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On safety, pharmacokinetics and dosage of bevacizumab in ROP treatment – a review

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Abstract:
Off label intravitreal use of the VEGF antibody bevacizumab for retinopathy of prematurity (ROP) increases despite lack of studies on safety, pharmacokinetics and dosage in developing individuals. Systemic absorption has been considered negligible. A literature search was performed with emphasis on potential adverse systemic effects in developing individuals. Intravitreal bevacizumab enters the general circulation, suppresses plasma VEGF levels and remains in the blood for more than 8 weeks in primates. Possible adverse effects on VEGF dependent development must be considered.

Key notes
Intravitreal bevacizumab enters the general circulation, results in prolonged VEGF inhibition and has a half-life of 1-2 weeks in primates. VEGF is critical for growth and development of vital organs such as kidneys, lungs and brain during the third trimester. After proper investigations of systemic effects, pharmacokinetics and dosage, anti-VEGF might be an opportunity for severe ROP. As an alternative to laser, its effects are presently too poorly known.

Introduction
The number of publications on the off-label use of intravitreal bevacizumab (Avastin®) for severe retinopathy of prematurity (ROP) is rapidly increasing. Although most authors agree that studies of pharmacokinetics and systemic safety are needed, no such studies on preterm infants have been published. A recent editorial expressed the opinion that it seems reasonable to assume that intravitreal bevacizumab is safe and that it should be the treatment of choice for zone I ROP (1). Others question the use of this medication without clinical trials with
meticulous evaluation of multiple variables which normally precedes the introduction of drugs in clinical use (2, 3).

One randomized controlled study of intravitreal use of bevacizumab (BEAT-ROP) has been published so far (4). The dose injected was 0.625 mg i.e. half the dose used in adults. Regarding safety, it was concluded that for assessment of mortality, 2800 infants would be required and assessment of local or systemic toxicity would require an even larger number of infants. It was stated that bevacizumab could not escape the eye more than in very small amounts due to its large size, unless laser therapy had destroyed the retinal barrier.

A prematurely born infant receiving treatment for ROP is at a stage when growth and differentiation normally are intense. Early development is characterized by critical periods of susceptibility when environmental factors effectively produce long lasting changes. Knowing that VEGF is essential for normal angiogenesis and, in addition, has neuroprotective effects, we set out to review studies on safety, pharmacokinetics and dosage of the drug in relation to the developmental stages of important organs during the third trimester and early postnatal life.

Bevacizumab is a recombinant humanized vascular endothelial VEGF antibody that prevents VEGF from binding to its receptors (5). Bevacizumab binds to all isoforms of VEGF (6), blocks VEGF induced angiogenesis and is approved by the U.S. Food and Drug administration for intravenous use for metastatic colorectal cancer. It is used off-label intravitreal to treat neovascular retinal disorders such as age related macular degeneration (7), diabetic retinopathy (8) and central retinal vein occlusion (9).

Metabolism and elimination of bevacizumab are similar to those of endogeneous IgG i.e. primarily via proteolytic catabolism in the whole body including endothelial cells and not mainly through the kidneys or the liver (FASS.se).
VEGF, a secreted glycoprotein, is an angiogenic as well as a vasopermeable factor which is secreted by fetal and adult epithelial and mesenchymal cells and exerts mitogenic effects on endothelial cells. In the fetus, VEGF is expressed in most tissues. In normal angiogenesis, VEGF activity often represents a rate-limiting step. Median plasma concentrations of VEGF in premature babies in one study showed a large variation but no significant difference between infants without and with ROP at 32 weeks postmenstrual age (PMA) (median 0.658 ng/ml, range 0.049-2.152 and median 0.904, range 0.142-2.349 respectively), and at 36 weeks PMA (median 0.437, range 0.089-2.367 and 0.344, range 0.066-1.334 ng/ml respectively) (10). In the human kidney, VEGF is highly expressed during glomerular development and also in the adult indicating roles for normal glomerulogenesis and for control of vascular permeability (11). A strong dosage sensitivity for VEGF-A in the developing glomerulus has been reported and dysregulation of VEGF has been found to play a pathogenic role in glomerular disease. A note of caution for clinical trials aimed at altering VEGF levels has been issued and careful monitoring of renal function with a particular emphasis on the glomerular filtration barrier is recommended (12).

In the human lung, primitive alveoli are first seen at PMA 29 weeks. With increasing gestation, the alveoli get thinner walls and at 36 weeks, all alveoli are thin walled (13). There is strong evidence that VEGF is necessary for alveolarization during normal lung development and that inhibition of VEGF during a critical period of growth contributes to bronchopulmonary dysplasia (BPD) (14). In a study of premature mice, VEGF increased surfactant synthesis and improved lung function and was considered a potential therapeutic possibility for respiratory distress syndrome (15).
In a study on human fetal and postnatal brains, VEGF expression was found in different locations during different time periods. Bevacizumab treatment for ROP mainly takes place at PMA 30-40 weeks. At that time, VEGF expression was found in some brain locations but not in others (16).

**Dosage, pharmacokinetics and safety**

*In vitro*

In the following, all concentrations of VEGF and bevacizumab in blood will be expressed as ng/ml for simplicity.

Wang et al. (17) studied bevacizumab induced inhibition of VEGF (50 ng/ml) mediated effects on human umbilical vein endothelial cells (HUVECs) in cultures. They found a dose dependent inhibition of VEGF induced HUVEC proliferation with an estimated half maximal inhibitory concentration (IC50) of 22ng/ml. The addition of 500 ng/ml of bevacizumab completely blocked VEGF-induced endothelial cell growth, suggesting that a molar ratio of bevacizumab to VEGF of 2.6:1 is needed for maximum inhibition. Also for blockage of HUVEC survival, nitric oxide (NO) production and permeability a ratio of 2.6:1 was efficient, while a ratio of 10:1 was needed to block migration.

Porchine VEGF binds to bevacizumab. In perfused organ cultures from pig’s eyes, where 0.35 ng/ml of VEGF was produced per hour, VEGF was completely neutralized for 16 hours by 0.25 mg/ml of bevacizumab and 0.125 mg/ml of ranibizumab. The efficiency of the two drugs was similar (18).
Mice and rats

Mice and rat VEGF lack affinity for bevacizumab (5) and studies on effects of anti-VEGF treatment have been performed using other methods. In newborn mice, partial inactivation of VEGF led to increased mortality, impaired general growth and growth of organs, especially the liver (19) and in 24 days old mice to disturbed cartilage remodeling (20).

Pharmacokinetic studies have shown that rhuMab VEGF (= bevacizumab) was cleared from the serum in a biphasic manner with an initial half-life of 1.2 hours in mice and 7 hours in rats and the terminal half-life was 1-2 weeks (5).

Two of the most common adverse effects seen in adults receiving bevacizumab for cancer are proteinuria and hypertension. In mice, local ongoing VEGF production of podocytes in the kidney are necessary for the functioning of the adult glomerular filtration barrier and altered glomerular permeability appeared to be a direct consequence of VEGF inhibition in one study (21).

In rats, serum concentrations of bevacizumab after intravitreal injections were higher in animals with branch retinal vein occlusion than in healthy animals (22), indicating that a break-down of the blood retinal barrier allows larger amounts of the drug to enter the general circulation.

Rabbits

Rabbits have a sparsely vascularised retinas and their VEGF has reduced affinity for bevacizumab; about a fifth of that of primates (Ferrari personal communication in (5)). Using radiolabeled rhuMab VEGF (= bevacizumab) intravenously in rabbits (5) the distribution indifferent organs could be studied. Radioactivity in plasma was found to be ten-fold higher than in tissues where the organs exhibiting the highest radioactivity concentration after 2
hours in decreasing rank order were kidney, testes, spleen, heart lung and thymus with lower
levels in brain and eye.

A few pharmacokinetic studies of intravitreal bevacizumab have been performed in rabbits of
which one deals with newborn animals (23-25). In rabbit pups, who received 1.25 mg of
intravitreal bevacizumab 8 days previously, serum concentrations were significantly higher in
2 weeks old animals (19300ng±8100 ng/ml) than in 6 weeks old animals (4400±1300
ng/ml)(Table)(23).

In male rabbits (1.7-2.0 kg), intravitreal injection of 1.25 mg bevacizumab in one eye resulted
in a peak concentration of 400 000ng/ml in the vitreous after one day, concentration declined
with a half-life of 4.32 days and >10000 ng/ml was maintained for 30 days (Table). In serum,
a maximum concentration of 3300 ng/ml was found 8 days after injection and it declined with
a half-life of 6.86 days. In the vitreous of the fellow eye, bevacizumab concentrations
increased from 0.35 ng/ml day one to 11.17 ng/ml at 4 weeks (24).

In a study by Nomoto et al. intravitreal injection of 1.25 mg in one eye of rabbits weighing 1.9
to 2.5 kg each, resulted in a maximal concentration in plasma of 2087±200.8 ng/ml at two
weeks with a half-life of 1.85 weeks (Table). In the fellow eyes, bevacizumab from the
systemic circulation resulted in concentrations in the retina/choroid which were maintained
above IC_{50} for 8.0 weeks (25).

In the eye, bevacizumab has been found to be tolerated well (26, 27) although a dose
dependent increase in apoptosis has been revealed in photoreceptors and other cells (28, 29).

Full retinal thickness penetration was found 24 hours after injection of 2.5 mg but not after
four weeks (27).
Nonhuman primates

Early pharmacokinetic studies of intravenous administration of rhuMab VEGF (=bevacizumab) in cynomolgus monkeys showed that the antibody was cleared from the serum in a biphasic manner with an initial half-life of 11-26 hours and a terminal half-life of 1-2 weeks and that the terminal phase was dominant (5).

Intravitreal injection of 1.25 mg bevacizumab in one eye of 3 male adult cynomolgus macaques (3.9-5.5 kg) resulted in maximum serum concentrations after one week of 1430±186ng/ml (Table). Reduction rate was low and at 8 weeks, serum concentrations were 67.1±24.3 ng/ml which was approximately 187 times higher than that of the aqueous humor of the treated eye. The effect on serum VEGF concentrations could not be studied since they were below the limit of detection (0.031.2ng/ml) throughout the experiment (Figure) (30).

In another study on cynomolgus macaques, intravitreal bevacizumab penetrated through the retina and was found in choroidal vessels throughout the experiment indicating substantial transfer of the drug to the blood circulation. Enrichment of bevacizumab was found in rod photoreceptors and endothelial cells of blood vessels (31). In addition, a reversible reduction in the number of choriocapillaris endothelial cell fenestration has been found for at least two weeks after intravitreal injection of 1.25 mg bevacizumab as well as choriocapillaries perfusion disturbances (32).

Interestingly, a recent report demonstrates pronounced sustained choroidal vascular involution during the development of ROP (33).

Humans

In adults with proliferative diabetic retinopathy (PDR), patients treated with intravitreal injection of 1.25 mg bevacizumab in one eye before vitrectomy had significantly lower serum VEGF seven days after treatment (34). In another study of adults with PDR, different doses
(0.0062, 0.0125, 0.062, 0.125, 0.625 and 1.25 mg) of bevacizumab were injected intravitreal and consistent biologic effects were noted at all doses. A possible therapeutic effect in fellow eyes was found in single patients receiving 1.25 mg in one eye, also indicating that systemic inhibitory concentrations can be achieved in adults (8). We have found no studies of bevacizumab or its effect on VEGF in serum or plasma in preterm infants or in immature animals except the one on rabbit pups by Wu et al. (23).

Discussion

Bevacizumab enters the general circulation and stays there for weeks to months. It also reaches the fellow eye in potentially therapeutic concentrations (Figure 1). Young age (23), possibly due to smaller size, and impaired blood-retinal barrier (22) increase serum concentrations. Intravitreal bevacizumab has been reported to reduce serum levels of VEGF in adults (34).

In the cell culture study by Wang et al. (17) 500ng/ml of bevacizumab was able to neutralize 50ng/ml of VEGF. Vitreous VEGF levels in type I ROP are unknown but in vascularly active stage 4 ROP eyes, Sonmez et al. found median (range) concentrations of 3.454 (0.774-8.882) ng/ml which were significantly higher than 0.316 (0.105-0.665) ng/ml in vascularly inactive stage 4 eyes and 0.059 (0.038-0.135) ng/ml in controls (36). In stage 5 ROP, levels of 0.119±0.66 ng/ml have been found (37). Bakri et al. (24) found intravitreal bevacizumab concentrations of 400000 ng/ml after one day and > 10000 ng/ml 30 days after injection of 1.25 mg. If similar concentrations are achieved in infant eyes, the doses currently used appear to be very high. Avery et al. found consistent effects on PDR of intravitreal injection of doses as low as 0.006 mg of bevacizumab (8).

In contrast to the adult healthy macaque, the preterm infant with proliferative ROP has a compromised blood retinal barrier that may allow more bevacizumab to enter the blood stream. Assuming that a preterm infant with type I ROP at 30-36 weeks is about half the size
of an adult macaque of 3.9-5.5 kg and that the serum concentrations reached after an
intravitreal injection of 0.625 mg would be similar or, more likely, higher than in the monkey
receiving 1.25 mg, serum concentrations after one week would be roughly 1400 ng/ml and
after 8 weeks 70 ng/ml in the baby (30). Systemic VEGF concentrations in preterm infants
show a large variation. Pieh et al. found median (range) plasma concentrations of 0.90 (0.14-
2.35) ng/ml and 0.34 (0.07-1.33) ng/ml at 32 and 36 week’s PMA respectively in infants with
ROP (10). One must therefore suspect that serum bevacizumab levels eight weeks after
intravitreal injection, still prevents VEGF from acting in preterm infants at a stage when
VEGF is needed for the development of kidneys, lungs, brain and other organs.

Very preterm infants at risk for severe ROP have subnormal functioning of many organ
systems for the rest of their lives. Anti-VEGF treatment may have the capacity to reduce their
reserves even further. These effects may not be obvious until decades after treatment. For
clinical off-label use of a drug, basic research and animal experiments are required to evaluate
its safety and to reveal potential adverse effects. It is important to note that, in most patients,
type I ROP regresses after laser, which has been used for many years with proven efficacy
except in the most severe cases. The effects of laser treatment are limited to the eye.
The antibody fragment ranibizumab (Lucentis®) which has a shorter half-life in serum in
monkeys 3.5 d (35) than bevacizumab (12.3±2.6 days) (30) and was developed due to
concerns of systemic adverse effects of bevacizumab, may be an alternative to study further
although 40-fold more expensive.
We suggest that

1. experimental studies on animal species with VEGF that binds to bevacizumab (primates, pigs) at developmental stages corresponding to the human third trimester regarding adverse effects on developing organs such as kidneys, lungs and brain, are performed.

2. infants with very severe ROP who are treated with bevacizumab after laser failure should be included in controlled pharmacokinetic, dose/efficacy and safety trials with close monitoring of serum concentrations of VEGF.

3. until the above mentioned studies have been performed, infants who can be treated successfully with laser should not receive anti-VEGF.

Full information about the lack of evidence for safety and efficacy should be given to parents before preterm infants are treated with intravitreal bevacizumab.

**Acknowledgements**

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**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>BPD</td>
<td>bronchopulmonary dysplasia</td>
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<tr>
<td>HUVEC</td>
<td>human umbilical vein endothelial cell</td>
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<tr>
<td>IC$_{50}$</td>
<td>half maximal inhibitory concentration</td>
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<td>NO</td>
<td>nitric oxide</td>
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<td>PDR</td>
<td>proliferative diabetic retinopathy</td>
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<td>PMA</td>
<td>postmenstrual age</td>
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<td>rhuMab</td>
<td>recombinant humanized monoclonal antibody</td>
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<td>ROP</td>
<td>retinopathy of prematurity</td>
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<td>VEGF</td>
<td>vascular endothelial growth factor</td>
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References


Table. Bevacizumab concentrations in different compartments after intravitreal injection in one eye.

<table>
<thead>
<tr>
<th>species</th>
<th>weight</th>
<th>age</th>
<th>dose</th>
<th>days after injection</th>
<th>compartment</th>
<th>bevacizumab concentration ng/ml</th>
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<td>rabbit</td>
<td>1.7-2.0kg</td>
<td>1.25</td>
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<td>vitreous</td>
<td>30</td>
<td>&gt;10 000</td>
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<td>rabbit</td>
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<td>1.25</td>
<td>8</td>
<td>serum</td>
<td>2087</td>
<td></td>
<td>Nomoto (25)</td>
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<tr>
<td>rabbit (pup)</td>
<td>?</td>
<td>2w</td>
<td>1.25</td>
<td>serum</td>
<td>19400 ± 8100</td>
<td></td>
<td>Wu (23)</td>
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<tr>
<td>macaque</td>
<td>3.9-5.5</td>
<td>1.25</td>
<td>8</td>
<td>serum</td>
<td>4400 ± 1300</td>
<td></td>
<td>Miyake (30)</td>
</tr>
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Figure legend

Bevacizumab concentrations in injected eye, un.injected eye and serum after intravitreal injection of 1.25 mg in one eye of adult cynomolgus macaques. From Miyake et al. Invest Ophthalmol Vis Sci 2010;51(3): 1606-8 with the publishers permission.