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ENHANCED RELEASE OF DRUGS FROM SILICONE ELASTOMERS (III): SUBCUTANEOUS CONTROLLED ADMINISTRATION OF MELATONIN FOR EARLY ONSET OF ESTRUS CYCLES IN EWES

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ABSTRACT

Implants were fabricated from swellable silicone elastomers to release hydrophilic melatonin at a controlled rate for the early induction of breeding season in ewes. Both the *in vitro* and *in vivo* release profiles of melatonin were observed to follow a linear Q vs. $t^{\frac{1}{2}}$ relationship. The *in vitro*/*in vivo* release flux ratio ranging from 0.415 to 1.452 was obtained depending upon the polyethylene glycol 400 concentration in the aqueous release medium used for in vitro studies. The release flux ($Q/t^{\frac{1}{2}}$) of melatonin from the implants was observed to increase as a function of the glycerol content in the silicone elastomers. When the ewes were treated with subdermal implants containing 25% w/w of melatonin for up to 49 days, blood melatonin levels above

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the target level of 450-900 pmole/l were achieved and maintained for at least 35 days.

INTRODUCTION

Melatonin, a biochemically active hormone from the pineal gland, has several important biological functions. For example, the oral administration of melatonin in food pellets was recently reported to induce an early onset of the breeding season in ewes (1 - 3). The advantages of this type of melatonin treatment are: (i) the estrus cycles of a flock can be synchronized, (ii) the estrus cycles of ewes can be induced two months earlier than normal, and (iii) the resulting early-born sheep can be pasture-fed during the summer. Therefore, melatonin treatment could have significant benefits to the sheep industry in the future.

Since melatonin is known to be relatively expensive, the current method of melatonin administration in food pellets is not economically sound. Furthermore, the amount of melatonin absorbed is rather difficult to control, because it varies with the amount of food intake. An attempt was recently made to develop an implantable device for the administration of melatonin (4, 5). The first such device was found to be rather primitive, which was constructed by simply sandwiching the crystalline melatonin solids between two sheets of Silastic® membrane (0.127 mm thick each), which were glued on all sides with Silastic adhesive. It would be costly and impractical in the scaling up of this procedure. Also, the reproducibility in the drug release rates from the devices produced by this procedure could be rather poor.

The objective of this investigation is to develop an implantable subdermal device which could release melatonin at a

desirable rate for the induction of estrus (heat) cycles in ewes. The device should be easy to fabricate and should also produce the release rates in a reproducible manner. In this report, the methods of fabricating the subdermal melatonin-releasing implants, the results of *in vitro* and *in vivo* evaluations, and the blood melatonin levels achieved in the ewes will be discussed.

EXPERIMENTAL

1. Equilibrium Solubility of Melatonin in Aqueous PEG Solutions

An excess amount of melatonin (*1) was dispersed in an aqueous solution containing up to 30% v/v polyethylene glycol 400 (*2). After equilibration in a shaking waterbath (*3) at 37 C for three days, the suspension was filtered through a Milipore filter (*4). The clear filtrate was diluted with methanol and the concentration of melatonin in the diluted solutions was determined by the UV spectrophotometric method (*5).

2. Fabrication of Subdermal Melatonin-releasing Implants

Following the same method reported in the earlier investigation of this series (7), subdermal melatonin-releasing implants were prepared by dispersing melatonin in a mixture of medical-grade silicone elastomer 382 (*6) and varying concentrations of glycerol. The loading of melatonin was 25% (w/w) in the *in vitro* evaluation in various aqueous PEG solutions and in the *in vivo* implantations in ewes. Under continuous stirring, catalyst M (*7) was added to the mixture, which was then deaerated in a dessicator under a vacuum. The resultant drug-polymer blend was extruded into sections of Tygon tubing and cured overnight at room temperature.

3. Determination of *In Vitro* Release Rates

Implants (3 cm in length by 0.32 cm in diameter), which contained 25% w/w of melatonin prepared from medical grade

silicone elastomer 382 having 20% w/w of glycerol, were suspended in 10 ml aqueous solutions containing 20, 30 or 40% v/v of PEG 400, which was added to achieve a sink condition, and were maintained at 37 C in a shaking waterbath. The in vitro release studies followed the same procedures previously reported (6, 7).

4. Enhancement of Melatonin Release by Glycerol

To evaluate the enhancing effect of glycerol on the release of melatonin, subdermal implants were prepared to contain 10% w/w melatonin in silicone elastomer 382 having up to 30% w/w of glycerol. The in vitro release profiles of melatonin were investigated as a function of glycerol.

5. Determination of Blood Melatonin Levels

Subdermal melatonin-releasing implants, which contained 25% w/w melatonin, were prepared from the silicone elastomer 382 having 20% w/w of glycerol as outlined in Section 2. They were implanted subcutaneously behind the ear in 35 Romney ewes. Blood samples were collected once every week, and melatonin levels were determined using the radioimmunoassay technique reported previously (5).

6. Determination of In Vivo Release Rates

In addition to determining blood melatonin levels, groups of five implants were removed from the treated ewes every week after implantation. After removal, each subdermal implant was sliced into tiny pieces and the residual melatonin content in each of the implants was extracted following the method reported earlier for the extraction of desoxycorticosterone acetate in subdermal implants (6). The melatonin content was determined by high performance liquid chromatography (*8) using a reverse phase Bondapak C_{18} column and a combination of 80% methanol and 20% distilled water, as the mobile phase, at the flow rate of 1 ml per minute. Melatonin was quantitatively determined by comparing the peak height of the samples to those of the reference standards determined at the wavelength of 254 nm.

RESULTS

1. Dependence of Equilibrium Melatonin Solubility upon PEG Concentration

In order to maintain an appropriate sink condition in the *in vitro* study of the release of melatonin from the subdermal implants, the equilibrium solubility of melatonin in the aqueous solutions containing various volume fractions of PEG 400, as the solubilizer for melatonin, was determined (Figure 1). The equilibrium solubility of melatonin was observed to increase exponentially in increasing the volume fraction of PEG 400 in the solution. From this linear relationship, an aqueous PEG solution can be prepared to provide the specific sink condition required in the *in vitro* release studies of melatonin.

2. Effect of Glycerol Content on Melatonin Release

Figure 2 shows the release profiles of melatonin from the silicone implants containing various weight fractions (w/w) of glycerol. Glycerol was added to the silicone elastomers to make the implants swellable, which resulted in the enhancement of drug release (7). At the same melatonin loading, it was observed that the higher the glycerol content in the silicone implant, the higher the drug release profile. Similar to the results observed earlier, a linear Q vs. $t^{\frac{1}{2}}$ relationship was established for all the glycerol levels studied (Figure 3). The fluxes of release ($Q/t^{\frac{1}{2}}$), calculated from the slope of the Q vs. $t^{\frac{1}{2}}$ linearity, are listed in Table I. When the release fluxes obtained from the glycerol-containing implants are compared with that measured in the virgin implants (containing no glycerol), the flux of release had increased by 3 to 21 times, as 5-30% of glycerol was incorporated into the silicone elastomer.

The semilogarithmic relationship established earlier (7) between release fluxes and glycerol concentrations was also

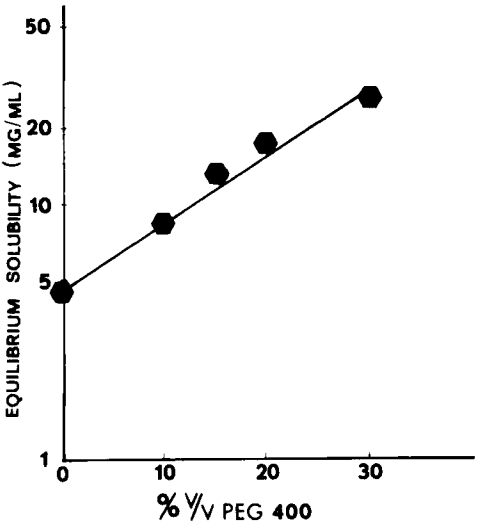

Figure 1. Semilogarithmic relationship between the equilibrium solubility of melatonin and the volume fraction of polyethylene glycol 400 in the aqueous solutions. Triplicate experiments were conducted with a standard deviation of less than 5%.

applied to analyze the enhanced release fluxes of melatonin from the silicone elastomers having various amounts of glycerol. Results from the linear regression analysis of the data in Table I indicated that the semilogarithmic relationship is also followed with a slope of 0.0326 and an intercept of 1.82 (correlation coefficient of 0.987)

3. In Vivo Release Rates of Melatonin

The mean values of melatonin released from the five subdermal implants removed after subcutaneous implantation in ewes for 7, 14, 21, 28, 35, 42, and 49 days, were 8.74, 10.61, 13.95, 12.83, 14.43, 14.57, and 15.34 mg/cm^2, respectively. The average coefficient of variance at each point in time was determined and found to be less than 10%. As observed earlier in the in vitro drug release studies, the in vivo release profiles of melatonin

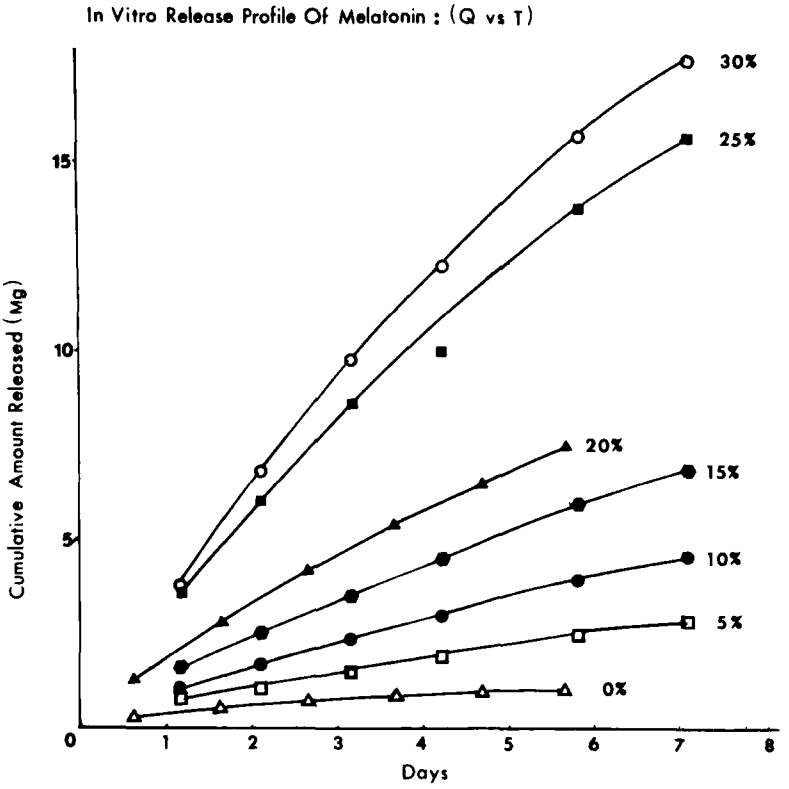

Figure 2. The Q vs. t plots for the in vitro release of melatonin from the swellable silicone implants in the 20% aqueous PEG 400 solution. Four implants from each formulation were tested. The number along each drug release profile designates the glycerol content in the formulation. Each of the formulations consisted of 10% w/w of melatonin. The standard deviation was found to be within 5% at each time point.

from the implants in the subcutaneous tissue were also found to be a linear function of the square root of time (Figure 4). The in vivo release flux, calculated from the slope of the linearity, was 1.46 mg/cm 2/day $^{\frac{1}{2}}$.

4. Correlation of In Vitro and In Vivo Release of Melatonin

When silicone implants were evaluated in the aqueous solution containing 20, 30, 40, 50, or 60% v/v of PEG 400, the in vitro

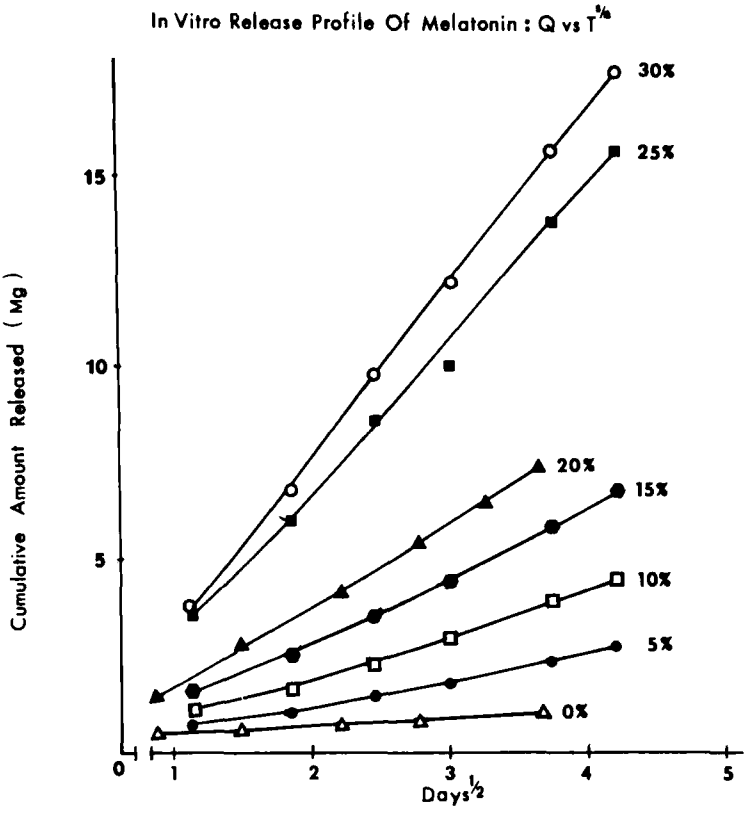

Figure 3. Linear Q vs. $t^{1/2}$ relationship for the in vitro release of melatonin from the subdermal implants. The data in Figure 2 were plotted.

release fluxes of melatonin from the silicone implants having 20% w/w of glycerol were 0.600, 0.987, 2.108, 2.120, and 1.853 mg/cm ^{2}/day $^{1/2}$, respectively. The in vivo release flux determined from the slope in Figure 4 is 1.46 mg/cm^2/day$^{1/2}$. Therefore, the ratio of in vitro/in vivo release fluxes was calculated to be 0.415, 0.676, 1.443, 1.452, and 1.269 for the release studies conducted in the aqueous solutions containing different volume fractions of PEG 400.

5. Blood Melatonin Levels in the Ewes

The blood melatonin levels in the ewes after subcutaneous implantation of the melatonin-releasing implants is shown in

Table I: Release fluxes of melatonin from silicone implants containing various weight fractions of glycerol.

Glycerol (% w/w)	Release Fluxes ($mcg/cm^2/hr^{\frac{1}{2}}$)	Enhancement Factor
0	28.32	1.0
5	90.40	3.2
10	149.58	5.3
15	218.57	7.7
20	253.81	9.0
25	506.17	17.9
30	587.09	20.7

Figure 5. The mean blood melatonin levels are 7.462, 4.033, 2.104, 1.237, 0.960, 0.323, and 0.371 pmole/l after 7, 14, 21, 28, 35, 42, and 49 days of implantation, respectively. The linear semilogarithmic relationship indicates that the blood melatonin level declines exponentially as a function of time (Figure 5), suggesting that first-order kinetics could be operating in the subcutaneous bioavailability of melatonin. The half-life of the decay in blood melatonin levels is estimated to be about eight days. The blood melatonin level at the end of the 5-week implantation was 960 pmole/l, which is above the target level of 450-900 pmole/l needed for effective induction of an early onset of estrus cycles in ewes.

DISCUSSION

Implantable polymeric controlled-release drug delivery systems have often been developed for veterinary uses. They are

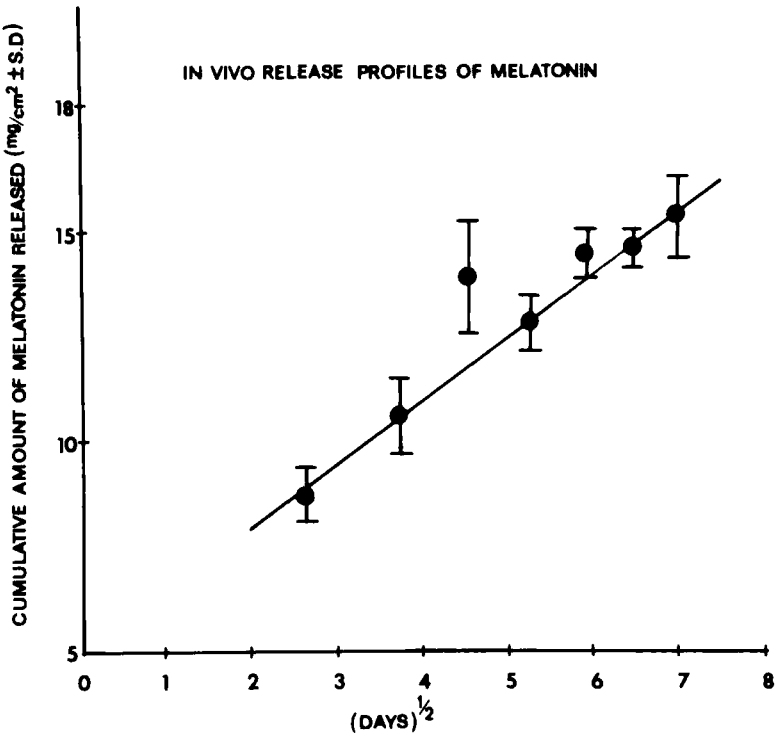

Figure 4. Linear Q vs. $t^{\frac{1}{2}}$ relationship for the subcutaneous release of melatonin from the subdermal implants. Each of the implants contains 25% w/w of melatonin in the silicone elastomer having 20% w/w of glycerol. Each data point is the mean value (± standard deviation) of five implants.

exemplified by the development of the estradiol-releasing Compudose® subdermal implant for growth promotion in cattle (8, 9) and the norgestomet-releasing Syncromate®-B implants for estrus synchronization in ewes (10). The subdermal melatonin-releasing implants developed in this investigation are designed for the early induction of estrus (heat) cycles, in addition to achieving the estrus synchronization in ewes. The results demonstrated that a blood melatonin level above the target level of 450-900 pmole/l can be achieved and maintained

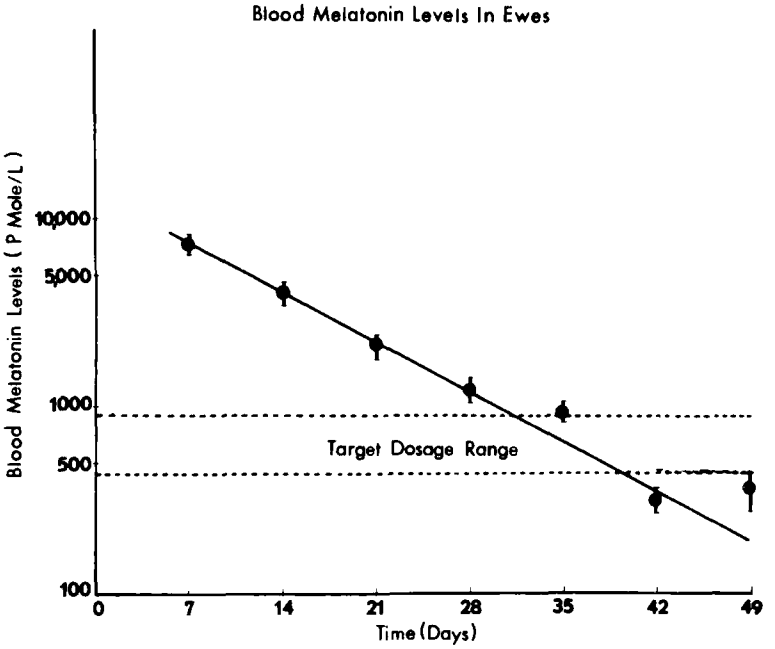

Figure 5. Blood melatonin levels achieved in the ewes receiving one subdermal implant containing 25% w/w melatonin in the silicone elastomer having 20% w/w of glycerol.

for at least five weeks (Figure 5). Since the implants are fabricated from a matrix diffusion-controlled drug delivery system, the release flux is expected to decrease in proportion to the sqare root of implantation time. Thus, the blood melatonin levels are reduced as a function of the duration of implantation. Although a high blood level of melatonin is observed in the early stage of implantation, it did not produce any adverse side effects in the ewes.

The unique features of the formulation used in the fabrication of subdermal melatonin-releasing implants in this investigation have been discussed in the first report of this series of investigations (7). The results obtained once again demonstrate that the addition of a water-soluble co-solvent enhances the release flux of hydrophilic drugs from the

lipophilic silicone elastomer matrix. At the same melatonin loading, the release flux is increased as a function of the weight fractions of glycerol in the polymer (Figures 2 and 3). It is rather encouraging to observe that, with a simple variation of the glycerol content in the formulation, the release flux of melatonin can be varied by as much as 20 times (Table I). Obviously, this type of swellable polymer formulation may also be useful in the delivery of polar compounds from hydrophobic silicone elastomers.

Melatonin, N-acetyl-5-methoxytryptamine, is the major secretory product from the pineal gland. The level of the enzyme hydroxyindole-O-methyl transferase, which is responsible for melatonin production in rats, has been reported to rise at night and fall during the day, as does the blood level of melatonin. This rhythmical fluctuation is known to be directly influenced by the level of darkness and light, the signals of which are transmitted through the nervous system connecting the pineal gland with the eye. The pineal gland appears to partially offer a chemical foundation for the operation of the so-called "biological clock". If a magnetically-controlled or other drug delivery system (11) could be designed to deliver melatonin in a rhythmical pattern, it may be possible to trigger at will the biological phenomena to simulate the biochemical function of melatonin.

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Footnotes

1. Sigma Chemical Co., St. Louis, MO.
2. Fisher Chemical Co., Fair Lawn, NJ.
3. Shaking waterbath Model 127, Fisher Scientific Co., Fair Lawn, NJ.
4. Millipore Corporation, Bedford, MA.
5. UV/Vis Spectrophotometer Model 559A, Perkin Elmer Co., Chicago, IL.
6. Dow Corning Co., Midland, MI.
7. Pump: Model 6000A solvent delivery system; Detector: Model 440 absorbance detector; Injector: Model U6K injector, Waters Associates, Inc., Milford, MA.

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