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Original Article

Injectable Estradiol Valerate, as a Substitute for Estradiol Pellets in Breast Cancer Animal Model

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The ability to maintain and study human tissues in *in-vivo* environment has proved to be a valuable tool in breast cancer research for several decades. The most widely tissues have been xenografts established human breast cancer cell lines into athymic nude mice. The aim of this study was to provide a new accurate and affordable method for the establishment of breast cancer xenograftin nude mice. Injectable estradiol valerate was assayed as a substitute for estradiol pellets that are rather expensive in order to create cancer tumors by MCF7 xenograft method in nude mice (B6). Twenty four healthy adult female nude mice (B6) were injected with different concentrations of pre-counted MCF7 cells. Then estradiol valerate and Matrigel B.D. were injected either alone or combined in two different groups of animals. In a period of 6 to 9 weeks, mice injected with increased amount of MCF7 cells, estradiol valerate and Matrigel (combined or alone) developed faster and larger tumors than animals which received MCF7 only or MCF7 and Matrigel combined. The results indicate that estradiol valerate which is way less expensive than estradiol pellets can be used as tumor proliferator to create animal breast cancer models.

Key Words: Etradiol valerate, nude mice, breast cancer, MCF-7

Breast cancer is the most leading type of cancer among women throughout the World (1). Current breast cancer chemotherapies, however, have not been satisfactory and researchers are constantly looking for newer protocols. Recently in-vitro studies on animal or human cell lines have led to introduce successful methods that can be helpful in pilot studies in cancer research (2-5). Ultimately, animal models such as mice are used to initiate an in-vivo breast tumor xenograft in mice in which their defense system are primarily destroyed (6-9). MCF7 cells are commonly used as a source of human breast cancerous cells. As most MCF7 cell lines possess estrogen receptor (ER+), estrogen is usually administrated to help tumor growth (7, 10). Estrogen pellets are often used as a source of estrogen (11-12). The estrogen pellets, however, are rather expensive and most research laboratories cannot afford them. Although some researchers have been tried less expensive agents namely estradiol cypionate (7) as an alternate to estrogen pellets. In this study, we used injectable estradiol

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valerate as a tumor proliferator and investigated its effect on tumor size and proliferation rate as well as its pathogenic feature.

Materials & Methods

Materigel was purchased from BD Biosciences (BD Biosciences, Belgium). Injectable estradiol valerate was purchased (Aburaihan Pharmaceuticals Co, Iran).

Cell line

MCF7 cell line was obtained from Pasteur institute of Iran, Tehran, Iran. The cells were cultured in RPMI 1640 medium (PAA, Austria) supplemented with 10% FBS and 1% antibiotics penicillin/streptomycin (Invitrogen, USA) at 37°C in an incubator with humidified atmosphere containing 5% CO2 and 95% air. After reaching 80- 90% confluence, cells were briefly trypsinized by addition of 0.05% trypsin and centrifuged at 1500 rpm for 5 min, and the pellet was refrigerated for further use.

Animals

Twenty four healthy adult female nude mice (B6) aged 7 to 9 weeks and weighting 20 to 25 g were obtained from North Research Center, Pasteur Institute of Iran. The animals were housed in polypropylene cages and maintained under standard conditions (12 h light: 12 h dark cycle; 25 ± 30°C; 35–60% humidity).

Table 1: MCF7 cell injection program

Groups	MCF7 Cell Counts ($\times 10^6$)	Other Injectable Materials
I	5, 10, 20	estradiol valerate 2mg/kg
II	5, 10, 20	-
III	5, 10, 20	Matrigel
IV	5, 10, 20	estradiol valerate 2mg/kg and matrigel



Figure 1: Tumor grown during 6 to 9 weeks post injection in a mouse preinjected with estradilol valerate.

Tumor induction

Normal healthy nude mice were randomly divided into four groups of six as indicated in Table 1. All mice were intramuscularly (IM) injected with different concentrations of pre-counted cells (5, 10, 20×10^6 cells) in their right thighs. Estradiol valerate (equaling to 1 mg/kg estradiol valerate) was IM injected into mice two days prior to MCF7 cells injection and once weekly. Other groups received either matrigel or a combination of estradiol valerate and matrigel, prior to MCF7 cells injection. A negative control group was injected with MCF7 cells without prior treatment. Table 1 represents the injection program of MCF 7 cells into each nude mice group.

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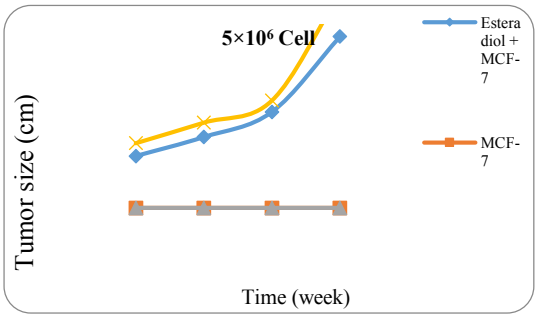


Figure 2: Kinetic of tumor development in different mice groups receiving 5×10^6 MCF 7 cells

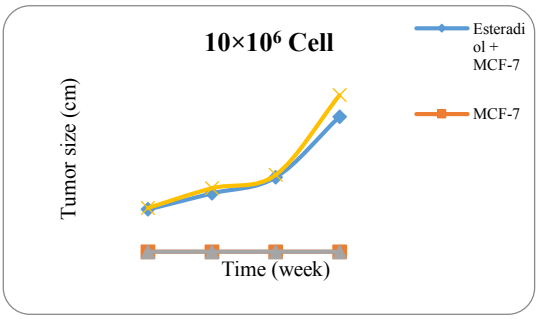


Figure 3: Kinetic of tumor development in different mice groups receiving 10×10^6 MCF 7 cells

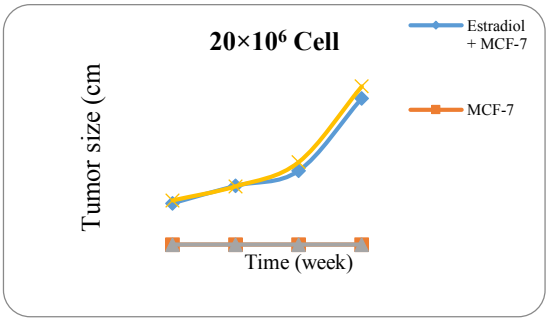


Figure 4: Kinetic of tumor development in different mice groups receiving 20×10^6 MCF 7 cells

Pathological Studies

Selected mice from different groups were sacrificed and their representative sections of tum--r with adjacent tissues were fixed in 10% neutral buffered formalin for 24 to 36 hours. Paraffin-embedded sections were prepared with 4µm thickness followed by standard H & E staining. Prepared tissues were examined using a light microscope.

Results

Mice who were not stimulated with estradiol valerate prior to MCF7 cells injection (groups II and III) did not develop any tumor. In other groups (I & IV) tumors sized 2 mm were observed during 6 to 9 weeks post injection (Figure 1) and all mice developed tumors. In group IV which received 300 µl matrigel, compared to group I, 2mm sized tumors appeared more rapidly. However, in both groups the time of tumor development decreased with the increase of the number of injected MCF7 cells while the rate of tumor growth increased. Figures 2 to 4 represent the kinetic of tumor development in different mice groups receiving 5, 10, 20×10^6 cells respectively.

Pathological observations

In examination of prepared tissues, tumoral lesions with wide necroses consisting of cells with large nucleus and course chromatins were observed. Most of the cells were vesicular and many possessed nucleolus and/or convex nucleolus. Minimal cytoplasm with indefinite boundaries which basically were accumulated or in a form of cellular disks was observed (Figure 5).

Discussion

Human breast tumor xenografts provide the opportunity to study various important interactions between the tumor and host tissues, stroma interactions. Our data clearly demonstrate that no tumor growth can occur in the absence of estradiol injection as estradiol is a potent tumor prolifator. However, the concomitant injection of estradiol and matrigel can accelerate tumor's formation without influencing its size or progression rate after reaching 2 mm, confirming including endocrinologic, immunologic and tumor the fact that materiel has the ability to enhance tumor growth (13). This suggests that stimulating agents or nutrients present in matrigel may help cell growth but are not sufficient by themselves in this animal model. Also, the initial amount of injected MCF7 cells influences the size of tumor.

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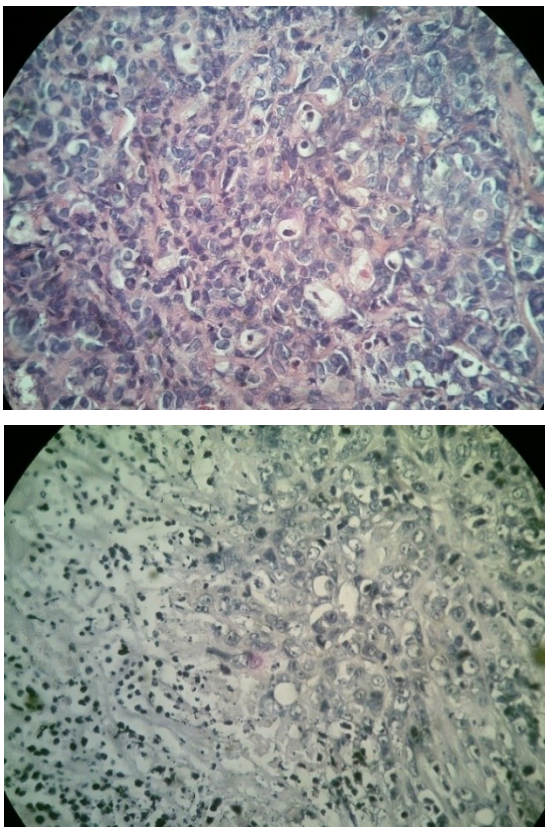


Figure 5: Hematoxylin & Eosin staining of tumor obtained from selected animals

Based on the findings in this study it can be concluded that the use of injectable estradiol valerate, can be substituted to other forms of estrogen as a tumor promoter reducing expenses in cancer research.

Conflict of interests

Authors declare no conflict of interest.

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