Prospective Evaluation

Opioids Inhibit Angiogenesis in a Chorioallantoic Membrane Model

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Background: Angiogenesis is an important characteristic of cancer. Switching from the avascular phase to the vascular phase is a necessary process for tumor growth. Therefore, research in cancer treatment has focused on angiogenesis as a drug target. Despite the widespread use of opioids to treat pain in patients with cancer, little is known about the effect of these drugs on vascular endothelium and angiogenesis.

Objectives: We aimed to investigate the efficacies of morphine, codeine, and tramadol in 3 different concentrations on angiogenesis in hens' eggs.

Study Design: This is a prospective, observational, controlled, in-vivo animal study.

Setting: Single academic medical center.

Methods: This study was conducted on the chorioallantoic membrane (CAM) of fertilized hens' eggs. The efficacies of morphine, codeine, and tramadol in 3 different concentrations were evaluated on angiogenesis in a total of 165 hens' eggs.

Results: Statistically significant differences were found between drug-free agarose used as a negative control and concentrations of morphine of 10 μM and 1 μM, a concentration of tramadol of 10 μM, and concentrations of codeine of 10 μM and 1 μM. Concentrations of morphine of 10 μM and 1 μM showed strong antiangiogenic effects. While codeine had strong antiangiogenic effects at high concentrations, at 0.1 μM it was shown to have weak antiangiogenic effects. However, tramadol at a concentration of 10 μM had only weak antiangiogenic effects.

Limitations: This is just a CAM model study.

Conclusion: In this study, we tested the effects of 3 different opioid drugs on angiogenesis in 3 different concentrations, and we observed that morphine was a good anti-angiogenic agent, but tramadol and codeine only had anti-angiogenic effects at high doses.

Key Words: Morphine, codeine, tramadol, opioid, bevacizumab, chorioallantoic membrane (CAM), angiogenesis

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Pain in cancer patients, particularly in patients with metastatic cancer, causes enormous suffering (1). The majority of advanced-stage cancer patients suffer from moderate-to-severe pain, and in 33% of patients, the pain persists despite curative treatment (2,3). The cause of the pain in more than 80% of metastatic cancer patients is direct infiltration of the tumor (1). Many medical and interventional treatment methods are applied to overcome the pain (4). When the pain is mild to moderate or severe, tramadol and

other opioids are agents that are often administered to achieve effective analgesia with medical therapy.

Angiogenesis is the name given to the formation of new blood vessels from preexisting vascular structures (5). This process—which is involved in wound healing, the female reproductive system, the formation of collateral vessels in chronically ischemic regions and embryological development—is firmly regulated in the body (6,7). For the maintenance of homeostasis, angiogenic arrangement is enabled by the continuation of the harmony between stimulators and inhibitors (8). However, pathological angiogenesis is associated with the deterioration of this harmony. Cancer, rheumatoid arthritis, and heart disease are examples of diseases that lead to the formation of pathological angiogenesis (5).

Angiogenesis is an important characteristic of cancer (9,10). The transition process from the avascular phase to the vascular phase is necessary for the growth of tumors, which is why research in cancer treatment has focused on angiogenesis as a drug target. Many angiogenesis inhibitors have been found and developed over the years, from endogenous angiogenesis inhibitors such as proteins, protein fragments, and microRNAs, to monoclonal antibodies (5).

The drugs that are most frequently used as angiogenesis inhibitors are vascular endothelial growth factor (VEGF) and VEGF receptor inhibitors. These inhibitors are monoclonal antibodies that bind to VEGF and VEGF receptors (11). Bevacizumab is a recombinant humanized monoclonal IgG1 antibody and a nonspecific inhibitor of VEGF. VEGF is the most potent and specific of all endothelial cell mitogens among angiogenic factors (12-13). Most produced by VEGF tumors is a heparin-binding growth factor specific to vascular endothelial cells (14). VEGF stimulates angiogenesis and lymphangiogenesis and increases vascular permeability (15). Moreover, it stimulates the migration of endothelial cells. All of these effects lead to tumor invasion and metastasis (16). VEGF represents a family of growth factors consisting of 7 members, the most important of which is VEGF-A. VEGF-A has the most powerful relationship with angiogenesis, is the most studied factor, and plays a role in pathological angiogenesis. Therefore, most anti-VEGF treatments focus on this factor (17). VEGF receptors include VEGF R-1, VEGF R-2, VEGF R-3, and the neuropilin-1 and neuropilin-2 receptors. VEGFR-1 has positive and negative angiogenic effects (18). Bevacizumab, which is the first anti-angiogenic agent approved for use in cancer treatment, was found to reduce serum VEGF levels to levels that could not be measured and to inhibit the growth of different tumors when combined with chemotherapy in phase I studies (19). Studies of the clinical effectiveness of bevacizumab in advanced colorectal cancers, breast cancers, non-small cell lung cancers, renal cell carcinomas, pancreatic cancers, ovarian cancers, and prostate cancers that did not respond to hormone therapy are ongoing (20).

There have been diametrically opposed and conflicting results in the literature about the effects of morphine on angiogenesis both in mammals (21-24) and in the chick embryo chorioallantoic membrane (CAM) model (25). For this reason, we believe that new studies are needed to eliminate speculation about the effects of morphine on angiogenesis. Moreover, a literature search found no studies investigating the effects of tramadol and codeine on angiogenesis.

In this study, we aimed to investigate the effectiveness of codeine, morphine, and tramadol, which are frequently used in the treatment of chronic pain in cancer patients, on angiogenesis using CAM as a model. To our knowledge, this study is the first in which the effects of codeine and tramadol on angiogenesis were investigated in chicken tissue. Additionally, we hope that this study might contribute to the elucidation of the existing confusion on the effects of morphine on angiogenesis.

Methods

Study Design

This study was designed as an in-vivo chicken animal study and was conducted on the CAM from fertilized hens' eggs in the pain center of a university hospital, following Institutional Review Board approval in February 2015. The study was planned as a prospective, observational, controlled trial.

Preparation of the Pellets

The pellets were prepared as in previous similar studies (26-27). Briefly, agarose was added to distilled water to obtain a 2.5% (weight/volume) solution. This solution was placed in an autoclave under one unit of atmospheric pressure and a temperature of 121°C to provide dissolution and sterilization for 15 minutes. Subsequently, the solution was left to cool in a sterile container to 37°C. The study drug was added at this stage. Codeine, tramadol, and morphine were used as the study drugs. While drug-free agarose pellets were used as a negative control, bevacizumab 1 μM, which

has been proven to be an anti-angiogenic agent, was used as a positive control. Three different concentrations were prepared for each drug (0.1, 1, and 10 μM per 10 μL pellet). One hundred sixty-five pellets were prepared, including drug-free agarose pellets and bevacizumab 1 μM pellets. Using a micropipette, 10 μL drops of this mixed solution were placed on previously sterilized, vertical, cylindrical, stainless steel rods, which were 5 mm in diameter, to obtain circular pellets with the same diameter. The pellets were then left to solidify at room temperature in a sterile setting.

Eggs Incubation and Experiments

A total of 165 eggs were used for this study. Fifteen eggs were used for each drug at 3 different concentrations. A total of 11 different groups were formed, including the positive and negative control groups. The fertilized hens' eggs were incubated in the horizontal position under environmental conditions of 37.5°C in temperature and 80% relative humidity. On the fifth day of the incubation period, 5 mL of albumen were obtained through the eggshell using a syringe from the bottom of the egg, and then an eggshell piece of 2–3 cm in diameter was removed gently from the top of the egg. Looking into the egg through the hole, whether development of the CAM was normal was verified (Fig. 1). If rotten or dead embryos were detected, the eggs were excluded from the study. The hole on the egg shell was sealed with stretch film, and the eggs were returned to incubation for 72 hours for the CAM to attain a diameter of 2 cm. On the eighth day, the stretch film that covered the egg was removed, and the pellets were placed on the CAM. The eggs were covered with stretch film again and placed in incubation for 24 hours. After 24 hours, final assessment was performed by an independent observer who was not yet involved in the study to assess the level of angiogenesis. At this stage, eggs containing an infected or dead embryo were excluded from the study.

Fig. 1. *Photo of the egg showing the inside to verify whether developmen of the CAM was normal.*

Outcome Measurements

Anti-angiogenic scoring

The inhibitory effects of the drugs on angiogenesis in the CAM were evaluated under a stereoscopic microscope and were assessed according to the scoring system used previously in several studies (28-29). In this scoring system, the change in the density of the capillaries around the pellet and the extent of the effect are evaluated. A score of 0 indicated the absence of any demonstrable antiangiogenic effects (normal embryo and no difference in surrounding capillaries), a score of 0.5 represented a very weak effect (no capillary-free area but an area no larger than the pellet area with a reduced density of capillaries), a score of 1 indicated a weak to moderate effect (a small capillary-free area or a small area with significantly decreased density of capillaries; less than double the size of the pellet involved), and a score of 2 indicated a strong antiangiogenic effect (a capillary-free area around the pellet, equal to or greater than double the size of the pellet itself) (Fig. 2). The following equation was used to calculate the average score of each group:

Average score

[(Number of Score 2 eggs) x 2)] + [(Number of Score 1 eggs) x 1] / Total number of eggs

According to this scoring system, a score less than 0.5 indicated that there was no antiangiogenic effect, a score of 0.5–1 indicated a weak antiangiogenic effect, and a score >1 indicated a strong antiangiogenic effect.

Statistical methods

All of the data were analyzed using the MedCalc Statistical Software, version 15.8 for Windows (MedCalc Software bvba, Ostend, Belgium). Repeated measure-

ments ANOVA (analysis of variance) parametric testing was used to compare the effects of different doses of drugs on angiogenesis with the positive and negative control groups. Additionally, the paired samples t test was used to compare the effects of different doses of drugs. $P < 0.05$ was considered statistically significant in all analyses.

Results

When the effects of drug-free agar were used as the negative control, and the effects of different doses of morphine on angiogenesis were compared, a statistically significant difference was found for the 10 μM and 1 μM concentrations of morphine, while no significant difference was found with the 0.1 μM concentration of morphine (*P* values < 0.007, 0.001, and 0.26, respectively). When the effects of bevacizumab 1 μM were used as the positive control, and the effects of different doses of morphine on angiogenesis were compared, no significant difference was found with any concentration of morphine (10, 1, and 0.1 μM) (*P* values < 0.78, 1.00, and 1.00, respectively) (Table 1, Fig. 3).

Similarly, when the effects of drug-free agar and different doses of tramadol on angiogenesis were compared, a statistically significant difference was found for the 10 μM concentration of tramadol, while no significant difference was found with the 1 μM and 0.1 μM concentrations of tramadol (*P* values < 0.02, 0.06, and 0.20, respectively). When the effects of bevacizumab 1 μM and different doses of tramadol on angiogenesis were compared, no significant difference was found for 10 μM of tramadol, whereas a statistically significant difference was found for 1 μM and 0.1 μM of tramadol (*P* values ≤ 1.00, 0.03, and 0.02, respectively) (Table 2, Fig. 4).

Again, when the effects of drug-free agar and different doses of codeine on angiogenesis were compared, statistically significant differences were found for the 10 μM and 1 μM concentrations of codeine, but no significant difference was found for the 0.1 μM concentration of codeine (*P* values ≤ 0.009, 0.01, and 1.00, respectively). When the effects of bevacizumab 1 μM were used as the positive control, and the effects of different doses of codeine on angiogenesis were compared, no significant differences were found for 10 μM and 1 μM of codeine, although a statistically significant difference was found for 0.1 μM of codeine (*P* values ≤ 1.00, 0.09, and 0.004, respectively) (Table 3, Fig. 5).

The results obtained after comparing the effects on angiogenesis of different doses of the same drug with each other are shown in Figs. 3, 4 and 5. In summary, no significant differences were found in any of

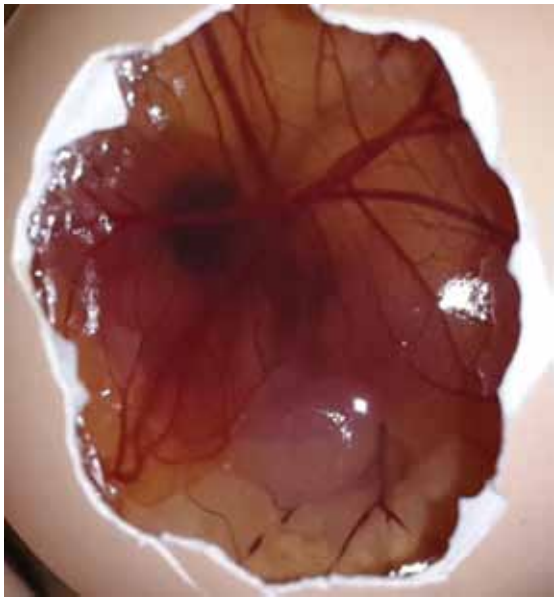

Fig. 2. *View of capillaries in yolk.*

Table 1. *Pairwise comparisons of controls and bevacizumab with different morphine doses.*

Factors			Mean difference	Std. error	*P*[a]	95%CI[a]
Control	-	M-10	-1.071	0.170	0.0074	-1.805 to -0.337
	-	M-1	-1.214	0.149	0.0018	-1.856 to -0.572
	-	M-0.1	-0.929	0.317	0.2624	-2.296 to 0.439
B-1	-	M-10	0.429	0.202	0.7814	-0.444 to 1.301
	-	M-1	0.286	0.286	1.0000	-0.948 to 1.519
	-	M-0.1	0.571	0.317	1.0000	-0.796 to 1.939

a Bonferroni corrected

Control indicates drug-free agarose, M-10 indicates 10 μM morphine, M-1 indicates 1 μM morphine, M-0.1 indicates 0.1 μM morphine, and B-1 indicates 1 μM bevacizumab.

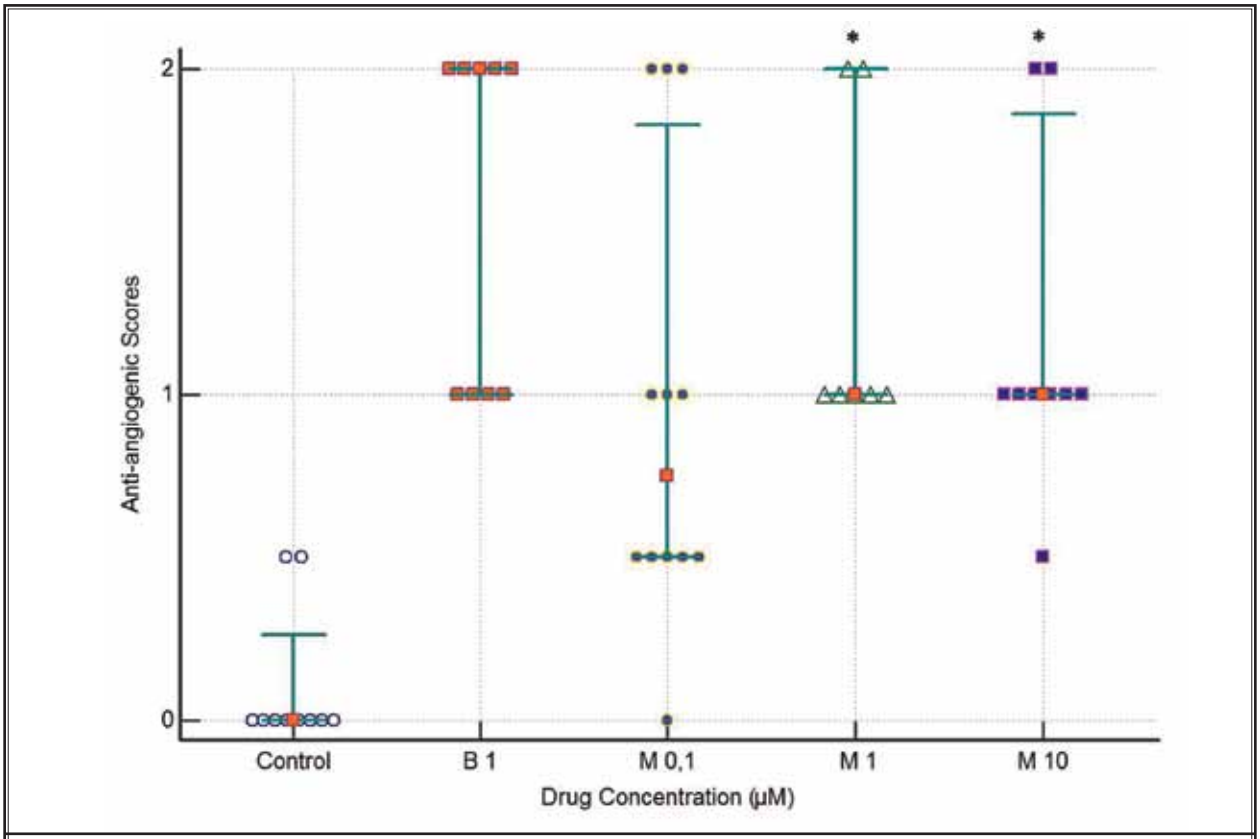

Fig. 3. *Effects of bevacizumab 1 µM were used as the positive control, and the effects of different doses of codeine on angiogenesis were compared, no significant differences were found for 10 µM and 1 µM of codeine.*

Table 2. *Pairwise comparisons of controls and bevacizumab with different tramadol doses.*

Factors			Mean difference	Std. error	*P*[a]	95% CI[a]
Control	-	T-10	-0.938	0.199	0.0218	-1.740 to -0.135
	-	T-1	-0.563	0.148	0.0660	-1.157 to 0.0319
	-	T-0.1	-0.438	0.148	0.2094	-1.032 to 0.157
B-1	-	T-10	0.438	0.305	1.0000	-0.793 to 1.668
	-	T-1	0.813	0.188	0.0342	0.0570 to 1.568
	-	T-0.1	0.938	0.199	0.0218	0.135 to 1.740

a Bonferroni corrected
Control indicates drug-free agarose, T-10 indicates 10 µM tramadol, T-1 indicates 1 µM tramadol, T-0.1 indicates 0.1 µM tramadol, and B-1 indicates 1 µM bevacizumab

the comparisons of morphine and tramadol among themselves, but a statistically significant difference was found when comparing codeine concentrations of 10 µM with 0.1 µM and 1 µM with 0.1 µM (*P* values of 0.01 and 0.01, respectively, for codeine).

When the average scores of the drugs were examined, the average score of drug-free agar was 0 (demonstrating no antiangiogenic effect), while bevacizumab 1 µM showed a very strong anti-angiogenic effect, as expected (score 1.56). Concentrations of morphine of 10 µM and 1 µM also showed strong antiangiogenic effects, whereas 0.1 µM was shown

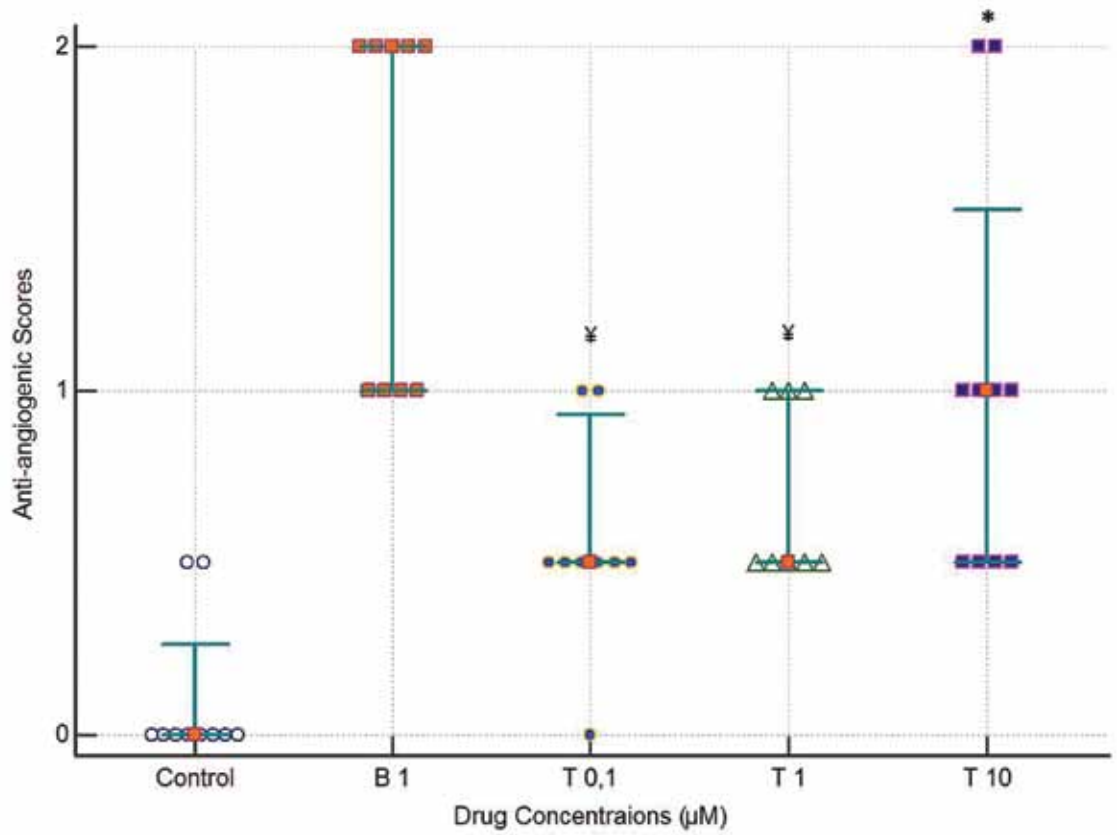

Fig. 4. Pairwise comparisons of controls and bevacizumab with different tramadol doses.

Table 3. *Pairwise comparisons of controls and bevacizumab with different codeine doses.*

Factors			Mean difference	Std. error	*P*[a]	95% CI[a]
Control	-	C-10	-1.125	0.206	0.0094	-1.955 to -0.295
	-	C-1	-0.625	0.125	0.0157	-1.129 to -0.121
	-	C-0.1	-0.250	0.164	1.0000	-0.909 to 0.409
B-1	-	C-10	0.250	0.250	1.0000	-0.757 to 1.257
	-	C-1	0.750	0.211	0.0935	-0.101 to 1.601
	-	C-0.1	1.125	0.183	0.0047	0.388 to 1.862

a Bonferroni corrected
Control indicates drug-free agarose, C-10 indicates 10 μM codeine, C-1 indicates 1 μM codeine, C-0.1 indicates 0.1 μM codeine, and B-1 indicates 1 μM bevacizumab.

to have a weak antiangiogenic effect. However, although the tramadol concentrations of 1 μM and 0.1 μM showed no antiangiogenic effects, it was revealed that the 10 μM concentration had a weak antiangiogenic effect. It was revealed that codeine had a strong antiangiogenic effect at high concentrations and a negligible level of antiangiogenic effect at concentrations of 1 μM. It could be said that it had no antiangiogenic effect at concentrations of 0.1 μM (Fig. 6).

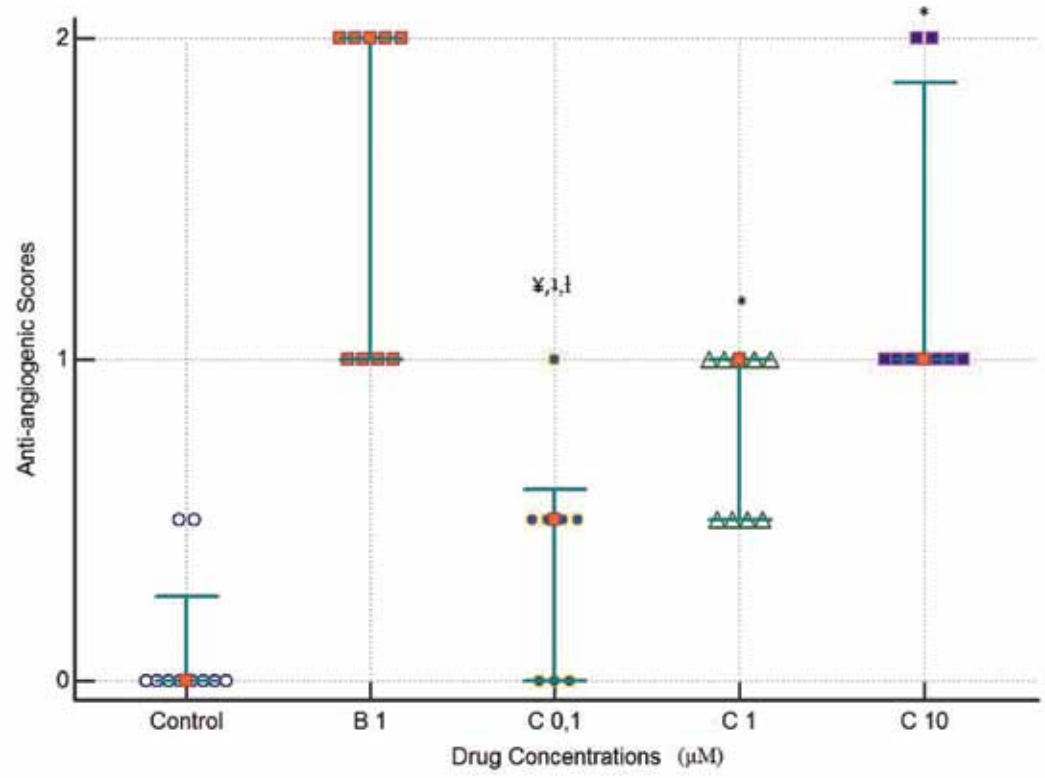

Fig. 5. *Pairwise comparisons of controls and bevacizumab with different codeine doses.*

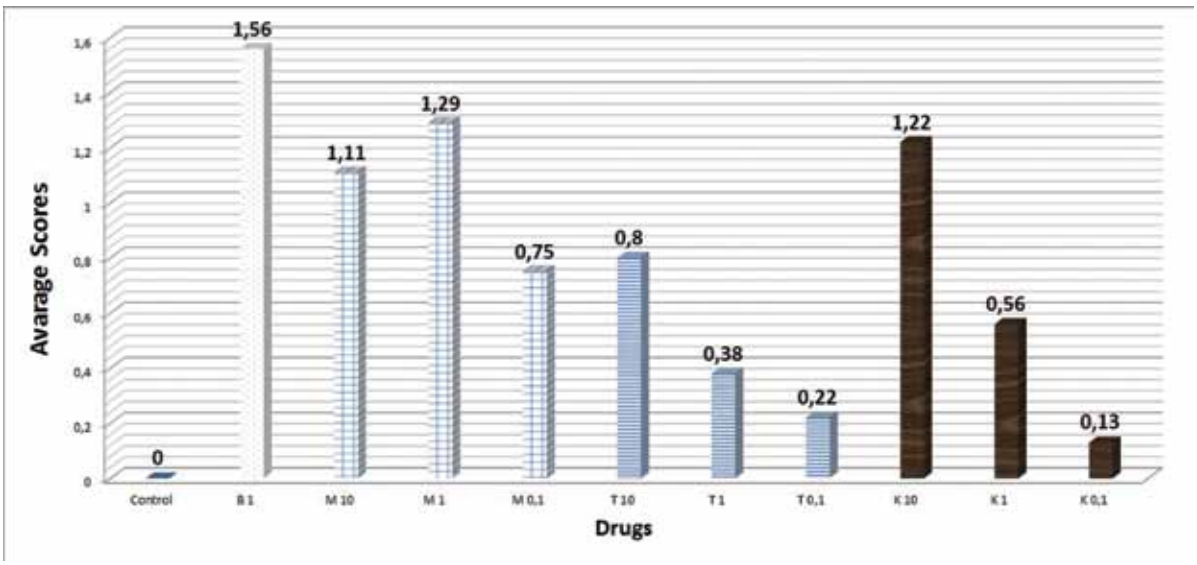

Fig. 6. *Average scores of drugs.*

Discussion

Angiogenesis, which is the formation of new blood vessels from pre-existing vasculature, is closely associated with the etiology and pathogenesis of several pathological conditions, including tumor progression and metastasis (21). Angiogenesis is required for invasive tumor growth and metastasis and it represents an important point in the control of cancer progression (22). Despite the widespread use of opioids to treat pain in patients with cancer, little is known about the effects of these drugs on vascular endothelium and angiogenesis (21).

Opioids are the most effective analgesics used for chronic cancer pain (22). Although the effects of opioids on the central nervous system have been well documented, what we know about their effects on extraneuronal systems is insufficient. In the central nervous system, opioids act via specific, well-defined receptors, such as mu, kappa, and delta, and they have been associated with several neuro-psychological effects, including analgesia, tolerance, and addiction (21,30). However, there is great confusion about the effect of opioids on the growth of cancer cells and cancer angiogenesis. The main reason for this confusion is that the results obtained from studies have been inconsistent.

Morphine, as a strong analgesic agent, has been shown to inhibit the growth of cancer cells in some studies (31-34), although it was claimed that, in contrast, that morphine did lead to the growth of tumor cells in other studies (35-36). Similarly, there have been conflicting results about the effects of morphine on angiogenesis in mammals, including humans, and in a CAM model. Some studies have claimed that morphine inhibited angiogenesis (23,25), but others have claimed that it stimulates angiogenesis (21-22). A study conducted by Koodie et al (23) in mice indicated that morphine treatment could potentially decrease leukocyte transendothelial migration and reduce angiogenesis-associated tumor growth. These researchers emphasized that the use of morphine for cancer pain management might be beneficial through its effects on angiogenesis. Similarly, in the study conducted by Pasi et al (25), the effects of beta-endorphin (beta-EP) and morphine sulfate (MS), in the presence and absence of naloxone (NX), on chicken chorioallantoic membrane assay were studied as a function of blood vessel proliferation. A 50% reduction in blood vessel proliferation was observed with 10 µg of beta-EP or 5 µg of MS per egg, compared to controls. In contrast, Gupta et al (21) showed that morphine in clinically relevant doses promoted tumor neovascularization in a human breast tumor xenograft model in mice, leading to increased tumor progression. In this study, it was also reported that naloxone itself had no significant effect on angiogenesis. These authors concluded that these results indicated that the clinical use of morphine could potentially be harmful in patients with angiogenesis-dependent cancers. Gupta et al (21) concluded that the results obtained by Pasi et al (25) showing that morphine inhibited angiogenesis were related to the use of high doses of morphine (1.65, 3.3, and 16.5 mM morphine [5, 10, or 50 g/4 L]). They indicated that, at high doses, morphine is cytotoxic to endothelial cells, and they stated that the serum/plasma concentration of morphine was only between 2 nM and 3.5 µM in humans. Therefore, they used 1 µM of morphine in their studies, stating that this dose was within the limits of clinical use, and they claimed that morphine at this dose was proangiogenic and stimulated angiogenesis.

In our study, we used 3 different doses (0.1, 1, 10 µM) to test the effects of morphine on angiogenesis. We found the angiogenesis score to be 0.75 at a dose of 0.1 µM. Although this score was not statistically significantly different compared to the control group, we found that, although it was weak, morphine still had an anti-angiogenic effect even at this dose. When testing the dose (1 µM) in the range of the serum/plasma concentration indicated by Gupta et al (21) in contrast to their claims, we found a significantly strong anti-angiogenic effect.

Similar to the study by Gupta et al (21) in a study recently published, Bimonte et al (22) demonstrated that morphine at clinically relevant doses did promote angiogenesis and did increase breast cancer progression in breast tumors in mice, compared to controls. Additionally, these researchers asserted that tumor-enhancing effects of morphine occurred after the administration of low daily doses or single doses of morphine, while tumor suppression occurred after chronic high doses of morphine. They suggested that these contrasting results might be associated with different concentrations and/or the time of administration of morphine. In contrast to their claims, in this study we achieved a significant anti-angiogenic effect, although we administered a single dose, as well as a low dose, of morphine.

In a very recent study conducted by Doornebal et al (24), unlike in the literature cited above, the authors claimed that morphine did not have any effects on tumor growth or angiogenesis. In this study, using

2 preclinical mouse models for metastatic invasive lobular and HER21 breast cancer, they showed that analgesic doses of morphine did not affect mammary tumor growth. Consistent with these findings, they also showed that the number and size of tumor-associated blood vessels, as well as the composition of tumor-infiltrating immune cells, were not altered by morphine. The researchers emphasized that opioid analgesics could be used safely for perioperative pain in patients with cancer, as a result of this study.

In this study, we investigated the effects of tramadol and codeine on angiogenesis, in addition to those of morphine. When we examined literature in English, we did not come across either in-vivo or in-vitro research that investigated the effects of tramadol and codeine on angiogenesis. In this regard, we believe that it is significant that our study was a first in terms of using these drugs. We observed that tramadol had some anti-angiogenic effects at a dose of 10 μM and found that it has no effects on angiogenesis at concentrations of 1 and 0.1 μM. We observed that codeine, however, significantly inhibited angiogenesis at high doses only, namely at doses of 10 μM. We therefore observed that among these 3 agents, morphine was a more potent inhibitor of angiogenesis than the others. We believe that these results will be significant in providing a preliminary idea for researchers who conduct studies in the future.

Our knowledge regarding how opioids inhibit angiogenesis is insufficient. However, the anti-angiogenic effect of opioids is considered to occur by the kappa opioid receptor ligands. The kappa opioid receptor agonists exhibit as an anti-angiogenic factor, hindering the angiogenic shift in angiogenesis by prohibiting the receptor's expression for VEGF. VEGF signaling plays a key role during de novo neovascularization seen in embryogenesis and tumor growth (37). It has been observed that the kappa opioid receptors—not the mu and delta opioid receptors—have been heavily expressed in vascular progenitors and endothelial cells, and that they have negatively regulated in-vitro vascular formation and endothelial cell differentiation by cAMP/PKA signalization inhibition. Activating kappa receptors by opioid agonists such as U50, 488H, and TRK820 inhibits the VEGFR2 and NRP1 receptors. For example, a significant vascular increase and density has not been encountered in mice without kappa opioid receptors during their early embryonic life stages (38).

So how can the mutually contradictory and inconsistent results of opioids on angiogenesis be explained? Yamamizu et al (38) showed in their studies that at low dosages such as 0.1 – 10 μg/kg twice a day, TRK820 (a Kappa agonist) exhibits an inhibitor effect on tumor angiogenesis and growth, whereas at quite elevated doses (150 μg/kg) it does not exhibit any affect on the growth of the tumor. They also considered that since this might be a result of developing a tolerance to the drug, the drug could also have a dual effect on tumor angiogenesis. The differing results of the effect of 3 different opioids on angiogenesis in our study can also be explained in this manner. For example, while morphine is a powerful kappa receptor agonist, codeine is a weak agonist. Tramadol, meanwhile, has almost no kappa receptor affinity. Additionally, another contradictory finding on this point is that opioids have the ability to "partially" stimulate angiogenesis by COX-2 activation. This increase causes the production of prostoglandin-E2, which on the one hand encourages angiogenesis, yet on the other hand also has a direct effect on the growth of a tumor (39).

Perhaps the greatest technical problem in angiogenesis studies has been determining the assay to use. Chick embryo CAM is an extraembryonic membrane that is commonly used in vivo to study both angiogenesis and anti-angiogenesis (40). The CAM has a rich vascular system that develops within the mesodermal layer and is served by paired allantoic arteries and paired allantoic veins. The surface area of the CAM increased from approximately 6 cm^2 at day 6 to 65 cm^2 by day 14. In addition to being an angiogenesis model, CAM assay has many applications, including in-vivo evaluation of drug delivery systems, tumor implantation, and toxicological studies (40-42). The main reason for our selecting CAM assay was our belief that a tissue having such an abundant vascular structure would provide us with a very good idea about the effects of drugs on angiogenesis. However, perhaps the most restrictive aspect of our study, in our opinion, was that it is not known what the exact results of our study would be in mammals, especially humans. In this respect, an important limitation of our study is the type of our study. In recent times, major efforts in the treatment of tumors have been directed toward completely putting a stop to angiogenesis, which is a very important event in tumor development. Unfortunately, after a period of time, resistance develops to drugs used for this purpose, and vascularization continues from the point where it last stopped (38). Because the subject of this study was not "tumor angiogenesis," unfortunately no thoughts can be provided on our study. In future studies, researchers

may make efforts toward this end. Apart from this, the most important characteristic of our study is to research the effect of opioids on only embryological angiogenesis. Because of this, the effect of opioids on tumor angiogenesis and tumor growth in human, especially, and in other mammals cannot be discounted from the study. This is because certain experimental studies being done into the subject have shown that opioids have several effects that facilitate the metastasis of cancer cells. In these studies, it has been claimed that morphine contributes to the proliferation of cancer cells and survival of them, activating the mitogen-activated protein kinase and AKT signaling paths in tumor cells (39). On the other hand, certain other studies have focused on the effect of mu opioid receptors on regulating the growth of and metastasis of tumors (43-44). In these studies it has been shown that mu receptors have an effect on the growth of and metastasis of tumors. In another study involving a small number of patients (a total of 28 patients), a significant increase in NK cell expression has been encountered in the group of patients not receiving opioids for anesthetic purposes, compared to the group of patients who were receiving opioids (45). As we have stated, however, the number of patients in this study is very small, and the study is very far from providing a definitive conclusion. Accordingly, there is no doubt that in order to eliminate the confusing results regarding the effects of opioids on cancer angiogenesis in human beings, there is a need for well-planned randomized controlled trial.

In conclusion, in this study in which we tested the effects on angiogenesis of 3 different opioid drugs at 3 different concentrations, we observed that while morphine at 10 μM is a very good anti-angiogenic agent that is like bevacizumab, morphine at 1 μM is moderate, and morphine at 0.1 μM is a poor anti-angiogenic agent. Additionally, tramadol and codeine are anti-angiogenic agents only at high doses. In order to fully know the effects of opioids on human tumor angiogenesis, there is a need for randomized controlled trials.

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The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

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