Review

Pharmacokinetics of Intravitreal Anti-VEGF Drugs in Age-Related Macular Degeneration

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Abstract: Intravitreal administration of anti-vascular endothelial growth factor (VEGF) antibodies has become the standard treatment for Age-Related Macular Degeneration; however, the knowledge of their pharmacokinetics is limited. A comprehensive review of the preclinical and clinical pharmacokinetic data that were obtained in different studies with intravitreal bevacizumab, ranibizumab, and aflibercept has been conducted. Moreover, the factors that can influence the vitreous pharmacokinetics of these drugs, as well as the methods that were used in the studies for analytical determination, have been exposed. These anti-VEGF drugs present different charge and molecular weights, which play an important role in vitreous distribution and elimination. The pharmacokinetic parameters that were collected differ depending on the species that were involved in the studies and on physiological and pathological conditions, such as vitrectomy and lensectomy. Knowledge of the intravitreal pharmacokinetics of the anti-VEGF drugs that were used in clinical practice is of vital importance.

Keywords: vascular endothelial growth factor/antagonists & inhibitors; ranibizumab; aflibercept; bevacizumab; Age-Related Macular Degeneration; pharmacokinetics; intravitreal
1. Introduction

Age-Related Macular Degeneration (AMD) is the leading cause of irreversible visual impairment among individuals over the age of 65 years all around the world (between 30 and 50 million people). It is expected that its prevalence will double in the next few decades, and it is estimated that 280 million people will be affected by 2040 [1,2].

The disease almost always begins as a non-neovascular form of AMD and it may progress to the neovascular form in one or both eyes [3]. A progressive deterioration in the macula characterises the non-neovascular form, which causes central vision loss. The neovascular form is caused by the abnormal development of blood vessels under the macula, leading to the leakage of fluid and blood causing inflammation. The latter form progresses more rapidly, and it can cause severe vision loss within a few months if left untreated [4].

The cause of the disease is multifactorial (i.e. age, ethnic origin and a combination of genetic and environmental factors) [5]. Several treatments for neovascular AMD have been widely studied, such as laser photocoagulation and photodynamic eye therapy with verteporfin, but nowadays the standard treatment consists of intravitreal injections of inhibitors of vascular endothelial growth factor (anti-VEGF). The anti-VEGF monoclonal antibodies that were used to treat AMD include the approved intravitreal administration of pegaptanib, ranibizumab, and aflibercept and the off-label intravitreal administration of bevacizumab and Ziv-aflibercept [6,7].

Nowadays, in clinical practice, it is very difficult to achieve adequate therapeutic drug levels in the vitreous humour through topical ocular or systemic administration, which is mainly due to the existence of physiological barriers. Oral treatments could be an attractive treatment option; however, they have failed to show benefits in combination with intravitreal anti-VEGF treatments and they are still being evaluated in monotherapy [8]. Therefore, intravitreal injections are still the most appropriate method for treating pathologies that affect the posterior segment of the eye. The frequency of administration plays a key role, as their administration is currently not standardised in clinical practice and therefore different administration schedules coexist. Fixed regimens were evaluated in pivotal studies [10–12], in which the patients received monthly or bimonthly injections on a continuous basis over the follow up months [10,11,13]. Fixed monthly injections offer the best visual outcome, but this regimen is not commonly followed outside clinical trials due to the increased number of required visits to the ophthalmologist [14]. In addition, the followed regimen can have significant economic repercussions due to the high cost of these treatments [15]. The two most commonly followed treatment regimens are the pro re nata (PRN), which consists of treating if reactivation, and the Treat and Extend (T&E) strategy. The latter consists of a proactive treatment regimen where the key is to treat the patient before the disease activity appears. It was created to reduce the frequency of injections and it is the most accepted treatment regimen [16,17]. T&E consists of a loading phase of three monthly injections, followed by a progressive lengthening of the treatment intervals by one or two weeks as long as no activity is detected [18]. If disease activity is detected during any visit, treatment intervals are reduced to the interval used prior to the extension. A recent meta-analysis has shown that the T&E regime has a mean of 6.9 fewer injections at 24 months when compared to monthly injections yielding similar visual acuity results. Moreover, when compared to the PRN strategy, T&E has revealed an improvement of 6.18 more letters than PRN in terms of visual gains, however a mean of 1.44 more injections was required for the T&E as compared to PRN regimen at 12 months [16].

Normally, the frequency of administration should be based on the half-life of the drug ($t_{1/2}$) in order to achieve a sustained therapeutic drug concentration in the vitreous. Direct determination of the vitreous drug levels requires invasive techniques, and for this reason, these type of studies are limited to the preclinical field [19,20]. Therefore, most clinical pharmacokinetics studies rely on indirect blood measurements, which have been mainly restricted to pivotal studies, which have not been studied in the great majority of post-authorisation trials [21,22]. For these reasons, the information that is available in this field is very limited.
The present review collects the most relevant aspects of the intravitreal pharmacokinetics of anti-VEGF drugs in AMD. For that purpose, an extensive review of the preclinical and clinical pharmacokinetic studies that have been published in this field was carried out. Moreover, information regarding the factors involved in the vitreous distribution and clearance, the methods for the quantification of anti-VEGF antibodies, and the utility of population models have also been compiled.

2. Pharmacokinetics of Anti-VEGF Drugs

It is very difficult to perform intravitreal pharmacokinetic studies on humans, given that taking vitreous samples is an invasive procedure; therefore, most of the studies have focused on preclinical research.

2.1. Ranibizumab

Ranibizumab is a fragment of a monoclonal antibody that does not contain the Fc region (heavy chains) and with affinity for all subtypes of VEGF-A (Table 1). It has been approved for the treatment of neovascular AMD based on the results of two clinical trials (ANCHOR and MARINA), where 0.5 mg was administered on a monthly regimen observing improvements in visual acuity with gains of 11.3 letters in the ANCHOR trial and 7.2 letters in the MARINA trial, as compared to the control groups [10,11].

Table 1. Properties of anti-vascular endothelial growth factor (VEGF) antibodies for Age-Related Macular Degeneration (AMD). Data from [23–25].

| Properties | Ranibizumab | Bevacizumab | Aflibercept |
|------------|-------------|-------------|-------------|
| Class      | Antibody fragment | Monoclonal antibody | Fusion protein |
| MW (KDa)   | 48          | 149         | 115         |
| Net charge | Negative    | Negative    | Slightly positive |
| Binding target | VEGF-A | VEGF-A | VEGF-A, VEGF-B, PIGF |
| K<sub>D</sub> for VEGF<sub>165</sub> (pM) | 46          | 58          | 0.49         |

M<sub>W</sub> = molecular weight; K<sub>D</sub> = equilibrium dissociation constant.

2.1.1. Animal Studies

In a pharmacokinetic analysis that was performed by Bakri et al., 0.5 mg of ranibizumab was intravitreally injected in the right eye of male Dutch-belted rabbits. While using a non-compartment model, they determined that the half-life of ranibizumab was 2.88 days in the vitreous humour and 2.84 days in the aqueous humour. The mean resident time (MRT) was 4 and 6.8 days in vitreous and aqueous humour, respectively, and no serum concentrations were detected [21].

Other studies have found vitreous half-lives within the same range. A study using I-124 labelled ranibizumab in rabbits and quantified by PET/CT obtained a similar vitreous half-life (2.81 days), but a two-compartment model was applied in this case [26]. Another study calculated a vitreous half-life of 2.75 days with a one-compartment model [27]. In all the aforementioned studies of the pharmacokinetics of ranibizumab in rabbits, the standard dose of ranibizumab (0.5 mg/0.05 mL) was injected in just one rabbit eye (unilateral injection). However, the authors of the following study decided to perform a bilateral injection, with a different dose to the standard. They injected 0.625 mg/0.05 mL in both eyes, obtaining a vitreous half-life of 2.9 days with a one-compartment model [28], which is comparable to the half-life that was calculated in the other conditions.

Other studies have tested ranibizumab pharmacokinetics in monkeys as a non-human primate model. Christoforidis et al. performed a study with owl monkeys with I-124 radiolabelled ranibizumab
in the same way as they performed the study in rabbits [26,29]. They obtained a vitreous half-life of 2.73 days after a single intravitreal injection of 0.5 mg/0.05 mL in one eye. Another study, in this case with cynomolgus monkeys, was performed with a bilateral single injection of ranibizumab, while testing two different injection doses: 0.5 mg/0.05 mL and 2 mg/0.05 mL [30]. They obtained vitreous half-lives with a one-compartment model of 2.63 and 3.95 days, respectively. The comparison between both groups suggests dose-linear vitreous pharmacokinetics. Aqueous humour, retina, and serum half-lives were also calculated (with a non-compartmental analysis), obtaining values of 2.54, 2.60, and 3.59 days for the injected dose of 0.5 mg, and 2.63, 2.28, and 3.47 days for the 2 mg injected dose, respectively. They also concluded that ranibizumab rapidly distributes to the retina, and it is removed from the vitreous humour through the anterior chamber (ranibizumab was found in the aqueous humour) and the posterior route (ranibizumab was found in both retinal layers). Moreover, they suggested that intraocular metabolism does not play a significant role in the elimination of ranibizumab from the vitreous chamber [30]. Even though Christoforidis and Gaudreault’s studies differ in the monkey species used and the uni/bilateral injection at the dose of 0.5 mg, the estimated vitreous half-lives are comparable (2.73 days vs 2.63 days) [29,30].

2.1.2. Human Studies

A population approach of non-linear pharmacokinetic modelling that is based on serum samples that were collected from patients with AMD enrolled in five clinical trials, receiving from 0.3 to 2 mg per eye of single or multiple intravitreal ranibizumab estimated a vitreous half-life of nine days [22]. Ranibizumab reached maximum serum concentration approximately 0.5 days after intravitreal administration and these concentrations were 90,000 times lower than those that were estimated to be found in the vitreous humour [22]. The estimated serum half-life was two hours, because it is not a full-length antibody and it lacks the FcRn (the neonatal Fc receptor for IgG) that protects from lysosomal degradation, ranibizumab is prone to systemic metabolism [22]. Another study performed three intravitreal injections of ranibizumab (0.5 mg) in AMD patients at monthly intervals. A serum half-life of 5.8 days was calculated after measuring serum levels. They also found that ranibizumab did not demonstrate systemic accumulation between the first and third dose, concluding that ranibizumab is very quickly cleared from the bloodstream [19,31].

Aqueous humour half-life was calculated in the study performed by Krohne et al. They included patients that were diagnosed with both clinically significant cataract and macular oedema secondary to AMD, diabetic maculopathy, or retinal vein occlusion. The patients received a single intravitreal injection (0.5 mg) within 40 days before surgery, in which the aqueous samples were obtained, obtaining an aqueous half-life of 7.19 days. Moreover, they performed axial length measurements of the ocular globe in order to correct ranibizumab concentrations for ocular volume, determining that volume differences did not significantly alter the aqueous half-life (7.15 days) [32].

There is no data available with regards to the vitreous levels of ranibizumab after intravitreal injection in humans, and consequently vitreous half-life values are no available. One group applied a mathematical model that was intended for intravitreal pharmacokinetics in rabbits to estimate the vitreous half-life of ranibizumab in humans. They calculated a vitreous half-life for ranibizumab of 4.75 days while using an experimentally determined mean half-life of bevacizumab in humans [33].

No significant reductions have been observed in the concentration of systemic VEGF levels between baseline and after intravitreal injections of ranibizumab [34–38] or drug accumulation between doses [31].

2.2. Bevacizumab

Bevacizumab is a full monoclonal antibody (with Fc fraction) with an affinity for all subtypes of VEGF-A and systemic indication in the treatment of different types of cancer (breast, colon . . . ) (Table 1). There is no current indication for AMD, but its mechanism of action and its administration at the level of the posterior chamber of the eye at a much lower dose (1.25 mg/0.05 mL) have promoted its off-label
use [39]. In addition, the possibility of splitting up the vial in the pharmacy departments reduces the cost of the treatment in comparison with the other two drugs that do have the indication [40].

2.2.1. Animal Studies

Bevacizumab vitreous half-life has been estimated at 4.32 days, following a 1.25 mg/0.05 mL unilateral intravitreal injection in rabbit eyes (non-compartmental analysis), with concentrations that remain above 10 µg/mL for 30 days [41]. The estimated half-life was 4.88 days and 6.8 days in the serum while using the same non-compartment model in the aqueous humour [41]. This data is consistent with the intravitreal pharmacokinetics that were analysed by molecular imaging with I-125-bevacizumab in a rabbit model ($t_{1/2} = 4.22$ days, two-compartment model) [26]. Bakri et al. found that bevacizumab concentration was higher in the aqueous humour of the fellow eye (uninjected) than in the vitreous, concluding that bevacizumab enters the fellow eye from the systemic circulation through the anterior route, reaching the aqueous humour first before diffusing into the vitreous, rather than entering through the choroidal blood flow [41]. However, very low concentrations of bevacizumab were found in the aqueous and vitreous humour of the uninjected eye, so this conclusion must be taken with caution.

Higher vitreous half-life values (5.95 days) were obtained in the study that was performed by Nomoto et al. in a rabbit model (unilateral injection). They also measured the amount of bevacizumab in iris/ciliary body and retina/choroids, obtaining half-lives in these tissues of 5.74 and 6.23 days, respectively. However, the higher half-lives that were obtained can be explained, because they performed an Enzyme-Linked ImmunoSorbent Assay (ELISA) that detected all bevacizumab, i.e., free bevacizumab, VEGF-bevacizumab complex and fragments of bevacizumab molecules, whereas the majority of the studies only measure free bevacizumab levels, as they have been considered as a good representation of total drug concentration. Moreover, their first sample taken was seven days after injection, so early data is missing in this study, which could counterfeit the results [42].

Another study has found even higher half-lives in vitreous, aqueous humour, and serum (6.61 days, 6.51 days, 5.87 days, non-compartmental analysis) when compared to the other studies of 1.25 mg/0.05 mL bevacizumab unilateral injection in rabbit models [43]. They used New Zealand rabbits instead of Dutch-belted rabbits, together with a high sensitivity ELISA (detection limit 0.001 ng/mL) detection kit, which could explain the differences that were found. However, when compared to Bakri’s work, both of the studies found that the maximum concentration was achieved at one day post-injection in the vitreous humour and after eight days in serum [41,43]. One great advantage of this study is that anti-bevacizumab antibodies in serum were also measured, concluding that these anti-bevacizumab antibodies cannot have an important effect on bevacizumab concentration due to their low concentration [43].

The intravitreal half-life estimated in owl monkeys of 1.25 mg/0.05 mL I-124-bevacizumab intravitreal injection was 3.6 days, with detectable concentrations up to 28 days [26]. The pharmacokinetics of bevacizumab in cynomolgus monkeys was also tested by ELISA, but in this case only aqueous humour and serum samples were collected, obtaining half-lives of 2.8 and 12.3 days, respectively [44], preventing the comparison of both studies.

No activity was detected in other parts of the rabbit body apart from the ocular globe in the studies that were performed by Christoforidis et al. with I-125 radiolabelled antibodies bevacizumab, ranibizumab, and aflibercept [26,45]. However, these results are inconsistent with other studies, where bevacizumab was detected in the brain, heart, and kidney after a single intravitreal injection [46].

2.2.2. Human Studies

Vitreous half-life was estimated to be 6.7 days following a two-compartment model. Patients received a single dose of 1.25 mg bevacizumab prior to vitrectomy. A peak concentration of 165 µg/mL was reached on the second day after the intravitreal injection [47]. Another author found that the vitreous half-life ranged between 2.5 and 7.3 days, with a mean of 4.9 days, after the administration of 1.25 mg/0.05 mL while using a one-compartmental model. The vitreous samples were
These results should be taken with caution, as only eleven [47] and three [34] patients, respectively, were used for the pharmacokinetic analysis. Moreover, the fact that vitrectomy was performed due to collateral complications, such as submacular haemorrhage and choroidal neovascularization [47] or cataract extraction [34] must also be taken into account. The latter study also evaluated the serum half-life of bevacizumab, determining it to be 11.3 days [34].

A serum half-life of 18.7 days [19,31] has been estimated after three-single intravitreal doses of 1.25 mg of bevacizumab. Moreover, its systemic exposure was found to be greater than that of ranibizumab or aflibercept, with a serum concentration of 1.58 nM, which is higher than the estimated inhibitory concentration (IC50) for VEGF factor (IC50 = 0.668 nM) [48]. This data suggests the possibility of adverse effects that are usually associated with the intravenous doses of these drugs, and that could appear in patients with AMD, macular oedema, etc. [49–51].

Aqueous half-life was estimated to be 9.82 days in humans by non-compartmental analysis. Patients received a single intravitreal injection of 1.5 mg bevacizumab and within 53 days after the injection, an aqueous humour sample was obtained during cataract surgery. The patients were diagnosed with cataract and recurrent macular oedema secondary to AMD. Bevacizumab concentration peaked on the first day, with a mean concentration of 33.3 µg/mL [52].

The same dose (1.5 mg) was administered in another study and was compared to a higher dose of 3 mg. The maximum concentration in the aqueous humour was obtained at one day post-injection for both doses, with an aqueous half-life of 7.85 and 11.69 days for the 1.5 and 3 mg doses, respectively, calculated by one-compartmental analysis. Double dosing induced a significant higher peak concentration at baseline, although the aqueous bevacizumab concentration was not significantly different after six weeks. Therefore, the administration of a double-dose does not significantly increase the duration of action. This study presents several limitations, as that the enrolled patients suffered from different retinal diseases and the injection volume was double for the dose of 3 mg in relation to 1.5 mg, which could affect the pharmacokinetic parameters [53].

Regarding the bevacizumab levels in the aqueous humour of the uninjected eye, the study that was conducted by Meyer et al. found that the concentration of bevacizumab was below the ELISA detection limit, so no significant levels are expected to be found in the fellow aqueous chamber [54].

2.3. Aflibercept

Aflibercept has a different mechanism than the other two. It is a recombinant fusion protein that consists of portions from the extracellular domains of the human VEGF receptors 1 and 2, which are fused with the Fc portion of the human IgG1. Aflibercept has a great affinity for VEGF A, B, and placental growth factor (P1GF) (Table 1). The data on the pharmacokinetics of aflibercept is scarce and mostly refers to animal models in comparison with the other two anti-VEGF drugs.

2.3.1. Animal Studies

Christoforidis et al. also performed studies with I-124 radiolabelled aflibercept, obtaining a vitreous half-life of 4.58 days after a single intravitreal injection of 2 mg/0.05 mL in Dutch-belted rabbits [45]. Another study found a vitreous half-life of 3.92 days in New Zealand white rabbits [55]. The differences could be due to the fact that the aflibercept concentration was quantified by an indirect ELISA and the dose injected was lower, 1.2 mg/0.03 mL. In this study, the aqueous humour and retina/choroid half-lives were also calculated, which obtained values of 2 and 2.425 days, respectively [55].

Studies in owl monkeys were also performed with I-124 aflibercept, obtaining a vitreous half-life value of 2.44 days [29], whereas the vitreous half-life value was 2.2 days in cynomolgus macaques where aflibercept concentration was measured by ELISA [56].
2.3.2. Human Studies

Serum half-life of aflibercept has been estimated at 11.4 days after three-monthly intravitreal injections of aflibercept (2.0 mg) in an AMD population. The authors found that aflibercept seemed to exhibit systemic drug accumulation between the first and third dose [19,31].

Some of the authors explored the relation between systemic exposure to intravitreal aflibercept injection and systemic pharmacodynamics (blood pressure). They included patients from four different clinical trials. Aflibercept plasma concentrations quickly decreased over a week to concentrations below the LLOQ (15.6 µg/L) once peak concentrations has been achieved within 1–3 days post-dose. Intravitreal administrations were not associated with common adverse effects of intravenous anti-VEGF [57].

The authors of this article suggest, that owing to the intermediate size of aflibercept (between ranibizumab and bevacizumab), the vitreous half-life of aflibercept could be hypothesised to be nine days since no intravitreal pharmacokinetic studies have been performed in humans with aflibercept [58]. A study conducted in five patients with AMD found an aqueous half-life of approximately nine days based on aqueous samples. They also found very low plasma levels, suggesting a lack of substantial plasma exposure [59].

The same author that calculated a vitreous half-life of ranibizumab in humans with a mathematical model, determining a vitreous half-life of 7.13 days for aflibercept following the same procedure [33].

A compiled list of the different pharmacokinetic parameters analysed in the studies of ranibizumab (Table 2), bevacizumab (Table 3), and aflibercept (Table 4) in different species (rabbit, monkey, and human) has been included at the end of this section. The compiled parameters include half-life \((t_{1/2})\), time taken to reach maximum concentration \((T_{\text{max}})\), maximum concentration \((C_{\text{max}})\), and area under the concentration-time curve (AUC). \(C_{\text{max}}\) is reached very early on due to the rapid distribution of the antibodies through the vitreous humour, so most of the studies assume that their first data point (normally one day post-injection) corresponds to \(T_{\text{max}}\). Therefore, the utility of defining \(T_{\text{max}}\) is sometimes controversial. However, \(T_{\text{max}}\) is shown in the pharmacokinetic tables in order to provide a time reference corresponding to \(C_{\text{max}}\).
Table 2. Pharmacokinetic parameters of intravitreal ranibizumab in different species.

| Model                  | Injected Dose | Determination | Sensitivity | PK Model | Sample | Time Points | Normal Eyes | Vitrectomy | Aphakia | Observations                  | Ref. |
|------------------------|---------------|---------------|-------------|----------|--------|-------------|-------------|------------|---------|-------------------------------|------|
| Dutch-belted rabbits   | 0.5 mg/0.05 mL| CLIA          | LLOQ = 0.375 ng/mL | NC       | VH     | 1, 3, 8, 15, 29 days | 2.88 days  | 1 day       | 162 µg/mL | No detection in serum         | [21] |
|                        |               |               |             |          | AH     |             | 2.84 days   | 3 days      | 17.9 µg/mL |                               |      |
|                        |               |               |             |          | Serum  |             |             | No detection in serum |      |                               |      |
| Dutch-belted rabbits   | 0.5 mg/0.05 mL| PET (I-124)   |             | 2C       | VH     | 0, 2, 5, 7, 14, 21, 28, 35 days | 2.81 days   | 0 h         | No detection in other organs | [26] |
|                        |               |               |             |          | AH     |             |             | No detection in other organs |      |                               |      |
|                        |               |               |             |          | Serum  |             |             | No detection in other organs |      |                               |      |
| Dutch-belted rabbits   | 0.5 mg/0.05 mL| PET (I-124)   |             | 2C       | VH     | 0, 2, 5, 7, 14, 21, 28, 35 days | 2.81 days   | 2.13 days   | 1.79 days |                               | [60] |
|                        |               |               |             |          | AH     |             |             | No detection in other organs |      |                               |      |
| New Zealand rabbits    | 0.625 mg/0.05 mL| ELISA           | 0.78 ng/mL | 1C       | VH     | 1, 8 h; 1, 2, 4, 7, 14, 21, 30, 42, 50, 60 days | 2.9 days  | 1 h         | 1280 µg/mL | Bilateral injection           | [28] |
|                        |               |               | 0.78 ng/mL | NC       | AH     |             | 3 days      | 48 h        | 57.1 µg/mL |                               |      |
|                        |               |               | 7.8 ng/mL  | NC       | Serum  |             | 24 h        | 0.055 µg/mL | No detection in other organs |      |                               |      |
| New Zealand rabbits    | 0.25 mg/0.025 mL| ELISA          | LLOQ = 0.375 ng/mL | 1C       | VH     | 1 h or 1, 2, 5, 14, 30, 30 days | 2.75 days   | 1 h         | 91.61 µg/mL |                               | [27] |
|                        |               |               |             |          | AH     |             | 2.51 days   | 118.01 µg/mL |               |                               |      |
|                        |               |               |             |          | Serum  |             | 1 h         | 20.38 µg/mL | 21.7 µg/mL |                               |      |
| Owl monkeys            | 0.5 mg/0.05 mL| PET (I-124)   |             | 2C       | VH     | 0, 1, 2, 4, 8, 14, 21, 28, 35 days | 2.73 days   |             |         |                               | [29] |
|                        |               |               |             |          | AH     |             |             | No detection in other organs |      |                               |      |
|                        |               |               |             |          | Serum  |             |             | No detection in other organs |      |                               |      |
| Cynomolgus macaques    | 0.5 mg/0.05 mL| ELISA         | 1.5 ng/mL  | 1C       | VH     | 6 h, 2, 3, 5, 8, 11 days | 2.63 days   | 6 h         | 169 µg/mL | Bilateral injection           | [30] |
|                        |               |               | 1.5 ng/mL  | NC       | AH     |             | 2.54 days   | 6 h         | 116 µg/mL |                               |      |
|                        |               |               | 15.6 ng/mL | NC       | Serum  |             | 3.59 days   | 6 h         | 150 µg/mL |                               |      |
| Cynomolgus macaques    | 2 mg/0.05 mL  | ELISA         | 1.5 ng/mL  | 1C       | VH     | 6 h, 2, 3, 5, 8, 11 days | 3.95 days   | 1 day       | 612 µg/mL | Bilateral injection           | [30] |
|                        |               |               | 1.5 ng/mL  | NC       | AH     |             | 2.63 days   | 1 day       | 478 µg/mL |                               |      |
| Cynomolgus macaques    | 2 mg/0.05 mL  | ELISA         | 15.6 ng/mL | NC       | Serum  |             | 3.47 days   | 6 h         | 616 µg/mL |                               |      |
| Cynomolgus macaques    | 0.25 mg/0.05 mL| ELISA         | LLOD = 156 pg/mL | 1C       | VH     | 1, 3 days; 1-8 weeks | 2.3 days  | 1 day       | 51.3 µg/mL |                               | [56] |
|                        |               |               |             |          | AH     |             | 171 days  | 41.8 µg/mL | 154 days |                               |      |
|                        |               |               |             |          | Serum  |             | 1.4 days   | 41.8 µg/mL | 154 days |                               |      |
| Model  | Injected Dose | Determination | Sensitivity | PK Model | Sample | Time Points | Normal Eyes | Vitrectomy | Aphakia | Observations | Ref. |
|--------|---------------|---------------|-------------|----------|--------|-------------|-------------|------------|---------|--------------|------|
| Human  | 0.5 mg/0.05 mL | ELISA         | 10-1000 ng/mL | 1C       | VH     | 1-37 days   | t_{1/2} = 7.19 days | T_{max} = 1 day | C_{max} = 56.1 µg/mL | t_{1/2} = 1 day | 32   |
|        |               |               |             |          | AH     |             |             |            |         |               |      |
|        |               |               |             |          | Serum   |             |             |            |         |               |      |
| Human  | Variable      | CLIA          | LLOQ = 0.3 ng/mL | 1C       | VH     | Variable    | 9 days      |            |         |                       | 22   |
|        |               |               |             |          | AH     |             |             |            |         |               |      |
|        |               |               |             |          | Serum   |             |             |            |         |               |      |
| Human  | 0.5 mg/0.05 mL | ELISA         | LLOQ = 15 pg/mL | NC       | VH     | 3 h, 1, 3, 7, 28 days | 5.8 days  | 0.11 nM | 0.46 h nM |                       | 19,31 |
|        |               |               |             |          | AH     |             |             |            |         |               |      |

CLIA = Chemoluminiscnt immunoassay; ELISA = Enzyme-Linked ImmunoSorbent Assay; PET = Positron Emission Tomography; LLOD = Lower Limit of Detection, LLOQ = Lower Limit of Quantification, 1C = One-compartment model; 2C = Two-compartment model; NC = Non-compartment model; VH = vitreous humour; AH = aqueous humour; t_{1/2} = half-life; T_{max} = time taken to reach maximum concentration; C_{max} = maximum concentration; AUC = area under the curve.
Table 3. Pharmacokinetic parameters of intravitreal bevacizumab in different species.

| Model                  | Injected Dose | Determination | Sensitivity | PK Model | Sample | Time Points | Normal Eyes | Vitrectomy | Aphakia | Observations                        | Ref. |
|------------------------|---------------|---------------|-------------|----------|--------|-------------|-------------|------------|---------|-------------------------------------|------|
| Dutch-belted rabbits   | 1.25 mg/0.05 mL | CLIA          | LLOQ = 0.0825 ng/mL | NC       | VH     | 1, 3, 8, 15, 29 days | t_1/2 = 4.32 days | C_max = 1 day | AUC = 400 µg/mL |            | [41] |
|                        |               |               |             |          | AH     |             | 4.88 days | 3 days | 37.7 µg/mL                       |      |
|                        |               |               |             |          | Serum  |             | 6.86 days | 8 days  | 3.33 µg/mL                       |      |
| Dutch-belted rabbits   | 1.25 mg/0.05 mL | ELISA         | LLOQ = 0.1 ng/mL  | VH       | 1, 2, 4, 12 weeks | t_1/2 = 5.95 days | C_max = 7 days | AUC = 59.7008 µg/mL |            | [42] |
|                        |               |               |             |          | AH     |             | 7 days | 37.6 ng/mL               |      |
|                        |               |               |             |          | Serum  |             | 12.95 days | 14 days | 2.0872 µg/mL                      |      |
| Dutch-belted rabbits   | 1.25 mg/0.05 mL | PET (I-124)   | 2C          | VH       | 0, 2, 5, 7, 14, 21, 28, 35 days | t_1/2 = 4.22 days | C_max = 400 µg/mL | AUC = 1 day | No detection in other organs | [26] |
|                        |               |               |             |          | AH     |             | 2.30 days | 2.08 days |                                  |      |
| Dutch-belted rabbits   | 1.25 mg/0.05 mL | PET (I-124)   | 2C          | VH       | 0, 2, 5, 7, 14, 21, 28, 35 days | t_1/2 = 4.22 days | C_max = 400 µg/mL | AUC = 1 day |                                  | [60] |
|                        |               |               |             |          | AH     |             | 2.30 days | 2.08 days |                                  |      |
| Dutch-belted rabbits   | 1.25 mg/0.05 mL | ELISA         | LLOD = 0.1 ng/mL | VH       | 2, 4, 10, 30, 35 days | t_1/2 = 6.69 days | C_max = 6.22 µg/mL | AUC = 69.2 d µg/mL |            | [61] |
|                        |               |               |             |          | AH     |             | 6.4 days | 6.22 µg/mL |                                  |      |
|                        |               |               |             |          | Serum  |             | 6.99 days | 6.22 µg/mL |                                  |      |
| New Zealand rabbits    | 1.25 mg/0.05 mL | ELISA         | LLOQ = 0.01 ng/mL | VH       | 1, 3, 8, 15, 29 days | t_1/2 = 6.61 days | C_max = 1 day | AUC = 406.25 µg/mL |            | [43] |
|                        |               |               |             |          | AH     |             | 6.51 days | 1 day | 5.835 µg/mL                       |      |
|                        |               |               |             |          | Serum  |             | 5.87 days | 8 days  | 0.413 µg/mL                       |      |
| New Zealand rabbits    | 1.25 mg/0.05 mL | ELISA         | LLOQ = 0.0825 ng/mL | VH       | 1 h, 1, 2, 5, 14, 30 days | t_1/2 = 7.06 days | C_max = 1 h | AUC = 1021.54 mg/mL |            | [62] |
|                        |               |               |             |          | AH     |             | 6.99 days | 1 day |                                  |      |
|                        |               |               |             |          | Serum  |             | 2 days | 121 mg/mL                  |      |
| New Zealand rabbits    | 0.025 mL      | PET (Zr-89)   |             | VH       | 5-60 min; 4, 24, 48, 120, 144 h | t_1/2 = 3.51 days | C_max = 1 day | AUC = 406.25 µg/mL |            | [63] |
|                        |               |               |             |          | AH     |             | 3.51 days | 406.25 µg/mL |                                  |      |
| Owl monkeys            | 1.25 mg/0.05 mL | PET (I-124)   | 2C          | VH       | 0, 1, 2, 4, 8, 14, 21, 28, 35 days | t_1/2 = 3.56 days | C_max = 2 days | AUC = 49.500 µg/mL |            | [29] |
|                        |               |               |             |          | AH     |             | 3.5 days | 2 days | 49.500 µg/mL                      |      |
|                        |               |               |             |          | Serum  |             | 3.5 days | 7.80 ng/mL |                                  |      |
| Cynomolgus macaques    | 1.25 mg/0.05 mL | ELISA         | LLOD = 0.156 ng/mL | VH       | 1, 3, 7 d, 2, 4, 6, 8 weeks | t_1/2 = 2.8 days | C_max = 2 days | AUC = 49.500 µg/mL |            | [44] |
|                        |               |               |             |          | AH     |             | 2.8 days | 1 day | 49.500 µg/mL                      |      |
|                        |               |               |             |          | Serum  |             | 12.3 days | 7 days  | 1.430 µg/mL                      |      |
Table 3. Cont.

| Model                  | Injected Dose | Determination | Sensitivity | PK Model | Sample  | Time Points       | Normal Eyes | Vitrectomy | Aphakia | Observations | Ref. |
|------------------------|---------------|---------------|-------------|----------|---------|-------------------|-------------|------------|---------|--------------|------|
| Cynomolugus macaques   | 1.25 mg/0.05 mL | ELISA         | 7.8–1000 pg/mL | VH       | 1, 3, 7 d, 2, 4, 6, 8 weeks | 1 day 10.8 µg/mL | 1.5 days |          |             | [64] |
| Human                  | 1.25 mg/0.05 mL | ELISA         | LLOQ = 313 pg/mL | AH       | 5.9 days | 42.2 ng/mL        |             |            |         |              | [19,31] |
| Human                  | 1.25 mg/0.05 mL | ELISA         | NC          | VH       | 3 h, 1, 3, 7, 28 days | 18.7 days 0.76 nM | 16.10 h·nM |         |             |      |
| Human                  | 1.25 mg/0.05 mL | ELISA         | 2C          | VH       | 1–101 days | 6.7 days 2 days 165 µg/mL | 2036 d·µg/mL |           |         |              | [47] |
| Human                  | 1.25 mg/0.05 mL | ELISA         | 6.25 ng/mL  | VH       | Variable  | 4.9 days 0.66 day |             |            |         |              | [34] |
| Human                  | 1.5 mg         | ELISA         | 1C          | VH       | 1–53 days | 9.82 days 1 day 33.3 µg/mL |             |            |         |              | [52] |
| Human                  | 1.5 mg         | ELISA         | 1C          | VH       | 1–60 days | 7.85 days 1 day 14.86 µg/mL |             |            |         |              | [53] |
| Human                  | 3 mg           | ELISA         | 1C          | VH       | 1–60 days | 11.69 days 1 day 27.74 µg/mL |             |            |         |              | [53] |

CLIA = Chemoluminescent immunoassay; ELISA = Enzyme-Linked ImmunoSorbent Assay; PET = Positron Emission Tomography; LLOD = Lower Limit of Detection; LLOQ = Lower Limit of Quantification; 1C = One-compartment model; 2C = Two-compartment model; NC = Non-compartment model; VH = vitreous humour; AH = aqueous humour; t1/2 = half-life; Tmax = time taken to reach maximum concentration; Cmax = maximum concentration; AUC = area under the curve.
Table 4. Pharmacokinetic parameters of intravitreal aflibercept in different species.

| Model                  | Injected dose | Determination | Sensitivity | PK Model | Sample | Time Points | Normal Eyes | Vitrectomy | Aphakia | Observations                                      | Ref. |
|------------------------|---------------|---------------|-------------|----------|--------|-------------|-------------|------------|---------|--------------------------------------------------|------|
| Dutch-belted rabbits   | 2 mg/0.05 mL  | PET (I-124)   | 1C          | VH       | 0, 2, 5, 7, 14, 21, 28, 35 days | t_{1/2} = 4.58 days, T_{max} = 0 h, C_{max} = No detection in other organs | [45] |
| Owl monkeys            | 2 mg/0.05 mL  | PET (I-124)   | 2C          | VH       | 0, 1, 2, 4, 8, 14, 21, 28, 35 days | t_{1/2} = 2.44 days | [29] |
| Cynomolgus macaques    | 2 mg/0.05 mL  | ELISA         | LLOD = 156 pg/mL | VH       | 1, 3 days; 1-8 weeks | t_{1/2} = 2.2 days, T_{max} = 1 day, C_{max} = 74 µg/mL, AUC = 174 µg.d/mL, AUC = 1.5 µg.d/mL, AUC = 68 µg.d/mL, AUC = 124 µg.d/mL | [56] |
| Human                  | 2 mg/0.05 mL  | ELISA         | LLOQ = 1000 pg/mL | VH       | 3 h, 1, 3, 7, 28 days | t_{1/2} = 11.4 days, T_{max} = 0.45 nM, C_{max} = 4.32 h nM | [19,31] |
| Human                  | 2 mg          | ELISA         |             | VH       | 4 h, 1, 3, 7, 14, 28, 35 days | t_{1/2} = 11 days, T_{max} = 4 h, C_{max} = 64.4 mg/L | [59] |

CLIA = Chemoluminescent immunoassay; ELISA = Enzyme-Linked ImmunoSorbent Assay; PET = Positron Emission Tomography; LLOD = Lower Limit of Detection, LLOQ = Lower Limit of Quantification, 1C = One-compartment model; 2C = Two-compartment model; NC = Non-compartment model; VH = vitreous humour; AH = aqueous humour; t_{1/2} = half-life; T_{max} = time taken to reach maximum concentration; C_{max} = maximum concentration; AUC = area under the curve.
3. Pharmacokinetic Considerations

Many factors are involved in the pharmacokinetics of anti-VEGF antibodies, from the physiological conditions of the eye, to the surgical procedures or the analytical methods, which allow for their determination.

3.1. Eye Physiological Factors

3.1.1. Distribution-Diffusion in the Vitreous Humour

The distribution of drugs in the vitreous humour is conditioned by its intrinsic characteristics, such as volume and composition, as well as by the properties of the drug (charge, molecular weight, and protein binding capacity). The vitreous humour occupies around 80% of the internal volume of the eye, which is around 4 mL in humans, 1.5 mL in rabbits, and 2 mL in monkeys [65]. It is an avascular structure that is mainly composed of a hydrophilic polymer of hyaluronic acid and collagen, which contributes to its consistency and attracts water, which is its majority component (98%). The central part of the vitreous humour has less of these components than the posterior part, which makes it more fluid-like. In addition, hyaluronic acid has a negative charge, meaning that the restrictive diffusion of positively charged molecules may occur [66]. Bevacizumab and ranibizumab are both negatively charged molecules under physiological conditions, therefore their movement should not be restricted in the vitreous humour [24]. However, aflibercept is considered to have a mild positive charge, which might affect its pharmacokinetic properties [25].

On other hand, molecular weight affects drug diffusion, which therefore affects the half-life. The anti-VEGF molecules are rather heavy molecules (149 KDa, 115 KDa, 48 KDa for bevacizumab, aflibercept, and ranibizumab, respectively), so they are expected to have a low intravitreal clearance when compared with small molecules that do not have steric hindrance (in general, intravitreal half-life increases as molecular weight rises above 10,000 Da) [67]. A comparison of the properties of the three anti-VEGF inhibitors can be found in Table 1 [23–25].

Moreover, the rheological properties of the vitreous humour change with age, in a process called liquefaction, in which the vitreous humour is turned into a more liquefied state. Liquefaction might increase drug diffusion, especially in those with high molecular weight [67]. When compared to plasma, the concentration and number of proteins in the vitreous humour is low (0.5–1.5 mg/mL in vitreous vs 60–80 mg/mL in plasma) [68]. The main proteins are collagen (a structural protein), albumin, and immunoglobulins (non-structural proteins) [69]. The binding of drugs to proteins might reduce its distribution through the vitreous humour, although this factor does not seem to affect the diffusion of anti-VEGF drugs [70].

3.1.2. Elimination of Drugs from the Vitreous Humour

The elimination of drugs from the vitreous humour can occur via two different routes, either by metabolism or by disposal into the systemic circulation. In the case of the anti-VEGF drugs, they do not appear to suffer metabolism nor degradation in the eye [58,70].

After intravitreal injection, the drugs can be removed to the systemic circulation by two routes: the anterior route or the posterior route. The anterior route consists of drug diffusion through the vitreous humour until it reaches the aqueous humour and it is then eliminated through its flow. All the drugs can be eliminated in this way. Various reports have considered that anti-VEGF drugs are mainly eliminated through the anterior route [21,28,32,52,71].

The posterior route consists of the secretion of the drug by the epithelium of the ciliary body, iris, or retinal pigment epithelium [70]. Peters and Heidushka tried to demonstrate that bevacizumab was also eliminated through the posterior route crossing the blood-retinal barrier. They observed that bevacizumab immunoreactivity after the intravitreal injection extended over time to the inner layers of the retina. However, they did not attempt to determine whether or not active transport is involved in
this process [72,73]. The effect of active transport through the retina is not yet clear, so the impact that this may have on the drug pharmacokinetics is yet to be defined [74].

3.2. Surgical Ocular Procedures (Lensectomy and Vitrectomy)

The distribution and elimination of anti-VEGF drugs from the vitreous are intimately related to several ophthalmic surgical procedures. Laude et al. suggest that cataract operated patients could have a faster clearance of vitreous drugs [67]. However, Krohne et al. found that ocular volume and lens status have no relevant impact on ocular pharmacokinetics and the duration of action of anti-VEGF drugs after comparing VEGF suppression times in phakic (natural lens) and pseudophakic (replaced crystalline lens) human eyes [75].

On the other hand, many patients with macular disease who are being treated with anti-VEGF drugs require surgical intervention for complications, such as bleeding in the vitreous. It is known that replacing the gel-like vitreous humour with a less viscous saline or aqueous humour facilitates the transportation of oxygen, as well as the clearance of VEGF inhibitors and cytokines, reducing oedema and retinal neovascularisation [76,77]. Additionally, the surgical procedure itself and the use of silicone oil as vitreous replacement can influence drug pharmacokinetics [78].

There are few studies that correlate the effect of vitrectomy with the pharmacokinetics and these give very different results. A study performed on rabbits with labelled mAbs with I-124 demonstrated a reduction in the half-life of anti-VEGF drugs after vitrectomy and lensectomy, going from 4.22 to 2.30 and 2.08 days, respectively, for bevacizumab and from 2.81 to 2.13 and 1.79 days, respectively, for ranibizumab [60]. The same author also quantified the serum concentration of bevacizumab in rabbits, finding that the serum levels initially increased following the vitrectomy, but determining that there were not any significant differences later on [61]. On the contrary, other authors did not find significant differences on the vitreous half-life on injected bevacizumab in non-vitrectomised vs vitrectomised eyes in rabbit eyes (7.06 days vs 6.99 days) [62], which suggested that VEGF is a complex molecule that is not restricted to the elimination by diffusion. However, they did find that vitrectomy affected the PK parameters in the initial distribution phase in a two-phase fitting [62]. Another study that was published by the same author on ranibizumab also showed no differences between the vitreous half-life in normal rabbit eyes (2.75 days) and following the vitrectomy (2.51 days) [27]. In this case, the parameters were established according to one-phase kinetics.

After a vitrectomy, filling the vitreous cavity with a tamponade, such as silicone oil, is a common procedure. The impact of silicone-oil filled eyes in the pharmacokinetics of injected bevacizumab was studied, observing longer $T_{\text{max}}$, smaller $C_{\text{max}}$, and relatively sustained bevacizumab levels in the ocular tissues in comparison with non-vitrectomised rabbit eyes [79].

Niwa et al. calculated the aqueous half-life of intravitreally injected ranibizumab and aflibercept in macaque eyes, even though the majority of the studies were performed in rabbit model. They found that the aqueous half-life was reduced after the vitrectomy (from 2.3 to 1.4 days for ranibizumab and from 2.2 to 1.5 days for aflibercept) [56]. However, these results must be taken with caution, due to the fact that the aqueous half-life might be not comparable to the vitreous half-life.

In summary, there is evidence of a decrease in the half-life of intravitreal injected antibodies after vitrectomy is performed, although it is not quite clear whether or not these differences are relevant enough to change the injection interval of anti-VEGF antibodies [64,76]. Moreover, this decrease is higher when the vitrectomy is performed in combination with a lensectomy. However, these results come from animal studies and their translation to humans is still controversial, which is mainly due to the anatomic and physiological differences between the species [76].

3.3. Analytical Methods Used in Pharmacokinetic Studies

The assessment of pharmacokinetic parameters for a drug administered by intravitreal route poses a challenge. It is not easy to obtain periodic samples of vitreous or aqueous fluids due to the invasive nature of the method. Moreover, when trying to assess drug systemic levels, the exposure may be low,
or the technique does not offer the sensitivity that is required to enable pharmacokinetic evaluations of antibodies. Most of the reported assays are based on ELISA (Enzyme-Linked ImmunoSorbent Assay) assays, which are considered the “gold standard” method used for the measurement of monoclonal antibodies [22,30,34]. Although there are a large variety of ELISA methods available for anti-VEGF antibodies determination, most of the work in this field relies on an indirect determination by VEGF, where factors, such as the type of VEGF, or the binding affinity, might have a big influence. Out of the three, ranibizumab is the one that requires a higher sensitivity and a more specific detection method, since the ranibizumab serum levels are often lower than the levels that can be detected by conventional methods [21,30]. The pharmacokinetic profile of Fab antibodies (such as ranibizumab) is characterised by a long elimination of the vitreous half-life and a rapid elimination from the systemic circulation [80]. Molecules containing a Fc region, such as bevacizumab or aflibercept, have greater systemic half-lives [58], because they are protected from proteolytic catabolism by binding to the neonatal Fc receptor (FcRn). However, the impact of FcRn receptor on the intravitreal pharmacokinetics is still unclear [70]. Additionally, high-sensitivity ELISA methods require for drug samples to be diluted within the detection range, which can add some inaccuracy.

Lowe et al. developed a novel electrochemiluminescence assay (ECLA) that allowed for a more sensitive determination of ranibizumab in serum. This assay was first used to support some clinical trials, offering 67 times more sensitivity than a conventional ELISA (20 ng/mL) [81] and with a reporting range of 0.3–24 ng/mL [82]. More recently, the same authors have presented another novel method that utilises a high-affinity monoclonal anti-ranibizumab-VEGF-complexes antibody (MARA) to measure ranibizumab in human serum. The assay format uses a semi-homogeneous solution that specifically binds to the ranibizumab-VEGF complex, but neither one alone. This new ELISA method has a lower limit of quantification of 15 pg/mL in human serum [83].

There are still a few studies have attempted to improve the detection method, even though most of the studies quantify the anti-VEGF drugs concentration by immunoassays. Dickmann et al. assessed the ability of fluorophotometry to measure the intravitreal pharmacokinetics of fluorescently-labelled ranibizumab in the rabbit and compared the results to those that were obtained using ELISA in previous publications, obtaining similar results [84].

Christoforidis et al. tried a different approach by labelling bevacizumab, ranibizumab, and aflibercept with a radionuclide, such as I-124, to evaluate the pharmacokinetics of the intravitreally injected anti-VEGF drugs by PET/CT [26,45,60]. The great advantage of this method in comparison to the traditional ones using ELISA is that the vitreous anti-VEGF antibodies levels can be controlled without needing to sacrifice the animals at determined time intervals or without taking invasive samples of the vitreous humour.

HPLC (High Performance Liquid Chromatography) is a fast and low-cost quantification method, however it is not commonly used in antibodies determinations in biological samples. Giannos et al. tried to correlate ELISA analytical methods to SE-HPLC (size exclusion high performance liquid chromatography) on in vitro studies, showing a close and significant correlation between them. Their SE-HLPC method uses a new marketed column designed for antibodies with a lower limit of detection (LOD) of 2.19 ng/mL and a lower limit of quantification (LLQ) of 8.79 ng/mL for bevacizumab and ranibizumab. Aflibercept LOD and LLQ were 8.79 and 17.578 ng/mL, respectively [85]. No in vivo studies were found that used HPLC as an analytical method for anti-VEGF drugs.

4. Outlooks

All of the pharmacokinetic studies centre their reports on the half-lives of the anti-VEGF drugs in different compartments (vitreous, aqueous humour, or serum) (Figure 1). Their objective is to explain the route of elimination of the drug from the eye, in the case of animal studies, or to relate the findings to possible adverse drug effects when entering the systemic circulation.
Only one study has compared the pharmacokinetics of the three anti-VEGF antibodies that were used in clinical practice [19]. Avery et al. compared the systemic exposure and the suppression of VEGF in plasma. Ranibizumab showed the least systemic exposure, whereas bevacizumab presented the highest with a 35-fold increase in AUC as compared to ranibizumab. These differences further increase after the third dose. Aflibercept appears to have the greatest suppression of free plasma VEGF out of the three, with serum concentrations that exceed its IC50 value (0.068 nM) at three hours post-injection and remain above this for seven days. In contrast, ranibizumab mean trough levels remain similar to the baseline [19].

However, no study has extensively examined the ocular pharmacokinetics of anti-VEGF antibodies in humans and their relation to the frequency of intravitreal doses. The establishment of the actual dosage regimens is mainly based on the activity of the disease that is assessed by OCT imaging or visual acuity and not on the pharmacokinetics of the drugs. An in vitro model in aqueous humour tried to associate the VEGF-A suppression times with the administration times, which suggested that individual dosing strategies are possible with a range of suppression of 26 to 69 days [86,87]. In humans, only one study determined levels of unbound aflibercept in a case series with seven patients that were treated over a six-month period with aflibercept and unbound VEGF-A in aqueous humour remained stable after every month and second month of intravitreal injections, supporting that bimonthly administrations may be enough in those patients that were treated with aflibercept [88].

Population pharmacokinetic analysis allows for the drug time-course profiles and the response dynamics over time to be characterised in a more precise manner. It also allows for the identification of the intrinsic and extrinsic factors that might be related to the observed drug exposure or response [86]. Population analysis, which is also known as non-linear mixed effects modelling, considers the structural pharmacokinetic or pharmacokinetic/pharmacodynamic models and stochastic models in order to account for inter-individual and/or inter-occasion variability and residual unexplained error [89–92]. Regulatory authorities have actually acknowledged the relevance of this discipline for drug approval and its value for an optimal dose selection in the special subgroups of the population [93,94]. However, these models are not used in the development of new agents for AMD, and only one author has applied the concepts of non-linear mixed effects modelling for the characterisation of the pharmacokinetic time-course profile of ranibizumab in this disease [22].

5. Conclusions

At present, the available pharmacokinetic data on anti-VEGF drugs after intravitreal administration are still limited, despite the fact that these molecules are the standard treatment for AMD. In recent years, many studies have been carried out in order to determine the main pharmacokinetic parameters of anti-VEGF antibodies in different animal species and humans, although the differences in the
methods of determination, in the samples analysed, in the time points taken, and the compartmental analysis, etc., make it difficult to attain standardised values for each anti-VEGF antibody. We believe that this comprehensive review will be of great use to research groups working on the pharmacokinetics of intravitreally administered VEGF inhibitors, although further studies are necessary in order to improve the knowledge in this area.

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