DELIVERY OF ESTEROGEN BENZOIATE (EB) AND EB *PLUS* PROGETERONE BY MEANS OF ZNCAP DRUG DELIVERY SYSTEM USING AN OSTEOPOROTIC RAT MODEL

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Introduction:

Currently, titanium, polyethylene, tricalcium phosphate and hydroxyapatite (HA) are widely used as biomaterials in medical implants. The addition of zinc in the ceramic formulations may prove to be effective in the development of orthopedic, dental, and medical implants. The use of zinc-phosphate-lysine devices (ZCAP) has shown to be a useful carrier for various steroid hormones for prolonged period of time. This suggested that EB filled ZCAP could be utilized in the long-term treatment of estrogen-deficient patients. The specific aims of this project were (i) to deliver EB and EB plus P at sustained levels by means of ZCAP drug delivery system using an osteoporotic rat model, and (ii) to subsequently evaluate the biochemical and morphological changes of hard and soft tissues associated with the sustained delivery of the aforementioned steroids.

Methods:

Experimental Design: a total of forty-two SD-adult female rats were used in this investigation. The animals were divided into six groups as indicated in Table 1.

Table 1. Experimental Design:

Groups	Day 0	Day 18	Day 42	Day 60
Group I (n=12)	Control (intact)		Necropsy (n=6)	Necropsy (n=6)
Group II (n=12)	OVX		Necropsy (n=6)	Necropsy (n=6)
Group III (n=6)	OVX + EB		Necropsy	
Group IV (n=6)	OVX	+ EB		Necropsy
Group V (n=6)	OVX + EB + P		Necropsy	

All animals in Groups II-V were ovariectomized (OVX) by following standard laboratory surgical procedures documented elsewhere. Groups III, IV and V were implanted with ZCAP delivery system loaded with either 50 mg of EB or a combination of 50 mg EB + 90 mg P. Vaginal smears were taken daily to evaluate the changes in reproductive organs prior to and during the experimental phase. All animals were acclimatized one week prior to surgery. Vaginal smears were taken by inserting 2 drops of sterile saline into the vagina and aspirating the samples. The smears were fixed and stained by a modified Papanicolaou method. Serum blood levels of the pituitary and steroid hormones and total body weights were recorded biweekly. Reproductive organs and other individual vital organs were weighed and tabulated upon termination of each phase of the experiment. During the entire duration of this study, the rats were kept on a 12-h day/night cycle, and were fed Purina Rodent Chow and water *ad libitum*.

Fabrication of Ceramic: The microcrystals of zinc calcium phosphate were prepared by following standard laboratory procedure in which is explained elsewhere. The sintered ZCAP mixed with EB or EB + P. The mixture was cold pressed into cylindrical form (final density of 1.79 +0.32 gm/cm^3) and surface area of (3.91 + 0.32 cm^2) using a 3/8" die set at a compression load of 2500 Kg .

Ceramic Implantation: Rats were anesthetized with a mixture of Zylazine/Ketamine, and their hind limbs shaved and scrubbed with providone iodine. The sterilized ceramics (gas/24 hours) were inserted intraperitoneally (IP) using standard surgical techniques. After implantation, abdominal muscle was closed by silk sutures and the skin was sealed with wound clips (9 mm), and the animals were injected with 0.1 ml of 200,000 units of Penicillin.

Tissue Processing: Reproductive and vital tissues were carefully dissected and placed in the embedding cassettes and dehydrated through a series of graded alcohol by following our standard laboratory protocols. Three-inch glass slides were coated with poly-L-lysine (Sigma, St. Louis, MO) to ensure tissue attachment to the surface of the glass slide. Tissues were sectioned using an American Optical microtome with a 5 µm setting. Tissues were floated on a water bath at 60°C and then placed on the coated slides. The slides were stored at room temperature until use. Hematoxylin and eosin (H&E) stained slides were prepared.

Bone Morphology and Biochemical Analysis: endocortical resorption, bone mineral density, and bone mechanical strength were performed by following standard methods reported elsewhere unless otherwise indicated.

Statistical Analysis: The data collected in this study were analyzed by means of analysis of variance at $p < 0.05$ using standard computer programs. (Jandel STAT Computes Software)

Summary:

The data obtained from this study demonstrated that XCAP delivery system was capable of releasing EB (7-9 pg/ml) , and EB (7-9 pg/ml)+ P (4.6-5.9 ng/ml) at sustained levels. Sustained delivery of EB alone or in combination with P resulted in marked changes in the female reproductive tract. The number of estrus cycles in the intact control animals varied from 2-8 times during the entire experiment. All of the EB treated animals exhibited a cytologic pattern of estrus for at least 5 times during the experiment phase. None of the OVX animals exhibited estrus and this was evidenced by presence of nucleated squamous cells (atrophy). Histopathological evaluation of vaginal smears obtained from the EB + P treated animals revealed that there were no estrus events observed during the duration of the experiment. This indicates the role of P in opposing the sustained levels of EB released from the ZCAP devices. However, the presence of thicker vaginal and cervical epithelium indicates the proliferative effect of the combination therapy of EB + P. The predominant cell types in the EB + P treated animals were found to be nucleated followed by low numbers of anucleated squamous cells. Results obtained from this investigation suggest that: (1) OVX animals exhibited an increase in body weight, endocortical resorption, and indices of cancellous bone turnover, as well as decreases in uterine weight, uterine epithelial cell height, bone mineral density, and bone strength, and (2) sustained delivery of EB or EB + P maintained homeostatic controls, and uterine functional capacity in OVX'd rats to the levels found in the age-matched intact rats.