

Research Article

Effects of Enoxaparin Emulsion on Dimethylbenzanthracene-induced Breast Cancer in Female Rats

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Abstract

Introduction: Enoxaparin is an anticoagulant medication. Anticoagulation inhibits tumor cell-mediated release of angiogenic proteins and diminishes angiogenic response. Angiogenesis is an important event in various cancers such as breast cancer. Angiogenesis provide oxygen and nutrients to tumor cells and causes tumor progression. The aim of the present study was to evaluate the anti-angiogenesis effect of an enoxaparin cream on breast cancer induced by dimethylbenzanthracene in rats.

Methods: In this experimental *in vivo* study, 50 Wistar female rats were divided into negative control (vehicle), positive control (cream base), and 3 groups with enoxaparin treatment (40, 60, and 80 mg/ml). After one month of treatment along with breast cancer induction by dimethylbenzanthracene, breast tissue samples were isolated and stained with hematoxylin-eosin, and tumor growth suppression rate was calculated. Tumor size (length and width) was measured using a clipper, and the tumor volume was calculated using the following formula: $V = (L \times W \times W)/2$, where V is tumor volume, W is tumor width, L is tumor length. The data were analyzed using one-way ANOVA and Tukey's post hoc test.

Results: Tumor suppression was significantly increased in enoxaparin treatment groups compared to the positive control group (40 mg/ml of enoxaparin treated versus positive control group; $P = 0.017$, 60 mg/ml of enoxaparin treated versus positive control; $P = 0.015$, 40 mg/ml of enoxaparin treated versus positive control; $P = 0.009$, 60 mg/ml of enoxaparin treated versus 40 mg/ml of enoxaparin treated; $P = 0.019$, and 80 mg/ml of enoxaparin treated versus 40 mg/ml of enoxaparin treated; $P = 0.011$ in a dose-dependent manner.

Conclusion: Enoxaparin inhibits breast cancer in a dose-dependent manner. The application of enoxaparin cream in patients with breast cancer may considerably reduce tumor growth.

Keywords: Angiogenesis, Breast Cancer, Dimethylbenzanthracene-induced, Enoxaparin, Rat

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1. Introduction

Enoxaparin is a low-molecular-weight heparin, belonging to the category of organic acid compounds known as mucopolysaccharides, affecting thrombosis and angiogenesis in various cancers. Enoxaparin has an inhibitory effect on venous thrombosis, pulmonary embolism, acute coronary syndrome, thrombosis and angiogenesis in various cancers [1, 2]. Angiogenesis in cancer is the process of new blood vessel formation around the tumor as endothelial cells multiply. It can contribute to the proliferation and metastasis of tumor cells by increasing the supply of oxygen and various nutrients to tumor cells [3, 4]. Breast cancer involves the abnormal multiplication of breast tissue cells due to stress, obesity, immobility, hormonal changes, alcohol consumption, and radiotherapy. It can lead to various emotional, psychological, and physical complications. Breast cancer cells can become metastatic through angiogenesis [5, 6]. Blood coagulation inhibitors prevent the proliferation of lung, digestive tract, and breast cancer cells [7, 8]. Furthermore, heparin may have an inhibitory effect on metastasis in breast cancer cells [9]. Enoxaparin can effectively prevent the proliferation of breast cancer cells through its anti-angiogenesis effect [10]. Angiogenesis is one of the factors contributing to malignancy in cancer [11, 12]. It affects a variety of cancers, such as breast cancer in woman [13, 14]. Angiogenesis supplies oxygen and nutrients to breast cancer tissues to promote tumor development and metastasis [15, 16]. It is a physiological process where epithelial cells proliferate to promote neovascularization. In diabetes patients, neovascularization impairs improvement in diabetic foot ulcers and causes infertility due to delayed endometrium maturation [17, 18]. Heparin can have an inhibitory effect on the angiogenesis process [19–21]. The low-molecular-weight heparin, enoxaparin, inhibits breast cancer through its anti-angiogenesis effect [22–28]. In contrast, some studies have shown that the antithrombotic effect of enoxaparin is different in various cancers and that the anti-metastatic effect of enoxaparin may not correlate with its dose [29–31].

There is a high prevalence of breast cancer globally [32, 33] and in Iran [34]. Moreover, breast cancer has clinical, psychological, and economic complications [36]. In light of contradictory results reported in relation to breast cancer [21–23, 26, 27], the purpose of our study was to assess the effect of enoxaparin on dimethylbenzanthracene-induced breast cancer in rats. The results of this study might be clinically applicable for the prevention of breast cancer.

2. Materials and Methods

In this *in vivo* experimental study 50 Wistar female rats (300–350 g, 12–13 weeks) were procured from Pasteur Institute (Tehran, Iran) and all animal procedures were approved by the ethics committee of Tehran University of Medical Sciences. They were housed in clean cages with unrestricted access to water and food, under a 12-h photoperiod and standard temperature. Rats were randomly divided [35, 36] into the following 5 groups with 10 rats each: positive control group, negative control group, and 3 groups receiving enoxaparin at 40, 60, or 80 mg/ml concentrations. Enoxaparin (Lovenox)

was purchased from Rhone-Poulenc Rorer (Collegeville, PA, USA), and prepared as a cream at concentrations of 40, 60 and 80 mg/ml. The FDA and Australian standards were considered in preparing the base cream. The base for enoxaparin emulsion preparation was according to the FDA as follows: lanolin (5%), white Vaseline (2.5%), stearic acid (4%), and propyl paraben (0.05%) were heated at 77–82°C to prepare the oil phase. Methyl paraben (0.1%), disodium EDTA (0.05%), propylene glycol (5%), triethanolamine (1%), and water were used as an aqueous phase and mixed until they were completely dissolved. The aqueous phase was added to the oil phase. The storage temperature for the base cream and enoxaparin cream was 18–25°C. To induce breast cancer in each group, dimethylbenzanthracene was injected subcutaneously in the breasts of rats for 2 weeks. After the first injection, only the base cream was used for the positive control group. Only the dimethylbenzanthracene solvent and the base cream were used for the negative control group. In the drug-treated groups, after dimethylbenzanthracene injection in each group, enoxaparin emulsion (40, 60, and 80 mg/ml) was applied. After 4 weeks, which tumors were visible, rats were killed. Breast tissue samples were isolated and their dimensions were measured using a Vernier caliper and fixed in 10% formalin. Tissue sections (5- μ m thick) were prepared from paraffin blocks, stained with hematoxylin and eosin, and observed under a microscope. Based on previous studies [23, 31, 37, 38], tumor dimensions in each group were measured and divided with those in the positive control group. As mentioned earlier, tumor size (length and width) was measured using a Vernier clipper, and the tumor volume was calculated using the following formula: $V = (L \times W \times W)/2$, where V is tumor volume, W is tumor width, L is tumor length.

3. Statistics

Data were analyzed using SPSS version 18. Normal distribution of data was assessed using the Kolmogorov-Smirnov test, and after ensuring the normal distribution of data, they were analyzed using one-way ANOVA. Then, Tukey's post hoc test was used to compare the difference between the groups. A P-value less than 0.05 was considered significant.

4. Results

Table 1 shows tumor sizes in the enoxaparin treatment groups (40, 60, and 80 mg/ml) compared with those in the positive control group.

According to Table 1, tumor sizes were significantly smaller in the enoxaparin treatment groups (40, 60, and 80 mg/ml) than in the positive control group. Tumor sizes in the groups which were treated with 60 and 80 mg/ml of enoxaparin were significantly smaller than those which were treated with 40 mg/ml enoxaparin. Moreover, a significant decrease in tumor size was observed in the group which was treated with 80 mg/ml of enoxaparin compared to the group which was treated with 60 mg/ml of enoxaparin. Tumor suppression in the groups with high dose was significantly greater

TABLE 1: Comparison of tumor size in the positive control and enoxaparin treatment groups at different concentrations.

| Groups | Tumor size (Mean \pm SD) | Positive Control | Enoxaparin 40 mg/ml | Enoxaparin 60 mg/ml | Enoxaparin 80 mg/ml |
|---------------------|----------------------------|------------------|---------------------|---------------------|---------------------|
| Positive Control | 1 \pm 0.021 | 1 | 0.019 | 0.015 | 0.009 |
| Enoxaparin 40 mg/ml | 0.67 \pm 0.015 | 0.017 | 1 | 0.019 | 0.011 |
| Enoxaparin 60 mg/ml | 0.33 \pm 0.025 | 0.015 | 0.019 | 1 | 0.019 |
| Enoxaparin 80 mg/ml | 0.16 \pm 0.015 | 0.009 | 0.011 | 0.019 | 1 |

The numbers represent the mean \pm standard deviation of tumor suppression.

than those with the lower dose of enoxaparin. Furthermore, the reduction in tumor growth was dependent on enoxaparin concentration in the emulsion.

Our microscopic figures also, confirmed the tumor size results in different groups. According to the figures, breast tumors in groups with enoxaparin treatment have been reduced in size in a dose-dependent manner. There is no sign of tumor as it is showed in the figure E, the group which was received 80 mg/ml of enoxaparin.

5. Discussion

The results of this study showed that enoxaparin can decrease breast tumor size in a dose-dependent manner in rats. In other words, cutaneous enoxaparin can shrink the tumor size and also, our results are confirmed by microscope which there was no sign of tumor at the high dose of enoxaparin. Our results were confirmed by other studies [7–9]. Enoxaparin in different forms has an inhibitory effect on various cancers [10–15]. Enoxaparin and other derivatives of heparin suppress various cancers [16–18]. However, enoxaparin is reported to have little effect on tumor size in breast cancer in women [26]. It is also reported to have no effects on breast tumor suppression and metastasis [28]. Controversy results may come from different methods of different form of enoxaparin which are applied. It may decrease thromboembolism and partial bleeding by binding to and potentiate anti-thrombin to form a complex that irreversibly inactivates clotting factor Xa, which transforms prothrombin to thrombin. Thus, enoxaparin reduces fibrin clots by reducing thrombin formation [39]. Low-molecular-weight heparins effectively inactivate factor IIa owing to their shorter duration of action than that of unsaturated heparin. Heparin inhibits coagulation factor IIa and tissue factor (TF) expression and activates tissue factor pathway inhibitor (TFPI), which reduces coagulation. Moreover, heparin releases tissue plasminogen and PAI-1 from endothelial cells to promote fibrinolysis [40]. Heparin is similar to glycosaminoglycan in the extracellular matrix. Heparin acts by stabilizing angiogenic growth factor in the extracellular matrix and reduces malignant cell growth by causing apoptosis and cell differentiation. Heparin also regulates the expression of some oncogenes, such as c-myc and c-fos [38, 41]. Since many studies documented that enoxaparin has an anti-tumor effects and also, some studies did not confirm that results. In other words, there are a controversy results in this subject, we aimed to conduct a research about the cutaneous effects of enoxaparin on breast cancer in rats. We wanted to focus on the

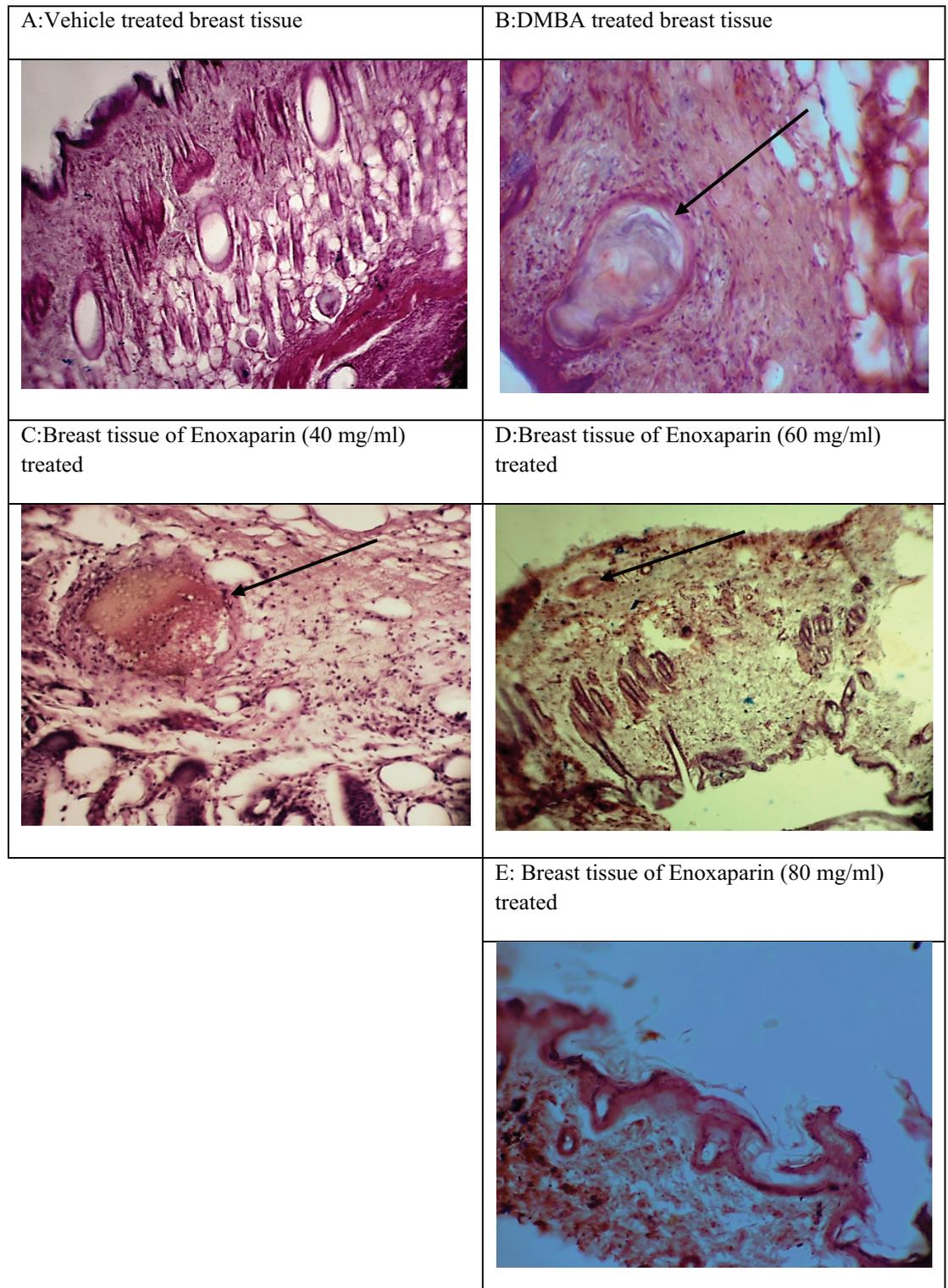


Figure 1: Fig1.A represent the healthy breast tissue, figure Fig1.B represent the positive breast tissue, Fig1.C represent a group that received 40 mg/ml of enoxaparin, figure Fig1.D represent a group that received 60 mg/ml of enoxaparin and Fig1.E represent a group that received 80 mg/ml of enoxaparin. According to the figures, breast tumors in groups with enoxaparin treatment have been reduced in size in a dose-dependent manner. As is showed in the figure E, which was received 80 mg/ml of enoxaparin, there is no sign of tumor. Arrows indicate the tumor place. The magnification was 400 X.

cutaneous absorbance and effects of enoxaparin emulsion on the breast cancer. We showed that the enoxaparin emulsion has its effect but it would be better to analysis the expression of tumor markers and other parameter related to tumor growth and tumor suppression. We are working on the molecular and cellular pathways which are involving in this subject and more studies will be need to clarify this effect.

6. Conclusion

Enoxaparin suppresses breast tumor growth in a dose-dependent manner in rats. Therefore, enoxaparin may be beneficial in suppressing tumor growth in patients with breast cancer. However, more studies are needed at the cellular and molecular levels to validate this tumor suppression effect.

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Conflict of Interest

None declared.

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