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Study of Angiogenesis-Related Phosphodiesterase Inhibitors

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ABSTRACT

Angiogenesis is the process through which new capillaries form from pre-existing capillaries and venules. Its occurrence depends on the migration of vascular endothelial cells which is inhibited by high levels of cAMP. Such levels can be regulated by the degradation caused by the phosphodiesterases (PDEs). Therefore, by inhibiting the action of PDEs it is assumed that angiogenesis can be inhibited with the prevention of migration of these cells. The aim of this study was to evaluate the effect PDE inhibitors on angiogenesis in mice by using non-specific inhibitors (aminophylline) and selective inhibitors of PDE4 (roflumilast) and PDE5 (sildenafil). BALB/c mice were used as a model; under anesthesia, the mice had a sponge of 0.5 x 0.5 cm introduced into their dorsal subcutaneous tissue; they were then divided into 4 groups and daily gavage treated: 1) control group (n=13) – treated with 0.3 mL of saline solution; 2) aminophylline group (n=16) – 50 mg/kg; 3) roflumilast group (n=14) – 5mg/kg; 3) sildenafil group (n=12) –100 mg/kg. After 7 days, with the animals anesthetized, a blood sample was drawn for hemoglobin (Hb) measurement, the sponge implant was removed, and its content was obtained in 2 mL of saline solution for hemoglobin measurement. Absorbance levels (A), the amount of Hb from the sponge (S) and the total blood Hb concentration levels from each mouse were evaluated. According to the results obtained, we concluded that aminophylline, roflumilast and sildenafil (phosphodiesterase inhibitors) did not cause any alteration in the angiogenesis evaluated by the sponge-implantation method.

INTRODUCTION

The concept of angiogenesis, or neovascularization, is established as the formation of new capillaries from pre-existing capillaries and venules (Bischoff, 1995). It plays an important role in many physiological processes. However, in some cases like neoplasias, it may be very harmful since it also promotes the vascularization of malignant tumors and contributes to the metastatic process (Netherton, 2005; Shibuya, 2008). Many types of cells, like vascular endothelial cells (VEC) which are present in all vessels of the body forming the most part of capillary structures, are necessary to make this process occur. Under stimulus, they migrate and trigger the neovascularization activity. This stimulus may be provided by substances released by tumors, activated lymphocytes and macrophages associated to the lesion. The balance between angiogenic and anti-angiogenic factors is responsible for the regulation of angiogenesis (Cao *et al*., 1996). Adenylate cyclase and guanylate cyclase are enzymes that catalyze the AMP transformations into cAMP and cGMP respectively. These second messengers play an important role in the migration of many cells like endothelial cells (Ghosh et al., 2010). By inhibiting the action of phosphodiesterases, an increase in cAMP levels occurs. As angiogenesis is inhibited by high levels of cAMP due to a decrease in the migration of endothelial cells, it is possible to imply that phosphodiesterase inhibitors may reduce angiogenesis (Herget et al., 2008; Francis et al., 2011). This study aimed to analyze the effects of phosphodiesterase inhibition on angiogenesis.

MATERIAL AND METHODS

The research was approved by the Ethical Animal Experimentation Committee of ABC Medical School (FMABC) in

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Santo André, São Paulo, under the number 013/12. A total of 55 BALB-c mice were provided by and kept at the Animal Facility of the same institution, where food and water were supplied *ad libitum*. The animals were anesthetized with ketamine and xylazine (80-100 mg/kg ketamine + 10 mg/kg xylazine IP). Under anesthesia, animals had the hair from the dorsal area removed and an incision of 0.5 cm was made, complying with all asepsis standards, exposing the subcutaneous tissue. A sterile sponge measuring 0.5 X 0.5X 0.5 cm was inserted into the subcutaneous tissue of each mouse as described by Feder *et al* (2013). The mice were followed up throughout a week, a period when they received analgesics (morphine 2.5 mg/kg IM-single dose) daily. Next, after sponges were implanted, the animals were divided into 4 groups:

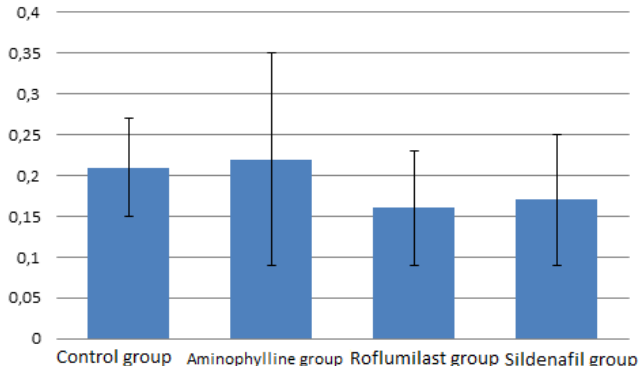
a) Group 1: control (n=13)-mice were daily gavaged with 0.2 mL of saline solution.
b) Group 2: aminophylline (n=16)-mice were daily gavaged with 50 mg/kg of aminophylline.
c) Group 3: roflumilast (n=14)-mice were daily gavaged with 5 mg/kg of roflumilast.
d) Group 4: sildenafil (n=12)-mice were daily gavaged with 0.7 mg/kg of sildenafil.

After 7 days the mice were anesthetized again and a blood sample was extracted for hemoglobin analysis. At the same time the sponges were removed and their contents were diluted in 2 mL of saline solution to measure hemoglobin levels by spectrophotometry 540 nm.

The relation between sponge hemoglobin and total blood hemoglobin, obtained from the different animals, was established by the variance test for independent samples using GB-STAT 9.0 software.

RESULTS AND DISCUSSION

The relation between sponge hemoglobin and total blood hemoglobin in the different groups is shown in graph 1.



Graph. 1: Mean and standard deviation of the values found in the sponge hemoglobin in relation to the values found in the total blood in the different groups.

The set of drugs used in this study showed a decrease in the relation between sponge and total blood absorbances. The most expressive decrease was that found in the group of animals treated with roflumilast, followed by the group treated with sildenafil. The aminophylline group had a mean value similar to the one found in the control group. Aminophylline is a non-specific phosphodiesterase inhibitor used in cases of asthma and COPDs (Essayan, 1999). Cekic *et al*, in a study with rats, showed that aminophylline targets A2BARs and attracts CXR3 cells, thus inhibiting not only tumor growth but also angiogensesis (Cekic et al., 2012). According to Adair, high levels of adenosine mediate the proliferation of endothelial cells, which contributes to the hypoxia-induced angiogenesis process. Aminophylline is also an adenosine receptor inhibitor, another possible mechanism for angiogenesis inhibition (Adair, 2012). However, despite this angiogenesis suppression demonstration, the experimental model with implanted sponges did not show the effects mentioned above.

Interestingly, opposed results were seen in the group treated with roflumilast. Such drug, used in COPD cases, is the most potent selective inhibitor of phosphodiesterase 4. Netherton and Maurice showed a great impact on the decrease of vascular endothelial cell migration as PDE 4 was inhibited, a fact that induces angiogenesis decrease. Pulamsetti *et al* demonstrated that PDE 4 is expressed in lung cancer, in hypoxia situations, and it plays a role in the progression of this kind of disease. As a result, it may be a therapeutic target (Pullamsetti et al., 2013).

As to the group treated with sildenafil, opposite results to the ones found in the aminophylline group were also observed. Sildenafil is a selective inhibitor of phosphodiesterase 5, and it is very much used in the treatment of erectile dysfunction. Its mechanism of action is the prolongation of action of NO in tissues: NO activates guanylate cyclase, turning GTP into cGMP, which is later degraded by phosphodiesterase. Upon inhibiting PDE 5, sildenafil allows for a greater time of action of cGMP (Rabe, 2011; Corona et al., 2011; Vidavalur et al., 2006). Some studies suggest that this drug can induce cardioprotective effect in infarction cases and it is of great help in cerebral vascular accidents due to the increase in angiogenesis. The main hypothesis is that the NO interacts with the VEGF (vascular endothelial growth factor), the major marker of angiogenesis process, and with the HIF (hypoxia inducible factor), promoting higher migration levels of endothelial cells in hypoxia situations (Sahara et al., 2010; Ferrara, 1999). Pyriochou *et al* (2007) showed, in chicken cells, that sildenafil increases angiogenesis levels.

All these drugs have the induction of inhibition of phosphodiesterase enzymes in common. These enzymes catalyze the hydrolysis of both secondary messengers cAMP and cGMP to 5'- AMP and 5'-GMP respectively. These messengers are paramount in functions like vision, muscle contraction, growth and cellular differentiation (Xu et al., 2000; Bingham et al., 2006).

A total of 11 known different types of this enzyme (numbered from 1 to 11) are spread throughout the different tissues of the body and classified according to their sequences and catalytic and regulatory potentials. Our results showed that the drug selectivity in a specific type of enzyme may reveal interesting results when angiogenesis is evaluated by a sponge-implantation model.

However, when it came to the complexity of the neovascularization process with the interaction of many substances other than phosphodiesterases, the response was quite unclear since no statistical significance could be observed in any of the studied groups. In conclusion, the phosphodiesterase inhibitors aminophylline, roflumilast and sildenafil did not cause any alteration in the angiogenesis when it was evaluated by the sponge-implantation method. Other models of angiogenesis evaluation should be used to shed light on the effects of this class of drugs in angiogenesis.

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