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RESEARCH ARTICLE

Effects of second-generation H1-antihistamine drugs on angiogenesis in *in vivo* chick chorioallantoic membrane model

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ABSTRACT

Background: Literature on the effects of second-generation H1-antihistamines on angiogenesis is limited.

Objectives: To investigate the effects of cetirizine, desloratadine, and rupatadine (second-generation H1-antihistamines commonly used in dermatology clinics) on angiogenesis in an *in vivo* chick chorioallantoic membrane (CAM) model.

Methods: The study was approved by the local ethics committee on animal experimentation. Forty fertilized specific pathogen free eggs were incubated and kept under appropriate temperature and humidity control. Drug solutions were prepared in identical concentrations by dissolving powders in phosphate-buffered saline (PBS). On the third day of the incubation, a small window was opened on the CAM and 0.1 mL desloratadine (1.5 μg/0.1 mL) in the first group, 0.1 mL cetirizine (1.5 μg/0.1 mL) in the second group, 0.1 mL rupatadine in the third group (1.5 μg/0.1 mL), and PBS (0.1 mL) in the fourth group were administered by injection. On the eighth day of incubation, the vascular structures of the CAMs were macroscopically examined and standard digital photographs were taken. The digital images were analyzed and data including mean vessel density, thickness, and number were compared between groups. $p < 0.05$ was considered statistically significant.

Results: Vessel densities were similar in the desloratadine, cetirizine, and control groups, whereas they were significantly less in the rupatadine group ($p = 0.01$). Furthermore, the rupatadine group had significantly lower vessel thickness and number compared with the other groups ($p < 0.05$ for both).

Conclusions: Rupatadine showed anti-angiogenic effects in the chick CAM model, compared with desloratadine and cetirizine. The anti-angiogenic effect of rupatadine could be due to its platelet-activating factor (PAF) receptor inhibition. Thus, rupatadine could be a treatment agent in pathological processes in which angiogenesis is responsible. Further studies with larger series are needed to clarify this potential.

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Introduction

Literature on the effects of second-generation antihistamine drugs on angiogenesis is limited. In addition, no study has evaluated the effects of these drugs on angiogenesis in the chick chorioallantoic membrane (CAM) model.

CAM is rich in vascular structures and is a convenient, low-cost, and practical *in vivo* experimental model to study angiogenic and anti-angiogenic factors [1–3].

In this study, we aimed to investigate the effects of cetirizine, desloratadine, and rupatadine, which are second-generation H1-antihistamine drugs commonly used in the clinic, on angiogenesis in an *in vivo* chick CAM model.

Methods

Forty fertilized specific pathogen free eggs were used in this experimental study, which was approved by the local ethics committee on animal experimentation. The eggs were randomly distributed into four groups of 10 eggs. All the fertilized eggs were placed in the incubator after their shells were sterilized and they were kept under appropriate temperature and humidity control.

The rupatadine (Sigma CDS022916), desloratadine (Sigma D1069), and cetirizine (Sigma C3618) were purchased from Sigma-Aldrich (St. Louis, MO).

Drug solutions were prepared in identical concentrations by dissolving powders in phosphate-buffered saline (PBS) in accordance with the manufacturer's requirements. PBS, which was also used in the preparation of drug solutions, was used as a control group.

On the third day of the incubation, after first sterilizing the egg shell, a small window was opened on the CAM and 0.1 mL desloratadine (1.5 μg/0.1 mL) in the first group, 0.1 mL cetirizine (1.5 μg/0.1 mL) in the second group, 0.1 mL rupatadine in the third group (1.5 μg/0.1 mL), and PBS (0.1 mL) inthe fourth group were administered by injection. The eggs were placed in the incubator by attaching a sterile film over the

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broken part of the shell. On the eighth day of incubation, the vascular structures on the CAMs were macroscopically examined and standard digital photographs were taken. Deformation of the embryo during extraction, bleeding, vascular rupture, or signs of infection was defined as exclusion criteria.

Images were obtained using a Canon EOS 600 D digital single-lens reflex camera (Canon USA, Melville, NY). Digital images obtained from five equal areas in every CAM were analyzed according to image J software [4], and differences between groups in terms of angiogenesis were evaluated. Data including mean vessel density, thickness, and number were compared between groups. SPSS version 18.0 (SPSS Inc., Chicago, IL) was used for the statistical analysis. A Kruskal–Wallis test was used for comparisons between groups. $p < 0.05$ was considered statistically significant.

Results

Eight eggs in each group were included in the further analysis. Vessel densities were similar in the desloratadine, cetirizine, and control groups, whereas vessel density was significantly less in the rupatadine group ($p = 0.01$) (Figure 1). The desloratadine, cetirizine, and control groups were similar in thickness and number of vessels. In contrast, vessel thickness and number were significantly lower in the rupatadine group ($p = 0.018$ *vs.* $p = 0.006$).

Discussion

H1 antihistamines are widely used drugs to treat histamine-dependent symptoms in various allergic and dermatological diseases. They are not receptor antagonists but are inverse agonists in that they produce the opposite effect on the receptor to histamine. First- and second-generation antihistamines have similar pharmacological effects and therapeutic applications, but second-generation antihistamines are more selective for peripheral H1 receptors and have fewer adverse effects [5].

Some second-generation antihistamines also have important additional anti-inflammatory effects. Several studies have shown the anti-inflammatory effect of second-generation H1-antihistamines result from downregulation of the activation of nuclear factor κ-light-chain-enhancer of activated B-cells (NF-κB), a ubiquitous transcription factor that regulates the production of a number of proinflammatory cytokines and adhesion proteins, including interleukin (IL)-1β, IL-6, IL-8, tumor necrosis factor-α (TNF-α), and granulocyte macrophage colony stimulating factor (GM-CSF) [6].

Angiogenesis, the formation of new vessels from existing ones, is a process involved in physiological conditions, such as development and wound healing, and also in pathological conditions, including cancer, infection, arthritis, and inflammatory diseases. It is a dynamic, multistep process involving endothelial cell proliferation, migration, and differentiation. Under physiological conditions, angiogenesis is active for short time periods and is subsequently inhibited, whereas inappropriate induction of angiogenesis is a hallmark of a wide range of pathologic conditions [7].

In contrast, controlled induction of angiogenesis has shown therapeutic efficacy in various conditions, including wound healing and revascularization of ischemic tissue. Angiogenesis is a dynamic process and various vascular-endothelial growth factor (VEGF)-related and VEGF-independent pathways have been shown to play a role in angiogenesis [7].

Rupatadine (8-chloro-11-[1-[(5-methyl-3-pyridinyl)methyl]-piperidin-4-ylidene]-6,11-dihydro-5H-benzo[5,6]cyclohepta[1,2-b] pyridine fumarate) is a selective, long-acting second-generation non-sedating H1-antihistamine. Rupatadine has a high affinity for the H1 receptor; this activity has been shown in various *in vitro* and *in vivo* models [8]. In addition to its

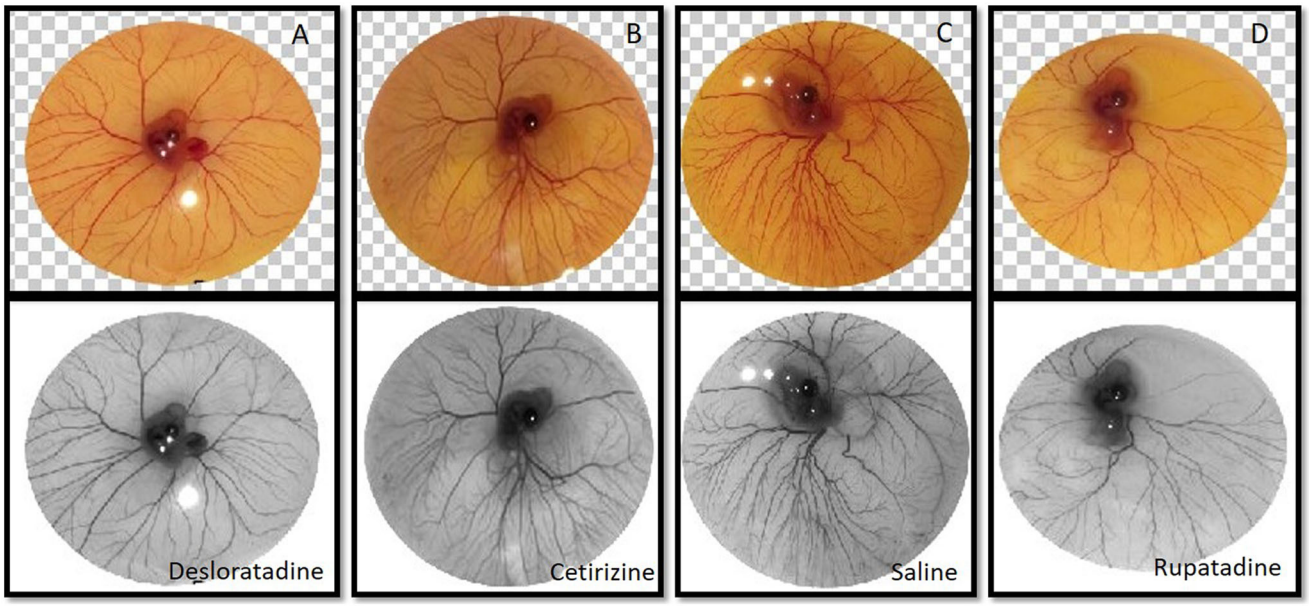

Figure 1. Vessel densities in the chick chorioallantoic membrane model. Vessel densities were similar in the desloratadine (A), cetirizine (B), and saline groups (C), whereas vessel density was significantly less in the rupatadine group (D) ($p = 0.01$).

histamine antagonistic effect, rupatadine has an additional antagonistic effect on platelet-activating factor (PAF) [8].

PAF (1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine) is an endogenous phospholipid mediator of inflammation that is released early by various cell types. Its biological actions are mediated through the activation of the G protein-coupled receptor PAF receptor found on most cells, including dendritic cells, platelets, monocytes, mast cells, granulocytes, B lymphocytes, and keratinocytes [9]. It is involved in cellular activation, intracellular signaling, apoptosis, and various inflammatory reactions [9].

PAF has been shown to upregulate the secretion of a variety of cytokines, including IL-1, IL-6, and TNF-α [9]. It has been implicated in the pathogenesis of various pathologic conditions, including asthma and other allergic conditions, inflammatory bowel disease, rheumatoid arthritis, multiple sclerosis, endotoxic shock, and dermal inflammation [9].

The involvement of PAF in angiogenesis has been shown in several studies. PAF enhances vascular permeability, directly stimulates the *in vitro* migration of endothelial cells, and promotes *in vivo* angiogenesis [10–17]. Some studies have suggested that PAF could contribute to angiogenesis by stimulating the production of VEGF, TNF-α, and hepatocyte growth factor [10–18].

In addition to antihistaminic and anti-PAF effects, rupatadine has also other anti-inflammatory and anti-allergic effects. It inhibits the release of leukotriene C4 from peritoneal rat mast cells and the release of TNF-α from human mast cells. Furthermore, rupatadine inhibits eotaxin-induced eosinophil chemotaxis and inhibits PAF- and LTB4-induced human neutrophil chemotaxis. In a study by Ramis et al. rupatadine was shown to be more effective in inhibition of PAF- and LTB4-induced human neutrophil chemotaxis than other antihistamines, such as cetirizine, fexofenadine, loratadine, and mizolastine [19].

In a study investigating the inhibitory effects of rupatadine, desloratadine, levocetirizine, and fexofenadine on proinflammatory cytokine (IL-6 and IL-8) secretion in human umbilical venous endothelial cells (HUVEC) activated by histamine, rupatadine showed the lowest IC50 value, followed by desloratadine, levocetirizine, and fexofenadine [20]. In addition, several studies have observed inhibition of IL-5, IL-6, IL-8, GM-CSF, and TNF-α secretion, as well as expression of the allergy-associated adhesion molecules (CD18 and CD11b) and NF-κB [21].

This study evaluated the angiogenic effects of second-generation antihistamines on the CAM angiogenesis model and found a significant anti-angiogenic effect of rupatadine. This effect was not observed with desloratadine and cetirizine at the same dose.

This anti-angiogenic effect of rupatadine could be due to the its anti-inflammatory and PAF receptor inhibition effects. Similarly, there are studies in the literature showing that PAF receptor inhibition reduces cancer-associated angiogenesis, angiogenesis in HUVEC, and corneal neovascularization [10–17].

The previous literature supports our study findings. This study findings could provide the basis for new studies on the inhibition of rupatadine on various pathological conditions, such as cancer angiogenesis.

The limitations of the present study are as follows:

1. The CAM model could be considered a screening test in terms of anti-angiogenesis and the results might need to be supplemented by more sensitive angiogenesis studies, such as HUVEC in hydrogel or cancer angiogenesis;
2. Chick embryos might not exactly match human tissue; thus, the results might not be generalizable to humans;
3. Although the number of embryos is sufficient for statistical analysis, it was not high due to ethical concerns. More sensitive results could be obtained with a larger sample size.

Conclusion

In conclusion, in our study, rupatadine showed anti-angiogenic effects on the chick CAM model. Thus, rupatadine could be a potential treatment agent in pathological processes (e.g. cancer angiogenesis, corneal neovascularization) in which angiogenesis is responsible, and further studies with larger series are needed to clarify this potential.

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Disclosure statement

The authors have no conflict of interest to declare.

IRB approval status

Reviewed and approved by Afyon Kocatepe University Animal Experimentation Ethics Committee (approval number: AKUHADYEK-256–17).

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