343 i.u. of hCG in 0.7 ml of water was made in the backs of 30 rats (group 2). The controls were 42 uninjected rats (group 3). The devices were excised just after sacrificing at a fixed time interval, freed from surrounding connective tissue, lyophilized, and weighed to estimate the degree of in vivo degradation from the weight ratio of the device before and after insertion.

The serum testosterone (T) level was measured by radioimmunoassay according to the method of Makino (8). The pharmacological effect of hCG with and without the use of PLA devices was evaluated by measuring the changes in weight of prostate-related organs, such as the ventral prostates (VPs), the right seminal vesicle (SV), and the right testis (9). On the other hand, the Leydig cells in the testis of rats administered the PLA device with and without hCG loading were observed immunohistochemically. For this purpose, the testes excised were first preserved in 20% buffered formalin (pH 7.4), followed by a paraffin embedding. After deparaffining, the specimens (sliced with a thickness of 3 μm) were digested with proteinase K (DAKO Co., Carpinteria, CA) for 6 min at room temperature. On the other hand, it is necessary to block endogenous peroxidase in the specimen by treating it with 0.3% methanol solution of H_2O_2 for 15 min. After this, the specimen was treated with rabbit antitestosterone (Biogenesis Ltd., Poole, UK) for 30 min at room temperature, then with DAKO Envision plus™ goat antirabbit immunoglobulin conjugated to peroxidase-labeled dextran polymer for 60 min at room temperature, and with chromogen 3,3′-diaminobenzidine for 3 min at room temperature. The specimen was finally counterstained with methyl green for 2 sec to observe microscopically.

RESULTS AND DISCUSSION

The degree of in vivo degradation of hCG-loaded PLA devices is shown in Fig. 1. We already reported that bulk PLA without the use of drugs completely disappears by the 70th day after the start of subcutaneous insertion, accompanied by a characteristic parabolic-type degradation pattern (6). Contrary to this, the degree of in vivo degradation while using the hCG-loaded PLA device reached 80% by the 14th day after device insertion, followed by perfect degradation within 21 days. This high percentage is closely related to the existence of water-soluble hCG in the device.

Changes in serum testosterone levels in immature rats administered using the controlled-release hCG-loaded PLA device (group 1), subcutaneous single injection of hCG only (group 2), and the control only (group 3) are shown in Fig. 2. Serum testosterone levels in group 3

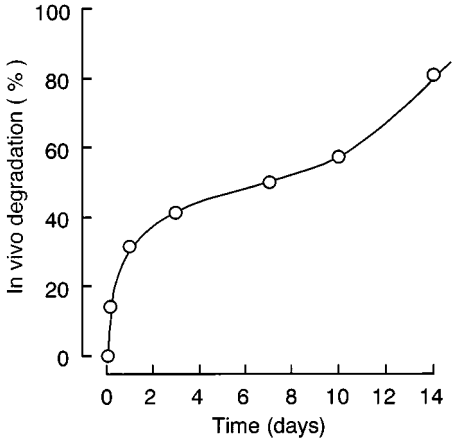

Figure 1. In vivo degradation pattern of hCG-loaded PLA device in fine cylinder form.

were about 0.2 ng/ml throughout the entire experimental period of 14 days because of immature rats. On the other hand, serum testosterone levels in group 2 showed a transient decrease on the first day after starting the experiment, as did those in group 3.

This phenomenon was observed in a previous experiment by Moger (10), who found that testosterone synthesis capacity is impaired by 4 hr after hCG administration, and this impairment becomes more prominent between 16 and 24 hr after administration. Sharpe (11) also reported that hCG causes accumulation of interstitial fluid in the testes beginning between 8 and 12 hr after injection; this fluid accumulation precedes the down-regulation of luteinizing hormone receptor and the reduction in the in vitro steroidogenic capacity of the testis that begins about 16 hr after administration of hCG.

It is believed that an increased concentration of estradiol in testicular tissue plays an important role in reduction of testosterone synthesis capacity. Serum testosterone levels, showing a transient decrease on the first day of the hCG administration, were then able to increase again in groups 1 and 2. However, in the case of group 2, serum testosterone levels rapidly dropped to those of immature rats on the fourth day at the start of the experiment, demonstrating that a single injection of hCG is not useful for maintaining effective serum testosterone levels over a relatively long period of time (e.g., 14 days). In contrast to group 2, serum testosterone levels remained constant at approximately 7 ng/ml over an experimental period of 14 days in group 1. This finding indicates that hCG is gradually released as the device degrades in vivo.

Zhou and Hutson (12) reported that continuous hCG stimulation induces the proliferation of Leydig cells within 24 hr, resulting in the synthesis of enough testosterone to produce almost normal spermatogenesis. To confirm this efficacy, the Leydig cell was immunohistochemically observed. It can be seen clearly in Fig. 3, which shows the Leydig cell in the testis stained by the

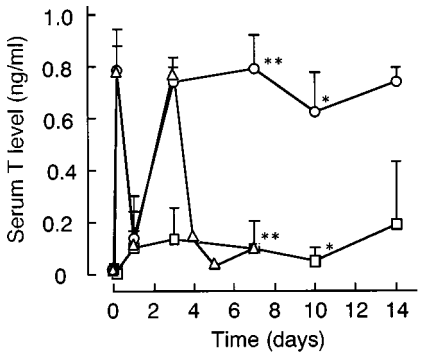

Figure 2. Changes in serum testosterone levels in immature rats administered using ○ controlled-release hCG-loaded PLA device in group 1, △ subcutaneous single injection of hCG only in group 2, and □ control only in group 3. *$p < .01$, **$p < .001$ compared with corresponding control group (Student t test, $n = 7$).

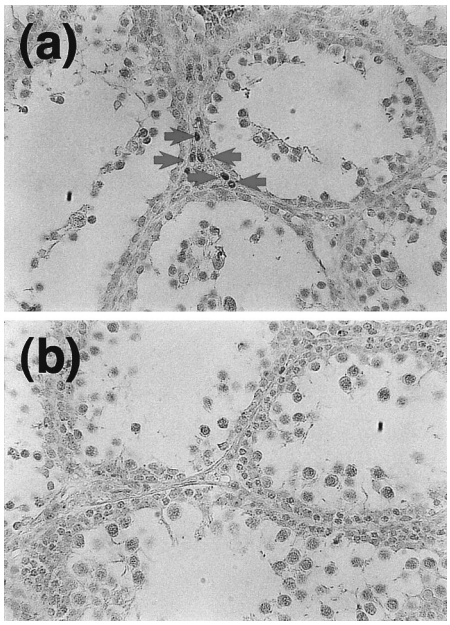

Figure 3. Optical microscopic views of Leydig cells in the testis of immature male rats, which were sacrificed on the third day after administered (a) with and (b) without hCG-loaded PLA devices, followed by a counterstain by the immunohistochemical technique. Arrows in figure are the Leydig cells, indicating testosterone production.

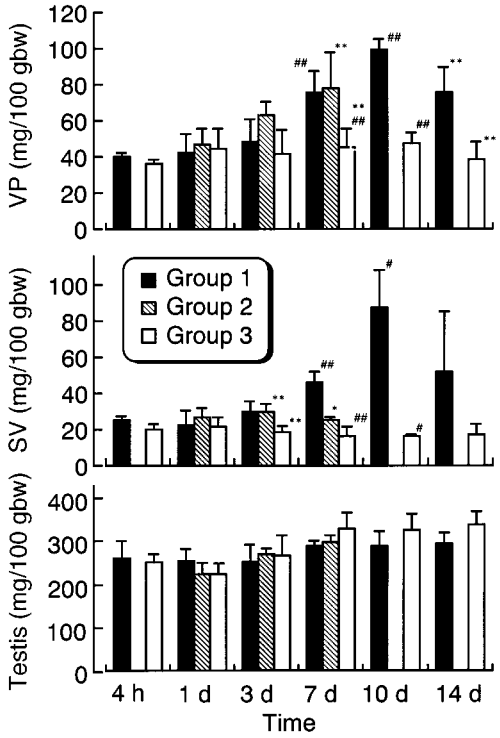

Figure 4. Changes in the weight of ventral prostates (VPs), right seminal vesicle (SV), and right testis in immature rats in groups 1, 2, and 3 given in Fig. 2. $*p < .05$, $**p < .01$, $\#p < .005$, $\#\#p < .001$ compared with corresponding control group (Student t test, $n = 7$).

immunohistochemical technique, for immature rats administered PLA devices with and without hCG loading. In the case of immature rats with hCG-loaded PLA devices, the Leydig cells were positively stained on the third day after the start of the device insertion (Fig. 3a), as testosterone is produced from Leydig cells. Contrary to this, in immature rats without hCG-loaded PLA device administration (control), no Leydig cells stained in close relation to testosterone production were observed even on the third week after the start of the insertion (Fig. 3b). However, most of the Leydig cells in such control rats were stained intensely 9 weeks after insertion because the rat reached sexual maturity (13). It is therefore reasonable to conclude here that this controlled-release hCG-loaded PLA device will be helpful in male infertility therapy.

Controlled release of hCG from the device was also evaluated by pharmacological effect. Figure 4 shows changes in the weight of prostate-related organs such as the VP, SV, and testis in immature rats in groups 1, 2, and 3. VP and SV weights in group 1 showed greater increases compared with those in groups 2 and 3. This increase in prostate weight was caused by increased testosterone production in the Leydig cells in the testis brought on by the effects of hCG. No change in the weight of the testis was observed in any group. The cause for this is not clear at present.

CONCLUSION

We fabricated a biodegradable PLA device for the controlled release of hCG by the melt-pressing technique. Serum testosterone levels in male immature rats remained constant at approximately 0.7 ng/ml over an experimental period of 14 days as a result of greater Leydig cell production of testosterone brought on by the effects of hCG. It is known that continuous hCG stimulation induces the production of normal spermatogenesis in close relation to the production of testosterone. Therefore, this controlled-release hCG-loaded PLA device is a useful drug delivery system for application in male infertility therapy.

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