Fundamental Toxicological Sciences (Fundam. Toxicol. Sci.)
Vol.4, No.2, 95-99, 2017

Original Article

Repeated-dose ocular instillation toxicity study: a survey of its study design on the basis of common technical documents in Japan

Masaaki Kurata, Yoshinori Yamagiwa, Yu Haranosono and Hideyuki Sakaki

Pharmacokinetics & Toxicology Laboratories, Senju Pharmaceutical Co., Ltd., 1-5-4, Murotani, Nishiku, Kobe, Hyogo 651-2241, Japan

(Received April 27, 2017; Accepted May 1, 2017)

ABSTRACT — A repeated-dose ocular instillation toxicity study is a type of general toxicity study having unique design characteristics of species selection and administration methods, because the test article, an eye drop formulation, is instilled in the eyes. The present survey was conducted to reveal the current status of the design of repeated-dose ocular instillation toxicity studies. Information on study design was collected from the common technical documents of 21 eye-drop drugs approved in the last decade in Japan. The species most frequently employed was rabbits, followed by monkeys, then dogs. The most frequently used breed of rabbit was New Zealand white, followed by Dutch-belted. Both sexes were used in almost all the studies. In most cases, the maximum concentration of test articles was set as 3- to 10-fold higher than the clinical doses, and dosing frequency per day was set as 1.5 to 2 times the clinical usages. In many cases, a single eye of each animal was instilled with one or two drops or a fixed volume (e.g., 0.050 mL/eye in rabbits, 0.030 mL/eye in monkeys, and 0.030 to 0.100 mL/eye in dogs) of the test article. As optional ophthalmological examinations, measurements of intraocular pressure and corneal thickness were integrated frequently. In conclusion, this survey revealed design characteristics of repeated-dose ocular instillation toxicity studies, which were different in some respects from systemic dose toxicity studies. The results can be used as a baseline when considering the study design of such studies.

Key words: Ocular Instillation, General Toxicology, Survey

INTRODUCTION

Repeated-dose ocular instillation toxicity studies (OITSs) are one type of repeated-dose general toxicity study, conducted mainly in the development of eye drop drugs. The studies are basically conducted in accordance with the Harmonized Tripartite Guideline (M3) of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH, 2009) and the ICH S4 guideline of Japan (Ministry of Health and Welfare, 1999), but have unique characteristics of species selection and administration methods unlike those of general toxicity studies for systemic drugs.

A guideline for non-clinical local tolerance testing of medical products in the EU (CPMP/SWP, 2001) exists, in which single- and repeated-dose ocular tolerance tests are described. In the USA, the Food and Drug Administration (FDA) has issued a draft guideline regarding nonclinical safety evaluation of reformulated drug products and products intended for administration by an alternate route, including ocular routes (FDA/CDER, 2008). Several reviews and books regarding ocular toxicology studies have been also published, including its methodology (Attar *et al*., 2013; Kurata *et al.*, 2016; Short, 2008; Weir and Wilson, 2013). However, to our knowledge, there is still little information available regarding detailed design features of OITSs. Such information would be valuable for the construction of a baseline to consider in the study design of OITSs. Therefore, the present survey of common technical documents (CTDs) in Japan was carried out to reveal the current status of OITS study designs, with particular attention to species selection and administration conditions (e.g., dosing level, volume, and frequency).

Correspondence: Masaaki Kurata (E-mail: m-kurata@senju.co.jp)

MATERIALS AND METHODS

Information on study design was collected from the CTDs of 21 eye-drop drugs that were approved between 2005 and 2016 in Japan, and was obtained from the Pharmaceutical and Medical Devices Agency (PMDA) website. The present survey was focused on ocularly instilled drugs (i.e., eye drop drugs), and ocular therapeutic drugs administered by other methods (e.g., intravitreal injection and ophthalmic ointment) were not included. The indications of the surveyed drugs are summarized in Table 1. The CTDs investigated were issued by 8 companies, including a few global companies.

RESULTS

Table 2 indicates the species selection in the surveyed OITSs. The species most frequently employed was rabbits (approximately 90% of the approved drugs), followed by monkeys (approximately 60%), and then dogs (approximately 25%). Regarding the rabbits, albino and pigmented breeds were employed equally (Fig. 1). The breed most frequently used was New Zealand white rabbits, and followed by Dutch-belted rabbits (Table 3a and 3b). Both sexes of animals were used in almost all of the studies (Fig. 2).

For dosing of test articles, the maximum doses varied from equal to approximately 30-fold higher than clinical dosage, but in most cases were set as 3- to 10-fold higher than the clinical dose (Fig. 3). As shown in Fig. 4, there was no OITS in which the frequency of dosing per

Table 1. The indications of the surveyed drugs.

Indications	Drugs
Control of intraocular pressure	11
Allergy	4
Conjunctivitis or keratitis with/without bacterial infection	4
Dry eye	2
Total	21

Table 2. Animal species used in ocular instillation toxicity studies of eye drop drugs approved from 2005 to 2016 in Japan.

Species used	Drugs	Rate (%)
Rabbit only	7	33.3
Rabbit and monkey	7	33.3
Rabbit, monkey and dog	4	19.0
Monkey only	2	9.5
Rabbit and dog	1	4.8

The information in this table was derived from the previous article (Kurata *et al.*, 2016).

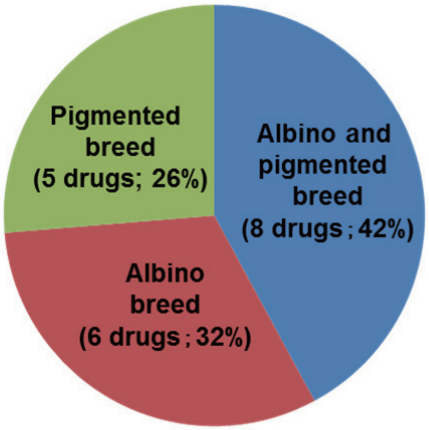

Fig. 1. Albino or pigmented breed of rabbits used. The number of drugs and their ratios are described in parentheses.

Table 3a. Breed of rabbit frequently used in ocular instillation toxicity studies of eye drop drugs approved from 2005 to 2016 in Japan.

Breed frequently employed	Drugs
NZW	11
Dutch	8
JW	3
F1 of NZW × NZR	3

The abbreviations were as follows; New Zealand white (NZW), New Zealand red (NZR) and Japanese white (JW).

Table 3b. Rabbit breeds used in ocular instillation toxicity studies of eye drop drugs approved from 2005 to 2016 in Japan.

Breeds	Drugs	Rate (%)
Dutch	4	21.1
NZW	3	15.8
NZW and Dutch	3	15.8
NZW and F1 of NZW × NZR	2	10.5
JW	1	5.3
JW and Dutch	1	5.3
NZW and JW	1	5.3
NZW and Himalayan rabbit	1	5.3
NZW and pigmented (uncertain)	1	5.3
HY/CR white	1	5.3
F1 of NZW × NZR	1	5.3
Total	19	100.0

The abbreviations were as follows; New Zealand white (NZW), New Zealand red (NZR) and Japanese white (JW).

day was less than that of the clinical dosage. The maximum ratio of frequency versus clinical usage was 4-fold. In most studies, the dosing frequency per day was set as 1.5 to 2 times the clinical usage.

Although there were OTISs in which the test article was instilled in both eyes, in most cases, test article was instilled in a single eye of each animal (Fig. 5). As shown in Table 4, the treating eye of each animal was instilled with one or two drops or a fixed volume (e.g., 0.050 mL/eye in rabbits, 0.030 mL/eye in monkeys, and 0.030 to 0.100 mL/eye in dogs) of the test article.

Table 5 indicates that measurements of intraocular pressure and corneal thickness were frequently integrated as optional examinations other than routine ophthalmological observations. The other optional measurements employed were observation of corneal endothelium, electroretinogram, blinking rate, tear volume, and flare-cells in anterior chamber.

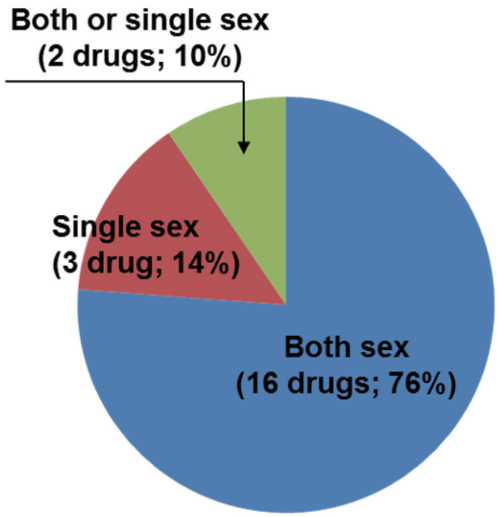

Fig. 2. Sex of animals used for studies. The number of drugs and their ratios are described in parentheses.

DISCUSSION

The present survey indicates that rabbits are the most commonly employed species in OITSs. The other species used are monkeys and dogs, which are non-rodent species. The reason these non-rodents are selected is probably because their ocular size is appropriate for eye drop instillation and ophthalmological examinations (Attar *et al*., 2013; Weir and Wilson, 2013). This is different from the species selection in general toxicity studies of drugs having systemic administration routes (oral and intravenous routes, etc.), in which one rodent and one non-rodent are selected (ICH, 2009). For rabbits, both albino and pigmented breeds are used, which is related with consideration of melanin-binding properties of test articles (Attar *et al*., 2013).

For administration of test articles, in most cases the maximum dose is set as 3- to 10-fold higher than the clinical dose. This dose level is considerably lower than that used in systemic general toxicity studies, in which the

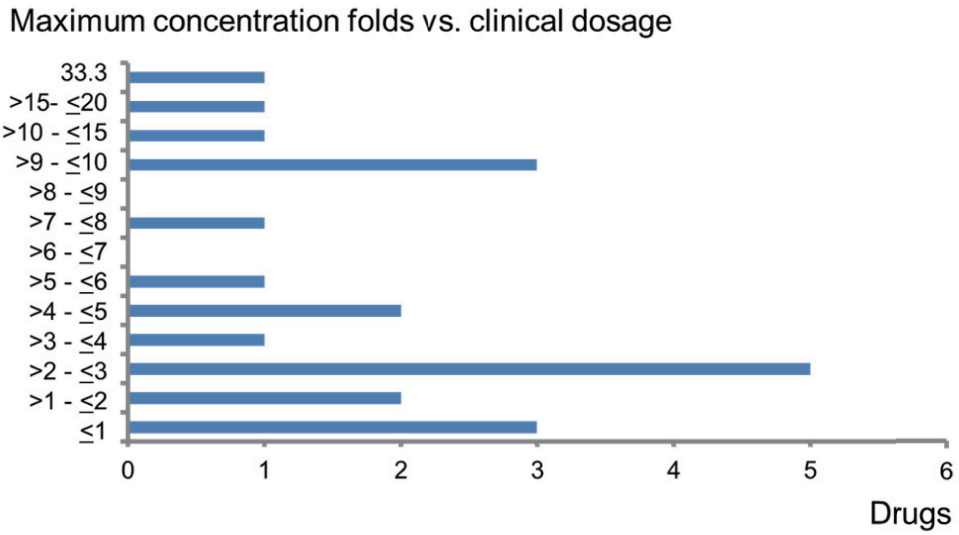

Fig. 3. Maximum concentration of test article in dosing formulation (vs. clinical dose).

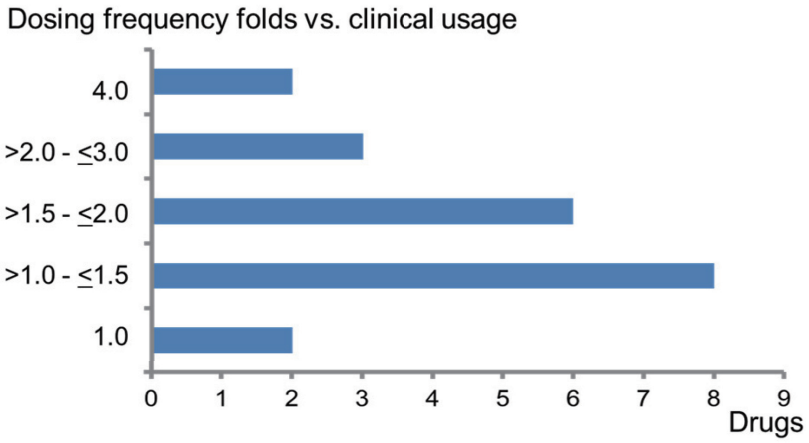

Fig. 4. Dosing frequency per day (fold vs. clinical usage).

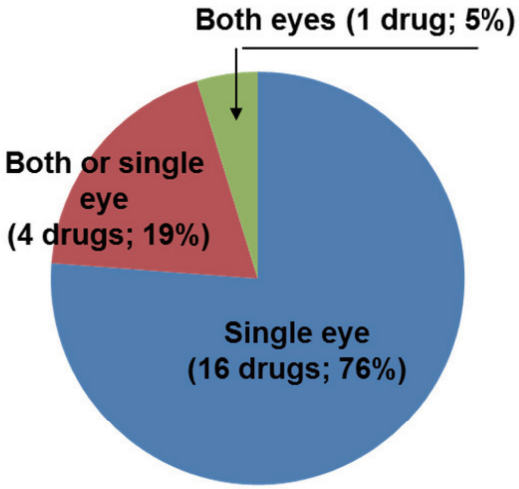

Fig. 5. Both or single eye administered with drugs. The number of drugs and their ratios are described in parentheses.

high dose level is set to a dose providing a 50-fold margin of human exposure, according to the ICH M3 guideline (ICH, 2009). The test article dose in OITSs is in many cases determined by the maximum feasible concentration, due to factors involved in the preparation of test article formulation (Attar *et al*., 2013; Kurata *et al*., 2016).

Attar *et al*. (2013) mentioned that the high dosing frequency in OITSs is intended to obtain higher exposure multiples, especially when the maximum drug concentration is difficult to increase due to physicochemical characteristics of the test article. Chrai *et al*. (1974) reported that repeated-instillation increases ocular exposure in rabbits. The present survey revealed that the instillation frequency per day in OITSs is set to be roughly twice that of clinical usage, which would effectively increase the ocular exposure.

Setting of administration volume is mainly classified into two methods: instillation (drop method) and fixed volume of formulation. Lederer and Harold (1986) reported an average drop volume of 0.039 mL. With this assumption, the volume of instillation in this survey varied from 0.02 mL to 0.08 mL (equivalent to two drops) across species. On the other hand, increases in the instillation volume are not proportional to the increases in ocular exposure (Whiston *et al*., 1993). Therefore, it is uncertain whether the variation of instillation volume affects the ocular exposure in OITSs.

If there is no particular reason otherwise, the test article should be instilled unilaterally from the viewpoint of the animal welfare. The present survey showed that in many cases instillation is performed in single eyes.

In OITSs, toxicities usually occur in the anterior segment of the eyes, because the concentrations of test articles are high at the site of topical administration (Kurata *et al*., 2016). Actually, adverse events have been reported on the ocular surface with eye drop drugs launched to the market (Fraunfelder *et al*., 2015a, 2015b). The present survey indicates that measurements of intraocular pressure and corneal thickness were frequently integrated as optional ophthalmology examinations, being in agreement with the target site of the eyes in OITSs.

Table 4. Administration volume (per eye) in ocular instillation toxicity studies of eye drop drugs approved from 2005 to 2016 in Japan.

Administration	Rabbit		Monkey		Dog	
Volume	Volume (/eye/point)	Drugs	Volume (/eye/point)	Drugs	Volume (/eye/point)	Drugs
Drop	2 drops	2	2 drops	2	1 drop	2
	1 drop	8	1 drop	4	-	-
Sub-total	-	10	-	6	-	2
Fixed volume	0.080 mL	1	0.080 mL	1	0.100 mL	1
	0.050 mL	6	0.035 mL	2	0.050 mL	1
	0.035 mL	1	0.030 mL	3	0.030 mL	1
	0.030 mL	1	0.020 mL	1	-	-
Sub-total	-	9	-	7	-	3
Total	-	19	-	13	-	5

Table 5. Optional ophthalmologic examinations added to the study in ocular instillation toxicity studies of eye drop drugs approved from 2005 to 2016 in Japan.

Ophthalmologic examinations	Drugs (among 21 drugs)
Intraocular pressure	13
Corneal thickness	10
Observation for corneal endothelium	5
Electroretinogram	4
Blinking rate	2
Tear volume	1
Flare-cells in anterior chamber	1

Conclusion

The present survey revealed characteristics of the study designs of repeated-dose ocular instillation toxicity studies. In some aspects, the study designs are different from those of systemic dose toxicity studies. The present survey results can be used as a baseline to consider in the design of repeated-dose ocular instillation toxicity studies.

ACKNOWLEDGMENT

We are grateful to Shingo Nemoto of Senju Pharmaceutical Co., Ltd., for his advice and support in the preparation of this manuscript.

Conflict of interest---- The authors declare that there is no conflict of interest.

REFERENCES

Attar, M., Brassard, J.A., Kim, A.S., Matsumoto, S., Ramos, M. and Vangyi, C. (2013): Safety evaluation of ocular drugs. In A Comprehensive Guide to Toxicology in Preclinical Drug Development (Faqi, A.S. ed.), pp.567-617, Elsevier, Amsterdam.

Chrai, S.S., Makoid, M.C., Eriksen, S.P. and Robinson, J.R. (1974): Drop size and initial dosing frequency problems of topically applied ophthalmic drugs. J. Pharm. Sci., **63**, 333-338.

CPMP/SWP (2001): Note for guidance on non-clinical local tolerance testing of medical products. Committee for proprietary medical products (CPMP) / Safety Working Party (SWP).

FDA/CDER (2008): Guidance for industry and review staff. Nonclinical safety Evaluation of reformulated drug products and products intended for administration by an alternate route (Draft guidance). Food and Drug Administration (FDA), Center for Drug Evaluation and Research (CDER).

Fraunfelder, F.T. (2015a): Ocular drug delivery and toxicology. In Drug-Induced Clinical Ocular Side Effects (Fraunfelder, F.T., Fraunfelder, F.W. and Chambers, W.A. eds.), pp.9-14, Saunders Elsevier.

Fraunfelder, F.T., Fraunfelder, F.W. and Chambers, W.A. (2015b): Drug used in ophthalmology. In Drug-Induced Clinical Ocular Side Effects (Fraunfelder, F.T., Fraunfelder, F.W. and Chambers, W.A. eds.), pp.290-343, Saunders Elsevier.

ICH (2009): ICH Harmonized Tripartite guideline M3, Guidance on nonclinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals M3.

Kurata, M., Atsumi, I., Yamagiwa, Y. and Sakaki, H. (2016): Ocular instillation toxicity study: current status and points to consider on study design and evaluation. Fundam. Toxicol. Sci., **3**, 217-232.

Lederer, C.M. and Harold, R.E. (1986): Drop size of commercial glaucoma medications. Am. J. Ophthalmol., **101**, 691-694.

Ministry of Health and Welfare (1999): Partial revision of guidelines for repeated-dose toxicity studies. Pharmaceutical and Medical Safety Bureau. Notification No. 655.

Short, B.G. (2008): Safety evaluation of ocular drug delivery formulations: Techniques and practical considerations. Toxicol. Pathol., **36**, 49-62.

Weir, A.B. and Wilson, S.D. (2013): Nonclinical regulatory aspects for ophthalmic drugs. In Assessing Ocular Toxicology in Laboratory Animals (Weir, A.B. and Collins, M. eds.), pp.259-294, Humana Press, New York.

Whitson, J.T., Love, R., Brown, R.H., Lynch, M.G. and Schoenwald, R.D. (1993): The effect of reduced eyedrop size and eyelid closure on the therapeutic index of phenylephrine. Am. J. Ophthalmol., **115**, 357-359.