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Safety of ranibizumab therapy in wet AMD and the role of vascular endothelial growth factors in physiological angiogenesis

Bezpieczeństwo terapii ranibizumabem w wysiękowej postaci AMD i rola naczyniowego czynnika wzrostu w procesie fizjologicznej angiogenezy

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Summary: Vascular endothelial growth factor – A (VEGF-A), is a major factor implicated in choroidal neovascularisation (CNV) and therefore a target for therapeutic agents in wet age related macular degeneration (AMD). Ranibiuzumab (Lucentis) blocks all active isoforms of VEGF-A and the products of their degradation. It penetrates through all layers of the retina in order to reach the target tissue. It is quickly removed from the system and it is characterised by low level of immunogenicity. The essence of angiogenesis is formation of new vessels by branching and expansion of already existing ones. Angiogenesis is an important physiological process that takes place during the healing of wounds, reconstruction of hypoxic injury and reproduction. However some diseases such as cancer, arthritis, diabetes and neovascular AMD are associated with persistent unregulated angiogenesis. There is an important question whether binding vascular-endothelial growth factors in wet AMD therapies using ranibizumab is correlated with the increase of the incidence of systematic adverse effects (AEs), such as cardiovascular episodes or thrombosis.
The aim of this article is to present ranibizumab as a safe drug in treating wet AMD patients. Even though the concentration of Lucentis administered in a dose of 0.3 or 0.5 mg into the vitreous body in the organism is very low, the incidence of AEs during the anti-VEGF therapy was traced. In MARINA and ANCHOR studies, occurrence of possible AEs was observed. No statistically significant differences were shown in the AEs frequency between the patients treated with ranibizumab and the control group, and in correlation with the general population of patients suffering from wet AMD.

Słowa kluczowe: ranibizumab, mokra postać AMD, patogeneza, mediatory nowotwórstwa naczyniowego, bezpieczeństwo.
Key words: ranibizumab, wet AMD, pathogenesis, vascular endothelial growth factors, safety.

Vascular endothelial growth factor – A (VEGF-A), is the main factor in the pathogenesis of age related macular degeneration – AMD and therefore it is fundamental for contemporary pharmacotherapy of this disease.

The formation of subretinal neovascular membrane is a multistage process:

- hypoxia and oxidative stress stimulate the secretion of inflammation factors and angiogenic cytokins,
- this results in the stimulation of abnormal growth of vessels and the formation of choroidal neovascularisation – CNV) underneath the retina,
- exudates and hemorrhages accompanying CNV, destroy the macula irreversibly and impair vision,
- disciform scar is the final stage of CNV (1).

1. Outline of wet AMD pathogenesis

In the course of wet AMD pathogenesis, the anatomical and functional relations between the complex of retinal pigment epithelium and choriocapillaries are disturbed. Insufficient supply of oxygen from choriocapillaries and RPE hypoxia cause intensified expression of VEGF-A genes and the production of growth factors, angiogenesis intensification and limiting the perfusion of choriocapillaries. These processes are particularly intense in patients older than 60 years of age, when they are combined with the atherosclerosis of vessels and thickening of their walls, increasing the vascular resistance and the following decrease in perfusion and the stimulation of pigment epithelium to produce VEGF-A. Additionally, VEGF-A activates the vascular permeability factors (VPF), which increase the permeability of the vessels and damage the walls of the vessels by inducing factors dependant on leukocytes (2).

Bruch's membrane should not be forgotten as it is the crucial barrier for the growth of CNV into the subretinal space. Druses and inflammation increase the sensitivity of Bruch's membrane to VEGF-A, they break its cohesion, which is conducive to the migration of new vessels. The hypothesis on the meaning of VEGF-A in the pathogenesis of wet AMD is supported by experimental studies conducted on transgenic mice, in which the growth of VEGF-A expansion in retinal photoreceptors resulted in the development of pathological neovascularisation.

On the other hand, the inhibition of VEGF-A in the experimental studies conducted on animals results in limiting the pathological neovascularisation and retinal ischemia (3-6).

2. Anti-VEGF therapy in wet AMD

Below are the main assumptions of anti-VEGF-A therapy:

- the level of VEGF-A is increasing in the vitreous body of an eye with wet AMD,
- VEGF-A stimulates vessel instability and it plays the key role in CNV pathogenesis,
- VEGF-A acts on the level of retina and choroidea,
- Anti-VEGF-A therapy limits both the angiogenesis and the lack of vascular tightness (7).

Ranibiuzumab (Lucentis) – inhibitor of VEGF-A as a drug of choice in the treatment of wet AMD is characterized by:

- The ability to block all active isoforms of VEGF-A and the products of their degradation,
- Penetration of all retinal layers in order to reach the CNV focus,
- Fast elimination from the organism,
- Low immunogenicity (8).

Ranibizumab (Lucentis) is a part of humanized monoclonal antibody. It is derived from a mouse anti-VEGF precursor – monoclonal antibody (Murine Mab 150kDa). The Fc part was removed in order to decrease its immunogenic potential in the human organism and to decrease the size of the molecule for the sake of easy retinal penetration. The idea of humanization is to join a fragment of mouse anti-VEGF-A antibody with a human Fab in order to obtain rhu Fab v1, a precursor of Lucentis molecules (48kDa). Mass production of Lucentis employs a vector technique based on E.coli. As a result of humanisation, the Lucentis molecule is characterized by 5-20x higher activity potential in comparison to a full-length antibody (9.10).

Following, are the features of ranibizumab, thanks to which it can be administered intraocularly:

- Its small molecule penetrates the retina. Research has show that the full-length antibody has no such property,
- Fast elimination from the organism (100x times faster than the full-length antibody),
- No Fc region prevents cellular cytotoxicity or cytotoxicity dependant on the complement system,
- Short half-life minimizes the exposition of ranibizumab in the system (8).

Lucentis joins and inactivates all biologically active isoforms of VEGF-A in the 86-89 site. Blocking those factors prevents the creation of CNV focus and the development of wet AMD. VEGF-A 165 is the most expressive isomer in human organism. 206 and 189 are typical for extracellular matrix. Isoforms are defined by the number of aminoacids in the molecule. The main isoforms VEGF-A are 121, 165, 189 and 206. 165 and bigger molecules have the heparine-binding domain, which is not present in the isoform 121. Several smaller isoforms and biologically-active derivatives of plasmin were identified as VEGF-A 110 (9.11).

The symptoms of neovascularisation in the course of wet AMD are: leakage of fluid, blood and cells into the subretinal space, serous detachment of retinal pigment epithelium and retina, retina edema and significant vision impairment. Lucentis blocks the symptoms accompanying CNV (9.11). Additionally, by means of optical coherent tomography (OCT) it was shown that there is a chance of coming back to the functional condition of retinal-choroideal complex in the eyes after Lucentis treatment.

3. Pharmacokinetics and pharmacodynamics of ranibizumab

The registration of Lucentis was proceeded by numerous clinical in vivo and in vitro studies. The in vivo pharmacology, pharmacokinetics and safety of Lucentis was tested on monkeys and white rabbits from New Zealand. The studies carried out on monkeys were of particular importance because VEGF in monkeys is homologous to human VEGF in 99%.

Lowe et al. described the effects that Lucentis exerts on the proliferation of endothelial cells (HUVEC, of human umbilical vein, which was stimulated by VEGF-A 165, VEGF-A 121 and the degradation product of VEGF-A 110. They noted that ranibizumab inhibits the proliferation of HUVEC. Alamar-blue fluorescein was used as the proliferation indicator. It was shown that only ranibizumab blocks the VEGF-dependant proliferation of endothelial cells in correlation with dosing. In this in vitro experiment different concentrations of ranibizumab were incubated in a solution of 10 ng/ml of each VEGF-A and HUVEC isoform for four days. On average the cultures contained 10% of human serum in the presence of Alamar-blue fluorescein. The total blocking of VEGF-165 was observed in the ranibizumab and VEGF-A165 concentration ratio of 1: 1 (12).

Gaudreault et al. investigated the vessel instability using the MILES test as a CNV marker. In this test Evans blue stain was used. It was administered to hairless guinea pigs. Vascular instability was induced by local administration of VEGF-A in standard concentration. Extravascular stain passage was a marker of vascular leak after administering certain amount of VEGF. The leak was then stopped by a known amount of VEGF-A inhibitor. It was noted that the vascular instability stimulated by VEGF--A 165 (100 ng/ml), VEGF-A 121 (205 ng/ml), or a product of VEGF-A 110 (189 ng/ml), degradation was limited by ranibizumab depending on dosing (0-1000 ng/ ml) (13).

In the *in vivo* study by Krzystolik et al. the safety and efficacy of ranibizumab was tested on monkeys. Monkeys were administered 0.5 mg of Lucentis intraocularly and placebo to the second eye in intervals of 2 weeks. On the 21st day, 9 foci of CNV were induced by photocoagulation with green argon laser in each eye. In the second phase of the study, both eyes were administered 0.5 mg of Lucentis on the 42nd and 56th day. The dynamics of CNV was monitored by ophthalmofunduscope, the pictures of the fundus of the eye were taken and fluorescent angiography was performed. In the first phase of the investigation the number of eyes with induced level 4 (very advanced, CNV changes was significantly lower in the group treated with Lucentis prior to CNV induction compared to the eyes treated with placebo. In the second phase the risk of level 4 CNV changes was statistically significantly decreased. The leak of already formed CNV membranes decreased as well. Summing up, the risk of developing advanced CNV changes was significantly lower in the eyes subjected to Lucentis injections in comparison to the control group. In each eye subjected to Lucentis injections there was a hemorrhage, possible inflammatory reaction that withdrew within a week after the injection and it was less intense after the following procedures (14).

ISSN 0023-2157 Index 362646

The preclinical tests based on Lucentis showed high potential of VEGF-A inhibition, decreasing the number of CNV foci and vascular instability in cases of experimentally evoked wet AMD in animal models. The results of experimental preclinical studies became the basis of using 0.5 mg dose in patients suffering from wet AMD.

4. The role of VEGF-A in angiogenesis

The essence of angiogenesis is the formation of new vessels through branching and expansion of the already existing ones. Angiogenesis is an important physiological process occurring when the wounds are healed as well as during the reconstruction of ischemic damage and reproduction. Simultaneously unregulated progression of angiogenesis may result in pathologies such as tumors, joint inflammation, diabetes and wet AMD. Angiogenesis is a multistage process. It is initiated by oxidative stress, which stimulates VEGF-A at most but other factors are also affected. This results in proliferation and migration of endothelial cells, proteolysis and penetration of new vessels through existing membranes.

VEGF-A has a number of properties:

- It is mitogenic for endothelial cells,
- It is necessary for the survival of newly formed vessels,
- It is a chemotactic factor for inflammatory cells,
- It deregulates the stability of vessels.

VEGF-A works through receptors localized on the surface of VEGFR-1, VEGFR-2 endothelial cells. The connection of VEGF-A with receptors results in the proliferation of endothelial cells, lack of tightness in the cell connections and the increase of vascular permeability (11). As a consequence of the fusion between photoreceptors and growth factors, the endothelium produces a number of cytokins including matrix metalloproteinase (MMPs), which degrades the basilemma. Migrating and proliferating endothelial cells break the structure of basilemma and new growth that takes form of vascular buds occurs. Then the vascular buds elongate and recompose to create new vessels. The process of maturation of the new vessels comes to an end when the layer of external pericytes is formed to support their walls. The increase in the lack of microcirculation tightness is currently seen to be the most important moment of angiogenesis coexisting with tubers and wound healing. Another important role of VEGF-A is the leak of proteins to the serum. It results in extracellular fibrin that is considered to be a substrate for the extravascular endothelial cells. The leak of serum components into the retina results in edema and creation of liquid spaces (15,16).

5. Safety of ranibizumab therapy

Even though the ranibizumab concentration in the organism after administering it into vitreous body is very low, systemic adverse effects (AEs) are observed. They can result from the fact that VEGF-A is blocked by ranibizumab and it can not take part in the processes of growth, reparation, regeneration and circulation. Antiplatelet Trialists Collaboration (APTC), carried out in detail analysis of the occurrence of adverse effects such as arterial hypertension or intravascular clots especially in arteries after general administration of anti-VEGF.

The complications classified by APTC as AEs are death, myocardial infarction, anemic stroke, hemorrhagic stroke without deadly result (17).

There were 875 participants in the ANCHOR, MARINA and PIER studies. In MARINA and PIER 299 control simulated injections were made, in ANCHOR there was a control group of 143 people treated with Visudyne.

In the MARINA study, 24 months of observation proved that the frequency of increases in the arterial blood pressure was analogous or lower compared to the control group during the first 12 months. Additionally, the systolic and diastolic blood pressure was reduced (opposed to the normal population) in the group treated with Lucentis (18).

No statistically significant differences were observed in the frequency of arterial thrombotic episodes between the control group and the group treated with doses of 0.3 or 0.5 mg. These episodes increased in numbers after 12 months of observation in the group treated with 0.5 mg of Lucentis compared to 0.3 mg group and the control group, however with no statistical differences. This difference in numbers disappeared after 24 months of examination (18).

The frequency of the occurrence of intravascular clots in arteries in MARINA study was compared to the general population of wet AMD patients (general population: frequency of infarctions 1.45%, strokes 1.86%. In the group treated with Lucentis 1.3-2.5% and 0.8-2.5% respectively).

During the first 12 months of MARINA study, there were three deaths in the group treated with Lucentis, however none of them was related to the treatment. In the 24 month observation in MARINA study, no differences were observed between the control group – 6 deaths, and the groups treated with 0.3 mg of Lucentis – 5 deaths and 0.5 mg of Lucentis – 6 deaths (18).

Less than 1% of the patients developed antibodies against ranibizumab after 12 months. The antibodies resulted from the treatment. Most likely they occurred because of anti-Fab. After 24 months, these values rose to 4.4% in the group treated with 0.3 mg of Lucentis, 6.3% in the group treated with 0.5 mg of Lucentis and 1.1% in the control group (18).

In the ANCHOR study, the 12 months observation showed that the frequency of rising arterial blood pressure was related or lower compared to the control group treated with Visudyne. Additionally, the systolic and diastolic arterial blood pressure was reduced (unlike in the general population), in the group treated with Lucentis (19).

No statistically significant differences in the frequency of arterial thrombotic episodes were observed between the control group treated with Visudyne and the group treated with 0.3 or 0.5 mg of Lucentis during the period of 12 months. These episodes increased in numbers after 12 months of observation in the group treated with 0.5 mg of Lucentis compared to the group treated with 0.3 mg and the control group treated with Visudyne, however with no statistical differences (19).

Conclusion

Clinical tests proved the safety and security of systemic therapies of wet AMD based on ranibizumab. Viterous administration of ranibizumab in the dose of 0.5 mg does not interfere with the physiological angiogenesis and reconstructive processes and it does not result in adverse effects of increased frequency of cardiovascular episodes or thrombotic episodes.

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The study was originally received 15.12.2009 (1181)/
Praca wpłynęła do Redakcji 15.12.2009 r. (1181)
Accepted for publication 20.03.2010/
Zakwalifikowano do druku 20.03.2010 r.

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ISSN 0023-2157 Index 362646