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

Anna-Lena Hård (annalena.hard@oft.gu.se), Ann Hellström
Department of Ophthalmology, Institute of Neuroscience and Physiology, The Sahlgrenska Academy at University of Gothenburg, Gothenburg, Sweden

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Correspondence
Anna-Lena Hård, Section of Pediatric Ophthalmology, The Queen Silvia Children’s Hospital, The Sahlgrenska Academy at University of Gothenburg, S-416 85 Göteborg, Sweden.
Tel: +46 313434720 | Fax: +46 31 848952 | Email: annalena.hard@oft.gu.se

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ABSTRACT
Off-label intravitreal use of the vascular endothelial growth factor (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.

Conclusion: 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.

INTRODUCTION
The number of publications on the off-label use of intravitreal bevacizumab (Avastin®, Roche, Basel, Switzerland) 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 that 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

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.

Abbreviations
BPD, Bronchopulmonary dysplasia; HUVEC, Human umbilical vein endothelial cell; IC₅₀, Half maximal inhibitory concentration; NO, Nitric oxide; PDR, Proliferative diabetic retinopathy; PMA, Postmenstrual age; rhuMab, Recombinant humanized monoclonal antibody; ROP, Retinopathy of prematurity; VEGF, Vascular endothelial growth factor.
assessment of local or systemic toxicity would require an even larger number of infants. It was stated that bev- 
acizumab could not escape the eye more than in very small 
amounts because of 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 
tense. Early development is characterized by critical peri-
ods of susceptibility when environmental factors effectively 
produce long-lasting changes. Knowing that vascular endo-
thal growth factor (VEGF) is essential for normal angi-
genesis and, in addition, has neuroprotective effects, we set 
out to review studies on safety, pharmacokinetics and dosage 
of the drug in relation to the development stages of impor-
tant organs during the third trimester and early postnatal life.

Bevacizumab is a recombinant humanized vascular endo-
thelial 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 ap-
proved 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 reti-
nopathy (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).

Vascular endothelial growth factor, a secreted glycopro-
tein, is an angiogenic as well as a vasopermanent factor 
which is secreted by foetal and adult epithelial and mesen-
chymal cells and exerts mitogenic effects on endothelial 
cells. In the foetus, 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 glomerulo-
genesis 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 func-
tion 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 alveo-
larization during normal lung development and that inhibi-
tion 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 foetal 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 bev-
acizumab 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 pro-
liferation with an estimated half maximal inhibitory concen-
tration (IC50) of 22 ng/mL. The addition of 500 ng/mL of 
bevacizumab completely blocked VEGF-induced endo-
thelial cell growth, suggesting that a molar ratio of bev-
acizumab 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.

Porcine 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 h 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 per-
formed using other methods. In newborn mice, partial inac-
tivation 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 remodel-
ling (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 h in mice and 7 h 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 is necessary for the functioning of 
the adult glomerular filtration barrier and altered glomeru-
lar permeability appeared to be a direct consequence of 
VEGF inhibition in one study (21).

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

**Rabbits**
Rabbits have a sparsely vascularized retinas and their VEGF has reduced affinity for bevacizumab, about a fifth of that of primates [Ferrari personal communication in (5)]. Using radiolabelled rhuMab VEGF (=bevacizumab) intravenously in rabbits (5), the distribution indifferent organs could be studied. Radioactivity in plasma was found to be tenfold higher than in tissues where the organs exhibiting the highest radioactivity concentration after 2 h 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 intravitreous bevacizumab 8 days previously, serum concentrations were significantly higher in 2 weeks old animals (19 500 ng ± 8100 ng/mL) than in 6 weeks old animals (4400 ± 1300 ng/mL; Table 1) (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 400 000 ng/mL in the vitreous after 1 day, concentration declined with a half-life of 4.32 days and >10 000 ng/mL was maintained for 30 days (Table 1). In serum, a maximum concentration of 3500 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 1 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 1.9–2.5 kg each resulted in a maximal concentration in plasma of 2087 ± 2008 ng/mL at 2 weeks with a half-life of 1.85 weeks (Table 1). In the fellow eyes, bevacizumab from the systemic circulation resulted in concentrations in the retina/choroid which were maintained above IC₅₀ 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 h after injection of 2.5 mg but not after 4 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 h 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 three male adult cynomolgus macaques (3.9–5.5 kg) resulted in maximum serum concentrations after 1 week of 1450 ± 186 ng/mL (Table 1). 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 humour of the treated eye. The effect on serum VEGF concentrations could not be studied because they were below the limit of detection (0.031.2 ng/mL) throughout the experiment (Fig. 1) (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

### Table 1  Bevacizumab concentrations in different compartments after intravitreal injection in one eye

| Species     | Weight (kg) | Age (weeks) | Dose (mg) | Days after injection | Compartment | Bevacizumab concentration (ng/mL) | References |
|-------------|-------------|-------------|-----------|----------------------|-------------|-----------------------------------|------------|
| Rabbit      | 1.7–2.0     |             | 1.25      | 1                    | Vitreous    | 400 000                           | 24         |
|             |             |             |           | 30                   | Vitreous    | >10 000                           |            |
|             |             |             |           | 8                    | Serum       | 3300                              |            |
| Rabbit      | 1.9–2.5     |             | 1.25      | 14                   | Plasma      | 2087                              | 25         |
| Rabbit (pup)| ?           | 2           | 1.25      | 8                    | Serum       | 19 400 ± 8100                     | 23         |
| Rabbit (pup)| ?           | 6           | 1.25      | 8                    | Serum       | 4400 ± 1300                       | 30         |
| Macaque     | 3.9–5.5     |             | 1.25      | 7                    | Serum       | 1430 ± 186                        |            |
|             |             |             |           | 8 weeks              | Serum       | 67.1 ± 24.3                       |            |

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**Figure 1** Bevacizumab concentrations in injected eye, uninjected eye and serum after intravitreal injection of 1.25 mg in one eye of adult cynomolgus macaques. From Miyake et al. (30) with the publisher’s permission.
2 weeks after intravitreal injection of 1.25 mg bevacizumab as well as choriocapillaris 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 7 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 (Fig. 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), 500 ng/mL of bevacizumab was able to neutralize 50 ng/mL of VEGF. Vitreous VEGF levels in type I ROP are unknown, but in vascularly inactive stage four eyes and 0.059 (0.038–0.135) ng/mL in type I ROP, levels of 0.119 ± 0.66 ng/mL have been found (36). Bakri et al. (24) found intravitreal bevacizumab concentrations of 400 000 ng/mL after 1 day and >10 000 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. (8) found consistent effects on PDR of intravitreal injection of doses as low as 0.006 mg of bevacizumab.

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 1 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. (10) 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. One must, therefore, suspect that serum bevacizumab levels 8 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®, Novartis, Basel, Switzerland), which has a shorter half-life in serum in monkeys 3.5 days (37) than bevacizumab (12.3 ± 2.6 days) (30) and was developed because of 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 the 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.

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