Fundamental Toxicological Sciences (Fundam. Toxicol. Sci.)
Vol.10, No.6, 249-258, 2023

The Japanese Society of Toxicology

Fundamental Toxicological Sciences
URL : http://www.fundtoxicolsci.org/index_e.html

Letter

Survey of toxicity study packages and designs of intravitreal drugs approved in Japan

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(Received October 2, 2023; Accepted October 10, 2023)

ABSTRACT — In contrast with standard systemic drugs, the toxicity study of ophthalmic drug has unique design and packaging characteristics. The present survey aimed to characterize the nonclinical toxicological strategy of intravitreal administration (IVT) drugs by summarizing the toxicity study packages and comparing toxicity findings with clinical side effects. Safety pharmacology studies, toxicity studies, and clinical adverse reactions of the following seven IVT drugs were surveyed: pegaptanib sodium, ranibizumab, aflibercept, brolucizumab, faricimab, triamcinolone acetonide, and ranibizumab biosimilar. The toxicity study packages for IVT drugs were constructed according to ICH guidelines, although there were several differences between the modalities, application categories, and drugs. The characteristics of toxicity study packages include the fact that many safety pharmacology and acute toxicity endpoints need not be conducted as separate studies owing to the low systemic exposure. In addition, local toxicity findings (especially intraocular inflammation) require caution due to the relatively invasive administration method necessitates, and monkeys were mainly used as animal species in the IVT studies. Notably, certain drugs were found to have the severe adverse reactions of retinal vascular lesions, probably owing to immunoreaction. Importantly, these immunoreaction-related adverse reactions were not detected in these nonclinical toxicity studies. Therefore, risk assessments using toxicity studies, immunogenicity, and host impurities are important for the prediction and control of adverse reactions. This survey provides valuable information for the construction of a toxicity study design for IVT drug development.

Key words: Toxicity tests, Intravitreal injections, Ophthalmic drugs, Study packages

INTRODUCTION

Posterior eye segment disease is a major cause of visual impairment (Pascolini and Mariotti, 2012). Age-related macular degeneration (AMD) and diabetic retinopathy are the leading causes of blindness and severe and moderate visual impairment globally in 2020, along with cataracts and glaucoma (Burton *et al*., 2021). However, the age-standardized prevalence of blindness due to AMD has exhibited a decreasing trend from 1990 to 2020. The widespread clinical introduction of anti-VEGF therapy for exudative AMD contributed to this trend (GBD, 2021). The method for delivering drugs developed for the treatment of posterior segment diseases, including AMD, to the posterior eye can be problematic. In the treatment of retinal and other posterior segment diseases, the various barrier functions of the eye (e.g. tears, cornea, conjunctiva, anterior and posterior chamber, sclera, choroid, and blood-retinal barrier) complicate conventional drug delivery, including systemic and eye drops, to the lesion (Nayak and Misra, 2018). Intravitreal administration (IVT) has been studied in-depth as a method for drug

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Table 1. Intravitreal administration drugs approved in Japan.

Generic name	Approved year	Modality	Indication[*1]	Product Category
Pegaptanib sodium[*2]	2008	RNA aptamer	· nAMD	New active ingredient
Ranibizumab	2009	Fab fragment of monoclonal antibody	· nAMD · RVO-ME [*3] · mCNV[*3] · DME[*3] · ROP[*3]	New active ingredient
Triamcinolone acetonide	2010	Small-molecule drug	[IVT] · Vitreous visualization duringvitreous surgery · DME[*3]	New administration route
Aflibercept	2012	Recombinant fusion glycoprotein	· nAMD · RVO-ME[*3] · mCNV[*3] · DME[*3] · Neovascular glaucoma[*3] · ROP[*3]	New active ingredient
Brolucizumab	2020	Recombinant single chain antibody	· nAMD· DME[*3]	New active ingredient
Ranibizumab biosimilar	2021	Fab fragment of monoclonal antibody	· nAMD · mCNV · DME[*3] · RVO-ME[*3]	Biosimilar drug
Faricimab	2022	Bispecific monoclonal antibody	· nAMD · DME	New active ingredient

*1: At September 2023. *2: Marketing was discontinued in 2020. *3: Indication added after initial approval.
Abbreviations: DME, diabetic macular edema; IVT, intravitreal; nAMD, neovascular age-related macular degeneration; mCNV, myopic choroidal neovascularization; ROP, retinopathy of prematurity; RVO-ME, macular edema following retinal vein occlusion.

administration to the posterior eye segment. From 2008, seven IVT drugs have been approved in Japan (Table 1), and are used to treat posterior eye diseases, including neovascular age-related macular degeneration (nAMD), diabetic macular edema (DME), myopic choroidal neovascularization (m-CNV), retinal vein occlusion macular edema (RVO-ME), retinopathy of prematurity (ROP), and neovascular glaucoma. Five of the seven IVT drugs are biotechnology-derived pharmaceuticals (BDPs). The other two are an RNA aptamer and a small-molecule drug.

Toxicity studies of ophthalmic drugs have unique design and package characteristics, unlike standard systemic drugs, including drugs to be administered orally or intravenously. Although most eye-injectable formulations are administered intravitreally, there are currently no ICH guidelines for nonclinical toxicity studies using this route. Despite several reviews of the design and packaging of nonclinical toxicity studies for eye drop drugs (Kurata *et al*., 2016, 2017) and safety evaluations of IVT drugs (Baumal *et al*., 2021; Falavarjani and Nguyen, 2013; Singh *et al*., 2022; Tolentino, 2011), few surveys have focused on the design and packaging of nonclinical toxicity studies for IVT drugs. The present survey aimed to characterize the nonclinical toxicological strategy of IVT drugs by summarizing the toxicity study packages and comparing toxicity findings with clinical adverse reactions.

MATERIALS AND METHODS

Safety pharmacology studies, toxicity studies, and clinical adverse reactions of the following five new IVT drugs approved between 2008 and 2022 in Japan were surveyed: pegaptanib sodium (PS) (Macugen, Bausch & Lomb Inc., Bridgewater, NJ, USA), ranibizumab (Lucentis, Novartis Pharma AG, Basel, Switzerland), aflibercept (Eylea, Bayer AG, Leverkusen, Germany), brolucizumab (Beovu, Novartis Pharma AG, Basel, Switzerland), and faricimab (Vabysmo, Chugai Pharmaceutical Co., Ltd., Tokyo, Japan). In addition, triamcinolone acetonide (TA) (MaQaid, Wakamoto Pharmaceutical Co., Ltd., Tokyo, Japan) and ranibizumab biosimilar (Senju Pharmaceutical Co., Ltd., Osaka, Japan), a drug with a new administration route and a biosimilar drug, respectively, were included in the survey (Table 1). Information on toxicity study packages, study design, results, and clinical adverse reactions was collected from the Common Technical Documents (CTDs), package inserts (Macugen: 4th edition, 2015; Lucentis: 1st edition, 2023; MaQaid: 1st edition, 2023; Eylea: 2nd edition, 2022; Beovu: 5th edition, 2022; Ranibizumab BS: 3rd edition, 2023; Vabysmo: 2nd edition, 2022), interview forms (IFs) (Lucentis: 16th edition, 2023; MaQaid: 11th edition, 2020; Eylea: 16th edition, 2022; Beovu: 5th edition, 2022; Ranibizumab BS:

Table 2. Toxicity study packages, test systems and routes of approved intravitreal administration drugs.

Study category	Pegaptanib sodium	Ranibizumab	Aflibercept	Brolucizumab	Faricimab	Triamcinolone acetonide	Ranibizumab biosimilar
Safety Pharmacology							
Cardiovascular system	Dog, IV	(Monkey, IVT)	Rat, Mouse SC; (Monkey, SC, IV)	(Monkey, IVT)	(Monkey, IV, IVT)	–	–
Respirator system	Rat, IV		Rat, IV	–		–	–
Central nervous system	Rat, IV		(Monkey, SC, IV)	–		–	–
Kidney function	(Monkey, IVT); (Dog, IVT)	–	–	–	–	–	–
Thrombus formation	–	–	Rabbit, IV	–	–	–	–
Wound healing	–	–	Rabbit, IV	–	–	–	–
General toxicity							
Acute toxicity							
Rodent	Rat, IV	–	Rat, IV	–	–	–	–
Non rodent	Rabbit, IVT; Monkey, IVT, IV	(Monkey, IVT)	Monkey, IVT	Rabbit, IVT; Monkey, IVT	(Rabbit, IVT, IV); (Monkey, IVT, IV)	Rabbit, IVT	–
Repeated-dose toxicity							
Rodent							
Subacute[*1]	–	–	–	–	–	Rat, SC	–
Subchronic[*2]	Rat, IV	–	Rat, SC	–	–	–	–
Non rodent							
Subacute[*1]	Dog, IVT	Monkey, IVT	Monkey, IVT, IV, SC	–	Rabbit, IVT, IV; Monkey, IVT, IV	Rabbit, IVT	–
Subchronic[*2]	Rabbit, IVT; Monkey, IVT	Monkey, IVT	Monkey, IVT, IV, SC	Monkey, IVT	Monkey, IVT, IV	Rabbit, IVT	–
Chronic[*3]	Rabbit, IVT; Dog, IVT	Monkey, IVT	Monkey, IVT, IV	Monkey, IVT	Monkey, IVT	–	Monkey, IVT[*5]
Genotoxicity							
in virto	Ames assay; Mouse lymphoma TK assay; Transformation assay	–	–	–	–	–	–
in vivo	Micronucleus assay	–	–	–	–	–	–
Developmental and reproductive toxicity							
FEED	Mouse, IV	–	–	–	(Monkey, IVT)	–	–
EFD	Mouse, IV; Rabbit, IVT	Monkey, IVT*4	Rabbit, IV	–	Monkey, IV	–	–
Prenatal development	–	–	Rabbit, SC	–	–	–	–
Fertility	–	–	(Monkey, IV)	–	–	–	–
Juvenile animals	–	–	Monkey, IV	–	–	–	–
ePPND	–	–	–	Monkey, IVT	–	–	–
Local irritation							
Eye	(Rabbit, IVT); (Dog, IVT); (Monkey, IVT)	Rabbit, IVT	(Monkey, IVT)	(Monkey, IVT)	(Monkey, IVT); (Rabbit, IVT)	Rabbit, ST*4	(Monkey, IVT)
Other places	(Mouse, IV)	–	Rabbit, IV, IM, SC	–	(Monkey, IV); (Rabbit, IV)	–	–

5th edition, 2023; Vabysmo: 2nd edition, 2022), and review reports obtained from the Pharmaceutical and Medical Devices Agency (PMDA) website (https://www.pmda.go.jp/PmdaSearch/iyakuSearch/). At the time of this survey, the package insert and IF of PS were not currently available on the PMDA website because the marketing of PS was discontinued. Moreover, information on ranibizumab biosimilar was collected from the package insert, IF, and review report as the CTD was not disclosed.

RESULTS

Nonclinical study packages of IVT drugs

Table 2 presents the approved safety pharmacology and toxicity study packages. Safety pharmacology core battery studies were conducted only for PS. Several separate safety pharmacology studies were conducted for aflibercept, and safety pharmacology studies were incorporated into general toxicity studies for other BDPs. For TA and ranibizumab biosimilar, safety pharmacology studies have not been conducted. Acute toxicities were

Table 2 (Continued). Toxicity study packages, test systems and routes of approved intravitreal administration drugs.

Study category	Pegaptanib sodium	Ranibizumab	Aflibercept	Brolucizumab	Faricimab	Triamcinolone acetonide	Ranibizumab biosimilar
Immunogenicity							
Lymphocyte stimulation assay	Human and mouse lymphocytes	–	–	–	–	–	–
ADA measurement	Mouse, SC (Rat, IV) (Rabbit, IVT)	(Monkey, IVT)	(Rabbit, IV, SC); (Monkey, IVT, SC, IV)	(Monkey, IVT)	(Monkey, IVT, IV); (Rabbit, IVT, IV)	–	–
Others							
Mechanism of toxicity	Rat tissues	–	–	–	–	–	–
Cross reactivity	–	Human tissues	Human tissue	–	Human tissue	–	–
Hemolysis, Hemocompatibility, and vitreous fluid suitability	–	Monkey serum/ plasma;Human serum/ plasma;Human vitreous humor	–	–	–	–	–
Hemolytic andaggregation in blood	–	–	Monkey blood/ serum/plasma; Human blood/ serum/plasma	–	–	–	–
Cytokine release	–	–	–	–	Human blood	–	–
Complement activation	–	–	–	–	Human blood	–	–
Combination with photodynamic therapy	–	Monkey, IVT	–	–	–	–	–
Combination with steroid	–	Monkey, IVT	–	–	–	–	–
New additives	–	(Rabbit, IVT); (Monkey, IVT)	(Monkey, IVT)	(Monkey, IVT)	–	–	–
Toxicity of putative metabolites	Ames assay; Chromosome aberration assay; Cell transformation assay; Rat, IV; Woodchuck, IV	–	–	–	–	–	–

–: Not submitted. (): Evaluated by results of repeated dose toxicity, local irritation, and/or developmental and reproductive toxicity studies. Underlined: GLP-compliant studies. *1: Studies with administration periods of 3 to 8 weeks. *2: Studies with administration periods of 9 to 16 weeks. *3: Studies with administration periods of 24 to 40 weeks. Administration period = Administration interval × Number of administrations. *4: Submitted when additional indications were approved. *5: Unclear whether GLP-compliant or not.
If both GLP-compliant and non-compliant studies were submitted for the same study category, only the GLP study are listed.
Abbreviations: ADA, anti-drug antibodies;; EFD, embryo-fatal development; ePPND, enhanced pre- and postnatal development; FEED, fertility and early embryonic development; IV, intravenous administration; IVT, intravitreal administration; SC, subcutaneous administration; ST, subtenon administration.

investigated in six drugs, and two of them were evaluated using the results of repeated-dose toxicity studies in non-rodents. In rodents, acute toxicity has only been investigated for two drugs. Repeated-dose toxicity was investigated via IVT for all drugs. Systemic administration general toxicity studies were conducted for four drugs, two of which were only conducted in rodents. Chronic or subchronic toxicity studies were conducted for all drugs via IVT, and monkeys were employed for all BDPs. Genotoxicity studies were not conducted for BDPs in accordance with ICH S6 (ICH, 2011) but were necessary for oligonucleotide agents. Although the systemic exposure via IVT was low, developmental and reproductive toxicity (DART) study was required to obtain approval for indications including RVO-ME, mCNV, and ROP, which young people suffer from. Separate DART studies were conducted for all new IVT drugs. Anti-drug antibodies (ADA) were measured in repeated-dose toxicity studies, DART studies, and/or immunogenicity studies of oligonucleotides and BDPs. Table 2 presents other studies.

Study design of IVT drugs

Table 3 summarizes the designs of the repeated-dose IVT studies. The animals most frequently employed for the studies were monkeys, followed by rabbits, and then dogs. For five of the seven drugs, IVT toxicity studies were conducted in a single species: monkey or rabbit. The longest administration period for pegaptanib, the BDPs excluding aflibercept, and TA was 40 weeks, 24–28 weeks, and 16 weeks, respectively. For aflibercept, the administration period was extended from 28 to 36 weeks because the toxicity findings occurred at the end of the chronic toxicity study. The recovery periods were not established for TA. Administration volumes in the toxicity studies were 50–100 μL, 30–90 μL, and 100 μL/eye in monkeys, rabbits, and dogs, respectively.

Table 3. Summary for designs of repeated-dose intravitreal administration studies.

Drug	Animal species	Dose/eye (mg)	Administration period[*1]	Number of administration	Administration interval	Recovery period	Volume (μL)	GLP compliance
Pegaptanib sodium	Rabbit	0, 0.1/2[*2], 0.3, 1	12 weeks	6	2 weeks	None	50	Yes
	Dog	0, 2	3 weeks	3	1 week	None	100	No
	Monkey	0, 0.1/1[*3], 0.25, 0.5, 1, 2	12 weeks	6	2 weeks	None	66	Yes
	Rabbit	0, 0.2, 0.67, 2	26 weeks	13	2 weeks	6 weeks	67	Yes
	Dog	0, 0.3, 1, 3	40 weeks	20	2 weeks	6 weeks	100	Yes
Ranibizumab	Monkey	0, 0.45, 1.8	6 weeks	3	2 weeks	4 weeks	50	Yes
	Monkey	0, 0.25, 0.5/0.75[*4], 0.5/2[*4]	16 weeks	8	2 weeks	4 weeks	50	Yes
	Monkey	0, 0.5, 0.5/1[*4], 0.5/1/2[*5]	28 weeks	14	2 weeks	8 weeks	50	Yes
Aflibercept	Monkey	0, 0.05, 0.25, 0.5	6 weeks	3	2 weeks	None	50	No
	Monkey	0, 0.5	14 weeks	7	2 weeks	None	50	No
	Monkey	0, 0.05, 0.25, 0.5	16 weeks	4	4 weeks	10 weeks	50	Yes
	Monkey	0, 1, 2, 4	16 or 18 weeks	3 or 4	4 or 6 weeks	4 or 10 weeks	50	Yes
	Monkey	0, 2	16 weeks	4	4 weeks	None	50	Yes
	Monkey	0, 0.5, 2, 4	36 weeks	9	4 weeks	4 months	50	Yes
Brolucizumab	Monkey	0, 0.5, 1, 2, 3, 6	10 weeks	2	5 weeks	6 weeks	50 or 100	No
	Monkey	0, 3	12 weeks	2	6 weeks	6 weeks	50	No
	Monkey	0, 0.5, 1, 3, 6	9 weeks	3	3 weeks	3 weeks	50 or 100	Yes
	Monkey	0, 1, 3, 6	24 weeks	6	4 weeks	4 weeks	50 or 100	Yes
	Monkey	0, 6	12 weeks	3	4 weeks	4 weeks	50	Yes
Faricimab	Rabbit	0, 1.5, 3, 6	4 weeks	2	2 weeks	None	50	No
	Monkey	0, 1.5, 3, 6	4 weeks	2	2 weeks	None	50	No
	Monkey	0, 1.5, 3, 6	12 weeks	3	4 weeks	4 weeks	50	Yes
	Monkey	0, 0.5, 1.5, 1.5/3[*6]	28 weeks	7	4 weeks	13 weeks	50	Yes
Triamcinolone acetonide	Rabbit	0, 2.4, 4.8, 7.2	2 weeks	2	1 weeks	None	30–90	No
	Rabbit	0, 1.8, 3.6, 7.2	8 weeks	2	4 weeks	None	90	Yes
	Rabbit	0, 1.8, 3.6, 7.2	16 weeks	4	4 weeks	None	90	Yes
Ranibizumab biosimilar	Monkey	0, 0.5, 1	26 weeks[*7]	Unknown	2 weeks	Unknown	50	Unknown

*1: administration period = administration interval × number of administration. *2: 2 mg was administered for the last 2 doses. *3: 1 mg was administered for the last 2 doses. *4: 0.5 mg was administered for the first dose. *5: 0.5 mg and 1 mg were administered for the first and second dose, respectively. *6: 1.5 mg was administered for the first dose. *7: Because the number of administrations is not disclosed, the administration period described in the review report is listed.

Table 4. Comparison of nonclinical intravitreal administration studies with clinical: dosage and administration interval.

Drug	Study category	Dose/eye			Administration interval		
		The highest dose in toxicity study (mg)	Approved dosage(mg)	Dose ratio (nonclinical/clinical)	Toxicity study	Approved dosage	Interval ratio (clinical/nonclinical)
Pegaptanib sodium	Single-dose	2	0.3	6.7	NA	6 weeks	NA
	Repeated-dose	3		10	1 – 2 weeks		3 – 6
	EFD	2		6.7	1 week		6
Ranibizumab	Repeated-dose	2	0.5	4	2 weeks	1 month or more	2
	Local irritation	5		10	NA		NA
	EFD	1		2	2 weeks		2
Aflibercept	Single-dose	2	2	1	NA	1 month or more	NA
	Repeated-dose	4		2	2 – 6 weeks		0.7 – 2
Brolucizumab	Single-dose	6	6	1	NA	4 weeks or more	NA
	Repeated-dose	6		1	3 – 6 weeks		0.7 – 1.3
	ePPND	6		1	4 weeks		1
Faricimab	Repeated-dose	6	6	1	2 – 4 weeks	4 weeks or more	1 – 2
Triamcinolone acetonide	Single-dose	7.2	4	1.8	NA	3 months or more	NA
	Repeated-dose	7.2		1.8	1 – 4 weeks		3 – 12
Ranibizumab biosimilar	Repeated-dose	1	0.5	2	2 weeks	1 month or more	2

Abbreviations: EFD, embryo and fatal development; ePPND, enhanced pre- and post-natal development; NA, not applicable.

Table 5. Findings in intravitreal administration toxicity studies.

Drug and cause		Finding
Pegaptanib		
	Test article-related change	Macrophage infiltration, vitreous body
	Manipulation-related change	Lymphocyte infiltration/redness/swelling, administration site; Fibrosis, choroid; Hemorrhage/hyperemia/inflammation/swelling, conjunctiva; Erosion, cornea; Adhesion to the administration site/fibrin deposit/fibrosis/hemorrhage, ciliary body; Edema/discharge/IOP increase/irritation, eye; Adhesion to the administration site/decreased response to mydriatic/hemorrhage/inflammation/miosis, iris; Capsule dilation/cataract/opacity, lens; Detachment/hemorrhage/scarring, retina; Bubbles/fibrosis/lymphocyte infiltration, sclera; Hemorrhage/scarring, tapetum; Cell infiltration/floater/fibrin deposit/hemorrhage/opacity, vitreous body; Penetrating fibrosis
	Endotoxin-related change	Flare, anterior chamber; Edema/hyperemia, conjunctiva; Inflammation/IOP increase, eye; Fibrin deposit/hemorrhage/inflammation/inflammatory cell infiltration, uvea; Hemorrhage/light reflex decreased/mixed mononuclear cell infiltration, iris
Ranibizumab		
	Test article-related change	Cell infiltration/flare/inflammation, anterior chamber; Neutrophil and monocyte infiltration, ciliary body and retina; Inflammatory cell infiltration, conjunctiva; Inflammation/IOP increase, eye; Inflammatory cell infiltration, eyelid; Abnormal reflexes, fundus; Inflammation, iris; White exudate/granulomatous lesion, optic disc; Inflammatory cell, plasma cell and lymphocyte infiltration/sheath/hemorrhage, retinal perivascular; ADA, serum; ADA/neutrophil and monocyte infiltration/flare/floater/inflammation, vitreous body
	Changes due to manipulation	Gray focus/inflammatory cell infiltration, administration/sample collection site; Inflammation, ciliary body; Inflammation/IOP increased or decreased, eye; Droplet, retina; Flare/floater, vitreous body
	Endotoxin-related change	Inflammation, eye
	Secondary change to inflammation	White inflammatory residue or pigment deposit, anterior chamber; Adhesion, lens to anterior capsule; Cataract, lens; Thickening, macular; Avascularity/thickening/swelling, optic disc; Vein dilation or torsion/beaded vein/thickening, retina; Exudate, retinal vessel walls/endothelial cells; Inflammation/inflammatory cell residue, vitreous body
Aflibercept		
	Test article-related changes	Cell infiltration/inflammation, anterior chamber; Lymphocyte infiltration, choroid; Cell infiltration/ERG response decrease/inflammation, eye; Erosion/ulcer, nasal respiratory epithelium; Lymphocyte infiltration, optic disc; Lymphocyte infiltration/perivascular sheath, retina; ADA/serum; Cell infiltration/lymphocyte infiltration, vitreous body
	Manipulation-related change	Cell infiltration/flare, anterior chamber; Lymphocyte infiltration, ciliary body; Edema, conjunctiva; IOP increase/ERG response decrease, eye; Floater, vitreous body
Brolucizumab		
	Test article-related change	Flare/fibrin deposit, anterior chamber; ERG abnormality/inflammation, eye; Degeneration, retina; ADA, serum; ADA/inflammation cell infiltration, vitreous body
	Manipulation-related change	Cell infiltration/flare, anterior chamber; Injury, choroid/ciliary body; Neovascularization, cornea; Inflammation, eye; Injury/lymphocyte infiltration, sclera; Cell infiltration/opacity, vitreous body
	Endotoxin-related change	Inflammation, anterior chamber; Opacity/pigmented cell infiltration, vitreous body; ERG abnormality, eye
Faricimab		
	Test article-related change	Fibrin and keratin deposit, anterior chamber; Hyperemia, conjunctiva; Edema/inflammatory cell infiltration, cornea; ERG abnormality/opacity/proptosis/inflammation/IOP increase/opacity/decreased response to mydriatic, eye; Necrotizing tissue fragment, lens; Inflammation, limbus; Mixed/plasma cell infiltration, optic disc; Degeneration/inflammatory cell, mixed cell and plasma cell infiltration/necrosis/perivascular sheath/scarring, retina; ADA/fibrinogen/AST/ALT increase, serum; Inflammation/inflammatory cell infiltration, uvea; Mixed/plasma cell infiltration/cell infiltration/floater/opacity, vitreous body; Reticulocyte count increase
	Manipulation-related change	Weight decrease, adrenal gland; Injury, eye; atrophy, thymus
	Secondary change to inflammation	IOP decreased, eye; Hemorrhage/degeneration/pigmented macrophage infiltration/retinal ganglion cell layer and blood vessel thickening, retina
Triamcinolone acetonide		
	Test article-related change	Increase: urine Na, Cl; plasma Na, K, Ca, total protein, α_1 globulin ratio, β globulin ratio, γ globulin ratio, cholesterol, glucoseDecrease: urine K; white blood cell, lymphocyte, eosinophil, and neutrophil count; plasma inorganic P, creatinine, albumin ratio, A/G ratio, α_1 globulin ratio, APTTWeight increase, kidney; Weight decrease, body/adrenal gland/spleen/thymus; Macrophage increase, cecal lymph node; Secretory granule increase, lacrimal gland; Vacuolization, lacrimal acinar cell;Lymphocyte decrease, mesenteric nude/submandibular nude/thymus/white pulp/; Atrophy, red pulp; Hypertrophy/thinning/vacuolar degeneration, zona fasciculata
	Manipulation-related change	Floater/lens tissue, anterior chamber; Redness, conjunctiva; Proptosis/semi-closure, eye; Thinning/partial defect/opacity, lens; Degeneration/hemorrhage/hemosiderin deposit, retina; Hemorrhage/lens tissue/opacity, vitreous cavity
	Ranibizumab biosimilar	No toxicity findings

Abbreviations: ADA, anti-drug antibody; AG, albumin/globulin; APTT, activated partial thromboplastin time; ERG, electroretinogram; IOP, intraocular pressure.

Table 6. Serious adverse reactions listed in the package inserts for approved intravitreal administration drugs.

Drug	Serious adverse reaction		Frequency (%)
Pegaptanib	Eye disorder	Intraocular inflammation	1
		Intraocular pressure increased	19.8
		Traumatic cataract	0.3
		Vitreous hemorrhage	1.3
		Retinal detachment	0.4
		Retinal tear	0.3
	Shock/anaphylaxis-like symptoms		Unknown
Ranibizumab	Eye disorder	Retinal hemorrhage, Vitreous detachment, Detachment of retinal pigment epithelium, Tear of retinal pigment epithelial, Vitreous hemorrhage, Rhegmatogenous retinal detachment, Retinal detachment, Retinal tear, Iatrogenic traumatic cataract, Blindness, Intraocular inflammation	1.5
	Stroke	Cerebral infarction, Intracranial hemorrhage	0.1
Aflibercept	Eye disorder	Intraocular inflammation	0.2
		Intraocular pressure increased	4.3
		Vitreous detachment	1.2
		Traumatic cataract	0.7
		Retinal hemorrhage	0.7
		Tear of retinal pigment epithelial	0.4
		Vitreous hemorrhage	0.4
		Retinal detachment	0.06
		Retinal tear	0.09
		Tear of retinal pigment epithelial	0.03
	Stroke		0.2
Brolucizumab	Eye disorder	Intraocular inflammation	0.5
		Intraocular inflammation (Uveitis etc.)	2.8
		Tear of retinal pigment epithelial	0.7
		Retinal detachment	0.2
		Retinal tear	0.6
		Retinal artery occlusion	0.4
		Retinal vasculitis	0.1
		Retinal vascular occlusion	0.4
	Arterial thromboembolism	Stroke	0.1
		Myocardial ischemia	Unknown
Faricimab	Eye disorder	Uveitis, Vitritis	1
		Tear of retinal pigment epithelial	0.4
		Intraocular inflammation	Unknown
		Rhegmatogenous retinal detachment, Retinal tear	Unknown
	Apoplexy	Ischemic stroke	0.05
		Thrombotic stroke	0.05
		Lacunar stroke	0.05
Triamcinolone acetonide	Eye disorder	Cataract	17.8
		Intraocular pressure increased	20
		Intraocular inflammation	Unknown
		Glaucoma	Unknown
Ranibizumab biosimilar	Eye disorder	Retinal hemorrhage, Vitreous detachment, Detachment of retinal pigment epithelium, Tear of retinal pigment epithelial, Vitreous hemorrhage, Rhegmatogenous retinal detachment, Retinal detachment, Retinal tear, Iatrogenic traumatic cataract, Blindness, Intraocular inflammation	1.5
	Stroke		0.1

Table 4 shows a comparison between nonclinical studies and the approved dosage. The highest doses per eye in repeated-dose IVT studies were 4–10 times higher than the clinical doses for the two drugs approved earlier, and 1–2 times higher than the clinical doses for the five drugs approved latter. The highest dose per eye in the acute toxicity evaluation was the same or lower than that in the repeated-dose toxicity studies. The highest doses per eye in IVT-DART evaluation was 6.7 times higher than the clinical dose for PS, and 1–2 times higher than the clinical doses for other drugs. The dosing intervals in repeated-toxicity studies were 0.7–2 times shorter than the clinical doses in BDPs and 3–12 times shorter than the clinical dose in PS and TA.

Nonclinical toxicity findings and the correlation with clinical adverse reactions

Table 5 shows the ocular and systemic findings of tox-

Table 7. Frequently occurred but not serious adverse reactions listed in the package inserts for each drug.

Drug	Frequency (%)	Adverse reaction	
Pegaptanib	1.0 ≤	Anterior eye	Punctate keratitis, Conjunctival hemorrhage, Anterior chamber inflammation, Cataract, Corneal edema, Conjunctival hyperemia, Lacrimation increased, Corneal erosion, Chemosis, Corneal epithelium disorder, Superficial keratitis, Ocular hyperemia, Conjunctivitis
		Posterior eye	Vitreous floater, Vitreous opacity, Vitreous disorder, Vitreous detachment, Retinal hemorrhage, Eye floater, Macular degeneration
		Others of eye	Eye discharge, Eye pain, Visual disturbance, Eye irritation, Foreign body sensation in eyes, Photophobia, Visual acuity reduced, Ocular pruritus, Eye discomfort, Blurred vision, Photopsia, Eyelid edema, Dry eye
		Cardiovascular system	Hypertension
		Neuropsychiatric disorder	Headache
Ranibizumab	5.0 ≤	Conjunctiva	Conjunctival hemorrhage
		Others of eye	Intraocular pressure increased, Eye pain
	1.0 – 5.0	Eye inflammation	Iritis, Vitritis, Iridocyclitis, Uveitis, Hypopyon, Anterior chamber inflammation
		Visual disturbance	Blurred vision, Visual disturbance
		Conjunctiva	Conjunctival hyperemia
		Vitreous body	Vitreous floaters
		Cornea	Punctate keratitis
		Others of eye	Eye irritation, Foreign body sensation in eyes, Lacrimation increased, Ocular pruritus, Ocular discomfort, Ocular hyperemia
Aflibercept	5.0 ≤	Anterior eye	Conjunctival hyperemia
		Others of eye	Eye pain
	1.0 – 5.0	Anterior eye	Ocular hyperemia, Punctate keratitis
		Posterior eye	Vitreous floaters
		Injection site	Pain, Hemorrhage
		Others of eye	Foreign body sensation in eyes, Eye irritation, Lacrimation increased, Blurred vision, Ocular discomfort
		Skin	Pruritus, Erythema
		Cardiovascular system	Hypertension, Systolic hypertension
		Neuropsychiatric disorder	Speech disorder, Headache
		Digestive system	Nausea
		Urinary system	Proteinuria
		Others	Urinary protein/creatinine ratio increased
Brolucizumab	5.0 ≤	Eye	Conjunctival hemorrhage
	1.0 – 5.0	Eye	Eye pain, Vitreous floaters, Intraocular pressure increased, Vitreous detachment
Faricimab	≤ 1.0	Eye	Intraocular pressure increased, Vitreous floaters, Ocular hypertension, Corneal abrasion, Eye pain, Ocular discomfort
Triamcinolone acetonide	5.0 ≤	Posterior eye	Intravitreal drug diffusion
		Others of eye	Eye floaters, Visual acuity reduced
		Metabolic abnormality	Blood glucose increased
	≤ 5.0	Others of eye	Blurred vision, Foreign body sensation in eyes
		Metabolic abnormality	Exacerbation of diabetes, Urinary glucose-positive, Blood triglyceride increased
		Blood	Basophil count increased, Eosinophil count increased, Platelet count decreased
		Body fluid/Electrolyte	Blood K increased
		Others	Blood lactate dehydrogenase increased
Ranibizumab biosimilar	5.0 ≤	Eye inflammation	Iritis, Vitritis, Iridocyclitis, Uveitis, Hypopyon, Anterior chamber inflammation
		Conjunctiva	Conjunctival hemorrhage
		Others of eye	Intraocular pressure increased
	1.0 – 5.0	Visual disturbance	Blurred vision, Visual disturbance
		Conjunctiva	Conjunctival hyperemia
		Vitreous body	Vitreous floaters
		Cornea	Punctate keratitis
		Others of eye	Eye pain, Ocular pruritus, Ocular hyperemia

icity studies via IVT. The findings were classified according to association with the test article, manipulation, endotoxin in the formulation, and secondary changes to inflammation. Notably, ocular inflammation occurred regardless of these causes, and systemic findings, including the appearance of ADA in the plasma and changes in blood biochemical values, were observed even with IVT.

Tables 6 and 7 present the serious and frequently occurring adverse reactions listed in the package inserts, respectively. Eye-related side effects were common, and

systemic side effects, including anaphylaxis, thrombus formation, and cardiovascular, neuropsychiatric, and metabolic abnormalities, occurred infrequently. Its safety in humans has recently been reviewed severally, particularly with regard to intraocular inflammation (Anderson *et al.*, 2021; Baumal *et al*., 2021; Singh *et al*., 2022).

DISCUSSION

The ICH S7A guideline states that safety pharmacology studies for locally applied agents and BDPs with highly specific receptor targeting may not be necessary or may be sufficient to evaluate safety pharmacology endpoints as part of toxicology and/or pharmacodynamic studies (ICH, 2000). This also applies to IVT drugs, which are locally applied agents including BDPs. In fact, separate safety pharmacology studies were not conducted for most IVT-BDPs. According to the CTD, safety pharmacology studies were not conducted for TA as blood concentrations in humans after IVT administration were 8−8339, 28–137, 5–39, 2–20, and 13−55 times lower than those of the approved doses of 40 mg intramuscular, 10–40 mg intra-articular, 0.4–1.6 mg inhaled, 0.1–0.44 mg intranasal, and 0.1% dermal drugs, respectively. This indicates that separate safety pharmacology studies are not necessary and can be incorporated into general toxicity studies of IVT drugs. The conditions include high specificity for the receptor, no distribution to other organs/tissues, sufficiently low systemic exposure, and the therapeutic class not being new.

Systemic acute toxicity studies were not conducted for four of the seven drugs. Only aflibercept has been used in separate GLP-compliant acute toxicity studies in both rodents and non-rodents. PS and TA have conducted separate GLP-compliant acute toxicity studies in rodents and non-rodents, respectively. For other drugs, acute toxicity was investigated in non-rodents only, incorporated into repeated-dose toxicity studies, and/or conducted under non-GLP compliance. Therefore, acute toxicity evaluation is required for any new IVT drug application. Acute toxicity evaluation was not conducted for ranibizumab biosimilar because the guidelines for ensuring the quality, safety, and efficacy of biosimilars (Ministry of Health, Labour and Welfare, 2020) indicate that nonclinical toxicity of biosimilar products can usually be evaluated in repeated-dose toxicity studies using one appropriate animal species.

For all BDPs, monkeys were used for chronic toxicity studies. Cynomolgus monkey (*Macaca fascicularis*) is a relevant animal species for anti-VEGF drugs because the DNA sequences of human and cynomolgus monkey VEGF show 99% homology, and amino acid sequences are expected to be nearly identical (Shima *et al*., 1996). In addition, the monkey eye is anatomically similar to that of humans in vitreous to lens volume ratio (Azhdam *et al.*, 2020; Atsumi *et al*., 2013; Hermans *et al*., 2009) and presence of macula and central fossa (Picaud *et al.*, 2019), and the monkey eye is large enough to allow IVT in terms of dose and frequency of administration. However, the use of monkeys for chronic toxicity studies of IVT-BDPs may change following the guidance issued by the Food and Drug Administration (FDA) in response to the shortage of nonhuman primate (NHP) supplies following the COVID-19 pandemic (FDA, 2022). The FDA states that if BDP is active in non-rodents, general toxicity studies should be conducted in non-rodents other than NHPs. The FDA also states that when a BDP is active in rodents and well-characterized on a case-by-case basis, conducting general toxicity studies in rodents only is appropriate. In addition, certain rodents and/or rabbits were not found to be relevant for chronic toxicity studies of the IVT-BDPs surveyed. Consequently, it is necessary to confirm the relevance of the employed animal species for toxicity studies of BDPs and to employ any other relevant non-NHP animal when relevant.

GLP-compliant repeated-toxicity via systemic administration was investigated of only aflibercept and faricimab, suggesting that investigation of chronic toxicity only using the clinical route, IVT, is acceptable. As described in ICH S4, the principle is to conduct general toxicity studies via the clinical route. Whether to conduct toxicity studies via systemic administration routes depends on each company's application strategy and agreement with the authorities. The fact that aflibercept is marketed as a systemic route anti-tumor drug (Zaltrap) may be related to the choice of the administration route. IVT dose ratios, nonclinical/clinical, appear to be lower than those in general systemic toxicity studies. When evaluating toxicity with IVT toxicity studies, the safety margin was calculated considering the differences in vitreous volumes between humans and cynomolgus monkeys (approximately twice as large in humans) and body weight ratio. Considering the species differences between IC_{50} for animals and humans would be necessary for calculation of the safety margin.

In IVT toxicity studies, ocular inflammation occurs for various reasons, including test article, immunoreaction, manipulation, and endotoxin. Because the cause of ocular inflammation was discussed in CTDs for six drugs excluding TA, the cause of the inflammation needs to be identified based on ophthalmological and histopathological findings, appearance of ADA, dose-response, and

the onset time of the inflammation. A recent review of intraocular inflammation after IVT administration classified intraocular sterile inflammation as acute or delayed onset (Anderson *et al*., 2021). Acute inflammation occurs immediately after dosing, and its severity often worsens after the first dose. In nonclinical studies, product quality attributes (PQA) such as endotoxins in the formulation or injury by administration cause acute-onset inflammation. Delayed-onset inflammation is often more severe after repeated-dosing, possibly because of an immune response related to ADA. However, the CTDs of ranibizumab, aflibercept and brolucizumab indicated that there was no correlation between the concentration of ADA in serum and toxicological findings, including ocular inflammation. This finding suggests that ADA expression alone does not cause ocular inflammation. In addition, the immunoreaction-induced intraocular inflammation observed in toxicity studies was not extrapolated to humans with CTDs. Moreover, adverse events, including retinal vasculitis and thromboembolism, could not be detected in nonclinical studies. The adverse reactions that might be attributed to immunoreaction and subjective symptoms, including pain, discomfort, and visual disturbance, were not predictable from the results of nonclinical studies. These adverse reactions should be noted in clinical trials, and in the case of high frequency, the cause should be investigated.

Summarily, we surveyed the toxicity study packages and clinical side effects of IVT drugs approved between 2008 and 2022 in Japan and characterized the construction, including dosing route, employed animal species, and evaluation items in each study. This survey provides valuable information for constructing of a toxicity study design for IVT drug development.

Conflict of interest---- K Yamada, Y Yamagiwa and Y Haranosono are employees of Senju Pharmaceutical Co., Ltd.

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