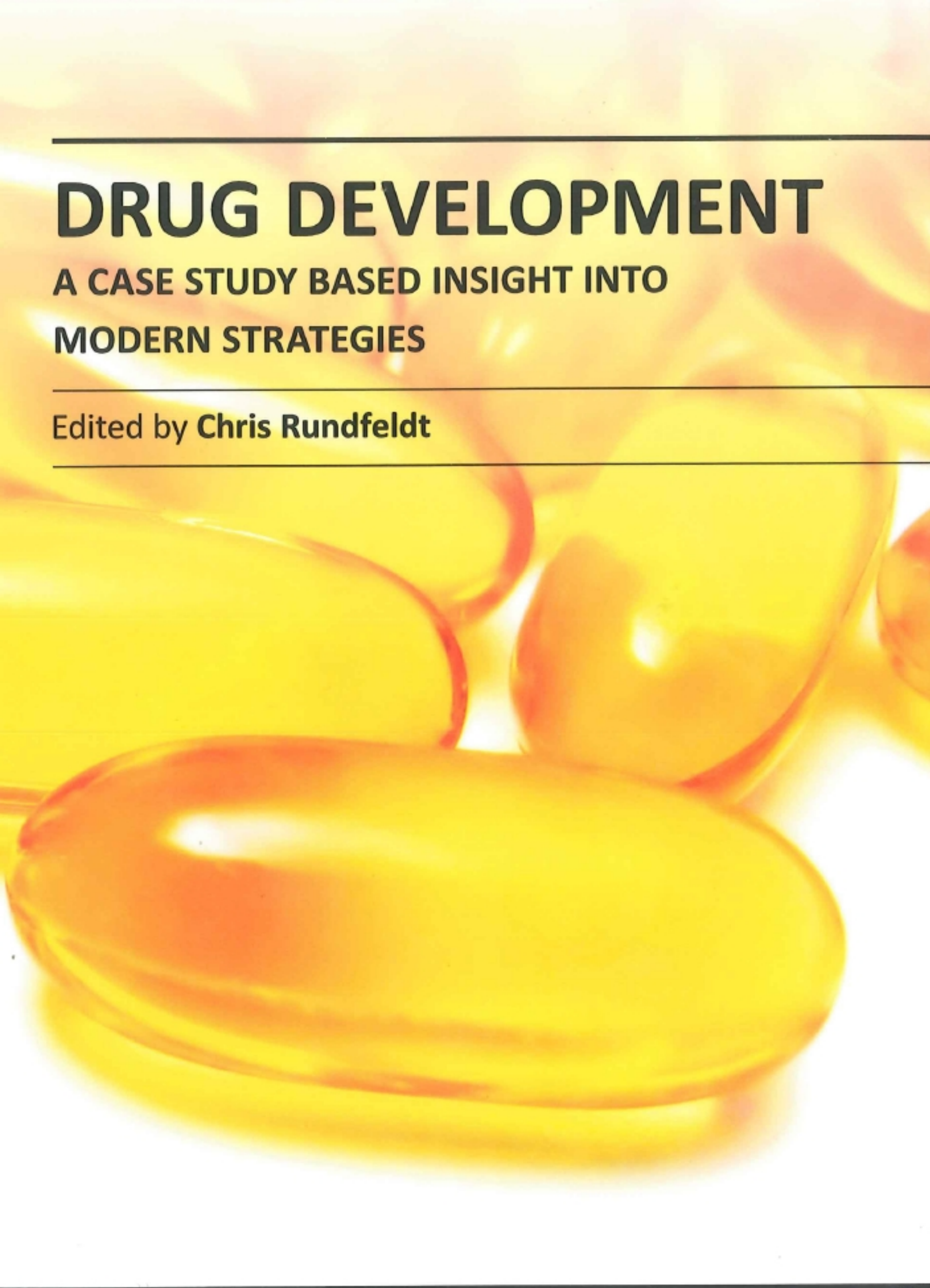
Annex: Studies on impact of preservatives used in ophthalmic dispensers

- Drug Development – A Case Study Based Insight into Modern Strategies, Edited by Chris Rundfeldt
- Prevalence and risk factors for ocular surface disease among patients treated over the long term for glaucoma or ocular hypertension - Baudouin et al 2013
- Allergy to Opthalmic Preservatives - Hong and Bilory 2009

DRUG DEVELOPMENT

A CASE STUDY BASED INSIGHT INTO MODERN STRATEGIES

Edited by **Chris Rundfeldt**



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20

Multi-Dose Container for Nasal and Ophthalmic Drugs: A Preservative Free Future?

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1. Introduction

The first nasal spray pumps were developed some 50 years ago and replaced step by step droppers and pipettes. Now they are widely used to moisturize the nasal mucosa using saline solutions (from a regulatory point of view considered medical devices under the EU directive) or as nasal preparations for the administration of topically acting drugs (e.g. nasal decongestants or nasal steroids) or for the non-invasive administration of substances which need to reach systemic circulation (anti-migraine medication, hormones). For chronic diseases like allergic rhinitis, multi-dose devices are very cost effective and convenient and provide the safety and precision regulatory bodies require. To date, most of the medications administered nasally contain a preservative to support long storage times and proper in-use stability.
For ophthalmic drugs, there was a similar development from simple droppers to more sophisticated devices. Squeeze bottles without metering function are still widely used for ophthalmic medications especially for chronic conditions. Artificial tears filled in multi-dose bottles are commonly used to relieve dry eye symptoms, these again being medical devices because the mode of action is purely physical. Looking at active drugs, the most successful in the ophthalmic field are drugs (e.g. beta-antagonists and prostaglandine-analogues) for the treatment of glaucoma and anti-histamines to treat allergic conjunctivitis.
The reader may wonder why nasal spray pumps together with multi-dose ophthalmic droppers are considered in the very same chapter. The answer is that both device classes share the same blueprint: they use a bottle made of plastic or glass attached to a pump or dropper which generates and delivers a more or less metered dose and on top of this serves as a closure. So the pumps or droppers support two general functions:

- Sealing of the container and protection of the content during storage and transportation.
- Delivery of a metered dose of liquid or viscous pharmaceutical formulation.

The major challenge during development is to reach a reliable delivery of the metered dose without having any negative impact caused by drug-device interactions.

2. Intranasal administration

Intranasal administration has a long tradition. Around three thousand years ago intranasal application of dried scabs was used to vaccinate against smallpox in India. Later intranasal administration of psychotropic drugs was and still is used for medical as well as recreational

purposes. Leaving these ancient therapeutic regimes behind, today the topical treatment of allergic rhinitis using anti-histamines (azelastine, levocabastine, olopatadine) or glucocorticoids (e.g. budesonide, fluticasone or ciclesonide) is well established. For this particular indication, drugs should act fast and just locally while systemic absorption should be as low as possible to avoid systemic side effects which are linked with typical oral formulations of comparable drug substances. Since allergic rhinitis is a chronic disease, only multi-dose bottles are used there.

	Preferred profile
Nasal decongestants	
e.g. oxymetazoline, xylometazoline, naphazoline, tetryzoline	Local activity only, low systemic adsorption to minimize side effects (headache and dizziness)
Intranasal steroids	
e.g. beclomethasone, budesonide, fluticasone, mometasone, ciclesonide	Local activity only, low systemic adsorption to minimize systemic side effects (cortisol suppression)
Antihistamines	
azelastine, levocabastine, olopatadine	Local activity only, low systemic adsorption to minimize systemic side effects (sedation)
Triptanes	
sumatriptan, zolmitriptan	Fast onset of action, high systemic adsorption to blunt migraine attacks
Analgesics	
fentanyl, ketorolac, (ketamine)	Fast onset of action but avoid high plasma concentration peaks

Table 1. Established drug classes for intranasal administration

But intranasal administration has much more potential. The nasal mucosa can be used for non-invasive systemic administration of drugs. The nasal mucosa in humans is around 150 cm^2, a surface which is well supplied by blood vessels. This ensures a rapid absorption of most drugs and can generate high blood levels. This administration route avoids the first pass metabolism which needs to be taken into account following oral administration. This bypassing of the gastric system even enables the delivery of peptide hormones. Calcitonin and desmopressin are on the market for years now, insulin and glucagon under clinical development for this administration route (Leary et al., 2008).

Intranasal administration is considered a non-invasive administration route and easy to do for self-administration or for care-givers with low potential for injuries or disease transmission (hepatitis B, HIV). This is of special importance if fast pain relief is required and patient's ability to deal with injections is impaired. Intranasal triptanes for migraine treatment, fentanyl to stop breakthrough pain and ondansetrone to relieve nausea take advantage of this administration route. For these indications, single dose devices or multi-dose devices with counting or lock-out mechanisms are available to reduce the risk of unintended overdosing or misuse.

A controversial discussion is ongoing regarding direct nose to brain drug delivery. This would open the door to a targeted treatment for various CNS diseases like Alzheimer, Parkinson, and epilepsies. Unfortunately, the available literature does not suggest consistent findings, more research is required here (Wen, 2011).

	Indication	Bioavailability [%]
Azelastine	Topical anti-histamine	40
Budesonide	Topical glucocorticoid	34
Fentanyl	Opiate analgesic	80-90
Sumatriptan	Anti-migraine	16
Calcitonin	Bone mineralization, polypeptide (32 amino acids)	~3
Desmopressin	Antidiuretic and anti-heamophilic, cyclic nonapeptide	10-20
Insulin	Antidiabetic (peptide hormone, 51 amino acids)	12-15%

Table 2. Nasal bioavailability of selected drugs (marketed or in late stage development)

For the successful development of a nasal spray it is important to understand the basic mechanisms of such a device to recognize the cliffs which must be avoided. Of course a product intended to be administered as a nasal spray should have no unpleasant smell, should not be irritating and even long term treatment should have no negative effects on the nasal mucosa (e.g. ulceration, loss of sense of smell). There should be also no safety concern, if a dose is unintentionally shot into the eyes.

For most pumps the dispensed volume per actuation is set between 50 and 140 µl, and an administered volume of 100 µl per nostril is optimum in adults. Higher volumes are prone to drip out immediately or to flow down into the throat (post nasal drip) and subsequent swallowing. So the anticipated dose should fit into a volume of roughly 100-200 µl when both nostrils are sprayed. Standard spray pumps will deposit most of the sprayed dose into the anterior region of the nasal cavity. Surface tension of the droplets and mucus layer will cause the immediate spread of the spray. Afterwards mucociliar clearance will distribute the liquid layer within the nasal cavity. Since the nasal mucus layer is continuously renewed and discarded into the throat, the nasal dwelling time of the administered drug depends on how fast it dissolves within the mucus layer and penetrates into the mucosa (Suman et al., 2002).

Although authorities require a lot of data to describe nasal spray devices, for deposition efficiency the plume angle and administration angle are critical factors, while many other spray parameters, including particle size, have relatively minor influences on deposition within the nasal cavity (Foo et al., 2007). These parameters will be discussed in more detail below.

3. Available technology and requirements

3.1 First steps to identify the right spray pump

Developing a suitable formulation with one or more active ingredients is a lengthy and challenging process. Rarely is a formulation just a simple water-based solution. Auxiliary compounds often have to be added, for example to enhance solubility and stability, to increase viscosity or to prevent microbiological contamination. Once the final formulation is available, the next step is to ensure that the ingredients do not affect the function and integrity of the container closure system (CCS). In the ICH guidelines it reads as follows: The suitability of the container closure system used for the storage, transportation (shipping) and use of the drug product should be discussed. This discussion should consider, e.g., choice of materials, protection from moisture and light, compatibility of the materials of construction with the dosage form (including sorption to container and leaching) safety of materials of construction, and performance (such as reproducibility of the

dose delivery from the device when presented as part of the drug product (ICH Guideline, 2005). The two topics we want to focus on are compatibility and performance.
To understand the potential problems an insight into the technology of a nasal spray pump system seems beneficial.
The manufacturer fills the drug formulation into multi-dose bottles made of glass or different plastic materials, which are closed by attaching the spray pump including a dip tube. The pump may be fixed by a screw closure, crimped on or simply snapped onto the bottle. Now the system should be tight and no leakage should be observed during subsequent handling. During shipment and storage, the drug formulation is only in contact with the bottle, the outer side of the dip-tube, the gasket between the pump and the bottle and the outer parts of the pump. It is important to recognize, that in this stage the formulation usually does not have contact with inner parts of the pump nor the actuator. So the bottle will provide the largest surface but also the gasket material should be considered when stability issues (e.g. drug sorption, leachables) occur.
Before someone can use the system, the pump must be primed which is normally done by the patient just before first use. A number of priming strokes are required to purge the air off the system and dip tube and to deliver the product at the targeted dose volume. Nasal spray pumps are displacement pumps. When actuating the pump, a piston moves downward in the metering chamber. A valve mechanism at the bottom of the metering chamber will prevent backflow into the dip tube. So the downward movement of the piston will create pressure within the metering chamber which forces the air (before priming) or the liquid outwards through the actuator and generates the spray. When the actuation pressure is removed, a spring will force the piston to return to its initial position. This creates a vacuum in the metering chamber which pulls the liquid from the container by lifting up the ball from the ball seat above the dip tube at the bottom of the metering chamber. It should be recognized, that the "next dose" may be under normal use conditions in contact with the materials for hours or even days when the pump is not regularly used.

Picture 1. Parts for a standard pump and how it looks like when assembled. On the right side: soaking of parts in formulation to check compatibility. For demonstration just parts for one pump are depicted.

It is recommended to evaluate effects on proper device function and compatibility of all parts which come in contact with the formulation early in the development process. This can be done in a simple way: disassembled pumps are stored for some days in the drug formulation under controlled conditions. This is done because some ingredients may cause swelling of plastic materials which may result in malfunction of the system. After appropriate storage time the parts are checked for discoloration and critical dimensions. Then the components of the pump system are cleaned and assembled and the proper function of the pump is evaluated. If the pump performs according to the agreed specification further problems are unlikely and the evaluation of performance parameters can start. These simple procedures can detect incompatibilities quite early and it is much better than to find out such issues later in the development stage or even after market launch due to complaints from consumers and patients.

3.2 General requirements from authorities

According to EU and FDA guidelines (EMEA guideline, 2006, FDA Guidance for Industry 2002) relevant information should be provided on the characteristics of each of the critical components of the CCS. Critical components are defined as:

- those that come into contact with the patient's mouth or nose or with the formulation
- those that affect the overall performance of the device and
- any additional protective packaging (FDA Guidance for Industry)

Within this chapter we cannot discuss all requirements in detail, but will provide an overview on the most important parameters (also see Table 3). For most parameters you just need to know that they are required and the pump supplier will disclose this information by providing the appropriate files or via referencing a drug master file type III for the US FDA.

Dimensions and used materials	
Dimensions	Critical dimensions for all individual components and drawings of the device and detailed description of all parts, manufacturing and assembly steps
Material used	Documentation on raw material, additives (e.g. colors, stabilizers) processing aids (e.g. lubricants) used for manufacturing including source and quality control
Performance	
Shot weight/dose weight	the quantity of drug substance/spray weight expected to be released from the device during actuation EU: mean shot weight/dose within ±15% of target dose US: mean shot weight/dose within ±10% of target dose For small doses (<20 µg) other criteria may be justified
Priming	Number of actuations needed to prime/re-prime the pump and obtain the full dose
Droplet size distribution	Description of droplet size distribution and quantification of fine particles (particles <10µm diameter)
Spray pattern/plume geometry	Parameters to describe size, shape and pattern of a delivered spray
Actuation force	Minimum actuation force to achieve desired spray characteristics

Table 3. Key requirements for nasal spray pumps

3.3 Overall general description of the device and compatibility issues

The pump manufacturer needs to provide technical drawings of the assembled device as well as for each individual part with critical dimensions which are controlled during production. Also the manufacturing of each part and the assembling process needs to be described in detail, additional operations like washing or coating steps, sterilization procedures and of course detailed quality control measures.

Since nasal sprays and drops are liquid medications, there is a high likelihood of interactions between the immediate packaging and the formulation. Therefore high quality standards for the used materials must be met. Preferably, only material is used which is described in a Pharmacopea or in foodstuff legislation (EMEA Guideline, 2005). Of course other materials may be also used but in such a case an extractable profile and complete documentation on safety and toxicology needs to be generated and submitted.

So all used materials must be identified including the monomers, any additives or colors used and the suppliers for the raw material must be disclosed. To deal with it, it is important, that the manufacturer of the devices has an established quality and change control process which documents all changes and gives notice to customers when required.

> **Definitions**
> **Extractables:** compounds which may be extracted from the container closure system by using stressful conditions.
> **Leachables:** compounds which may leach from the container closure system into the formulation under normal conditions of storage and use.

Even when material of high quality is used for the devices some interactions with the formulation can occur.

To ensure proper function of the pump, steel springs and balls are used which may be in contact with the formulation. Any metal components like balls and springs are prone to cause problems. Even if they are made of non-corrosive material, the surface can discolor the formulation due to impurities or contamination with lower grade material during the manufacturing process. Another risk coming from metal parts are remaining lubricants, which can contaminate the product.

Polyoxymethylene (POM), a widely used material for gaskets and pump parts can release formaldehyde into the drug. Materials like polyethylene or polypropylene are less likely to cause problems, but some monomers or additives can leach into the formulation. This problem of leachables coming from the pump is sometimes overlooked, because during the standard stability studies with non-primed pumps no interaction will be seen with parts inside the pumps. When the pumps are tested for dose consistency, the bottle will be emptied within a few hours. So it is recommended to evaluate compatibility of all parts which come in contact with the formulation as described above early in the development process.

3.4 Performance parameters

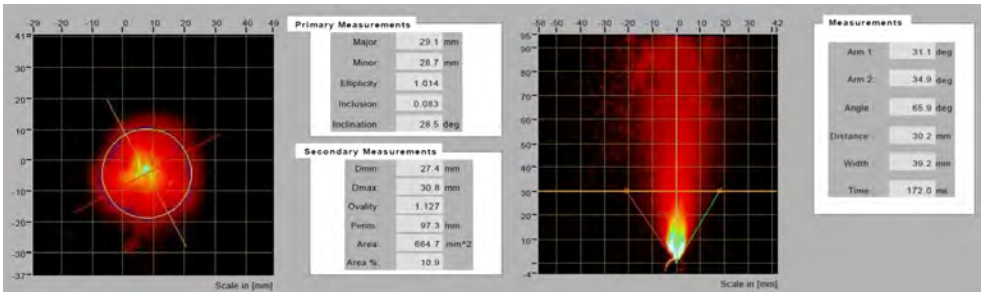
As already mentioned, plume geometry and administration angle have the most significant influence onto nasal deposition but some more parameters are required by authorities for nasal sprays and will be discussed briefly.

It should be clear that the parameters discussed below depend on the experimental conditions and the equipment used for the characterization. So it is important to use well

controlled conditions (e.g. automated actuation at defined speed or force) and procedures for the measurement of performance parameters. From experience it can be told, that the combination of a particular spray pump with a formulation will result in a quite unique performance profile which is hard to meet when considering the use of another pump. The US-FDA recognized that and recommends for generic nasal sprays to use the same brand and model of devices (particularly the metering valve or pump and the actuator) as used in the reference product to get equivalence on the basis of in vitro tests (FDA Guidance for Industry, 2003).

3.4.1 Spray pattern and plume geometry

The spray pattern is best described as a horizontal cut through the spray plume at a defined distance from the orifice, in most cases set at 30 mm. It can be assessed by simply spraying a stained formulation on a sheet of paper or thin layer chromatography (TLC) plates. For TLC plates some chemical reactions may be used to develop the pattern, which is considered more accurately. A higher sophisticated method is the use of a laser beam for the measurements, with systems e.g. from Proveris Scientific, Malvern Instruments, Oxford Lasers or InnovaSystems to name the most widely known providers. Then the shape (e.g. round or ellipsoid shape) and dimensions (e.g. shortest and longest axis) are evaluated and the spray angle may be calculated from this data.



Picture 2. Spray pattern (left) and plume geometry (right) measurements and derived parameters using a laser-based system (Equipment: Proveris Scientific SprayVIEW® and analysed by Proveris Scientific Viota software).

The plume geometry describes the shape and size of the plume (length, width, spray cone angle) at a defined time point following actuation, preferably during the fully developed phase. These parameters are closely linked to a good nasal deposition but are also important for evaluating the performance of the pump. It is recommended to test a range of pumps for a new formulation, because various factors affect the spray pattern and plume geometry, e.g. the size and shape of the nozzle, the design of the pump, and the size of the metering chamber. On the other side, these parameters are quite sensitive to changes in dimensions within the spray pump as well as changes in the formulation. For these reasons, the spray pattern is often used for quality control purposes.

3.4.2 Particle-size distribution

The nose is a very effective filter and most particles and droplets will be caught within the nasal cavity. Only particles less than 10 µm median aerodynamic diameter, so called fine

particles, can reach the lower airways during nasal breathing (Stuart 1984). Most spray pumps will generate an aerosol with a mean particle size in the range of 20-100 µm which are recognized as fine mist and will deposit well in the nasal cavity. During the formation and dissipation phases much larger droplets (>300 µm) can be formed. Droplet size distribution for nasal sprays is assessed by laser diffraction methods. Results are typically expressed as a size in microns below which 10%, 50% or 90% of the volume (D_V or mass D_M) of material exist (e.g. D_V10, D_V50, D_V90, span) and percentage of droplets less than 10 µm. Although a wide range of particles will certainly deposit in the nose, authorities require their characterization because it is a sensitive parameter to detect changes in pump quality or in the formulation.

3.4.3 Actuation force

Nasal spray pumps are normally actuated by a fast movement of thumb, index and middle finger. More recently also pumps with side actuation became available. When actuated, the pumps should deliver the whole dose as fine mist within a fraction of a second. Actuation forces should be in the range of 30-80 N (~3-8 kg). As a rule of thumb, higher viscosity will require higher actuation force. Of course there is also a great impact coming from the device side. Geometry of the metering chamber, design of the valve mechanisms, internal friction, spring forces, dimensions of the swirling chamber have a great influence on this parameter. Some of them can be adapted by the manufacturer to a certain degree to meet the requirements of a particular formulation. Beside this, it is always recommended to test several pump types with a new formulation to find the best match and to meet requirements of the target group (children, elderly).

Picture 3. Standard nasal spray pump on the left: This pump is actuated by pressing the actuator in a longitudinal movement towards the bottle. On the right side a nasal spray pump (Latitude®) designed for lateral actuation with the thumb which is preferred by some people.

3.4.4 Shot weight and delivered dose

Methods for the measurement of shot weight or delivered dose and acceptance criteria are well described in the appropriate guidelines. It should be recognized, that there are some differences between European and US guidelines which should be considered during development.

The volume of the metering chamber will define the delivered dose for a primed pump. This works normally fine for water or saline when the right actuation parameters (force, stroke acceleration) are used. Depending on surface tension and viscosity of the product, air may be trapped in the dosing chamber influencing priming and dose accuracy. Similar problems may occur when air bubbles come into the dip tube. Also too low actuation forces may lead to partial metering. To overcome the problem of partial metering, so called "user-independent" spray pumps were developed but are not widely used by the industry yet.

4. Bottles

Bottles or containers are an integral part of drug delivery devices and will also influence the general appearance of the final product. Special shapes may be used to differentiate a product from competitors. Glass bottles are less prone to cause interactions and will give good protection to the formulation even during long storage intervals. Sometimes the glass can influence the stability of the formulation (change in pH, release of trace metals). This depends of course on the quality of the glass which is described by its hydrolytic class (hydrolytic class I-III is normally used for pharmaceutical products). The disadvantages which glass bottles may have are the higher weight and the risk to break when dropped.

Bottles made of plastic material (e.g. polyethylene, polypropylene, PET) are sometimes used for nasal sprays but are mandatory for ophthalmic droppers because squeezing the bottles is needed to dispense the product. Pump supplier will most likely not manufacture these bottles themselves because a complete different technology is used. Parts for spray pumps or droppers are quite exclusively made by injection molding which gives high precision. Plastic bottle manufacturers use a process called blow-molding. The general principle is to make a hollow raw part and then blowing up the material to the final dimensions. The most important disadvantage for all bottles made of plastic material is evaporation/weight loss. Plastic materials are not a perfect barrier for gas or water evaporation. This problem can be tackled using laminated materials but these are more expensive. Another potential risk has to be considered: inks and adhesives from labels may migrate through the bottle wall and leach into the formulation.

Pure mechanics but critical for all types of bottles: the bottle opening must fit the delivery device exactly. It needs to be tested and dimensions need to be controlled because variations may cause leakages or destroy the closure during final assembly. To avoid troubles, consultation of the delivery system supplier is highly recommended as these companies are experienced in managing this interface. The pump supplier should be able to recommend a range of suited bottles from suppliers which provide reliable quality. Before switching to another bottle or bottle supplier, the compatibility with the device should be checked in advance.

5. Use of preservatives in multi-dose products

Multi-dose dispensers are widely used for the administration of liquid nasal, ophthalmic or dermal drugs because they are convenient and cost effective. To prevent degradation of the

product and to avoid the spread of potentially harmful microbes the content has to be kept sterile, both during storage and while in use by the patient or consumer. This requirement is in most cases met by a closely controlled manufacturing and filling procedure and by placing a suitable preservative or a combination of multiple preservatives in the formulations. Benzalkonium chloride (BAC) is by far the most widely used preservative, but thiomersal, chlorhexidine, chlorobutanol and phenylethanol, and parabens can also be found in topical medications (Freeman and Kahook, 2009).

Two general issues are linked to the use of these preservatives, one of which is the choice of materials. This is certainly of primary importance for the manufacturer only. Traditional glass containers do not interact with preservatives, but more and more used plastic containers and dispensing devices pose problems such as permeation of preservatives through the container or interaction with the plastic materials used in these. Rubber also reacts with preservatives but it is still used for closures, which in such events have to be pre-treated with the preservatives to minimize subsequent uptake during storage.

The issue of significance for the patient and consumer, however, is the high incidence of local side effects attributed to preservatives. The discussion is controversial, and published preclinical and clinical studies are not always consistent. It seems to be clear that short-term use of preparations containing preservatives at low concentrations is well tolerated, but preservatives can cause serious inflammatory effects with long-term use. The responses may include chemical irritation, hyperreactivity and true allergies (Hong and Bilory, 2009). The German Authorities (BfArM) addressed the use of BAC for nasal sprays in 2003 (BfArM notice, 2003) which pushed the preservative free systems for this administration route. Only recently, European authorities encourage the use of unpreserved multi-dose dispensers even for ophthalmic use (EMEA public statement, 2009).

Systems allowing multi-dose use of unpreserved formulations have the potential to solve some of the problems faced by pharmaceutical manufacturers (e.g. incompatibilities), as well as offer marked benefits for patients in need of chronic treatment. The technology starts to get widely known as PFMD devices (preservative free multi dose).

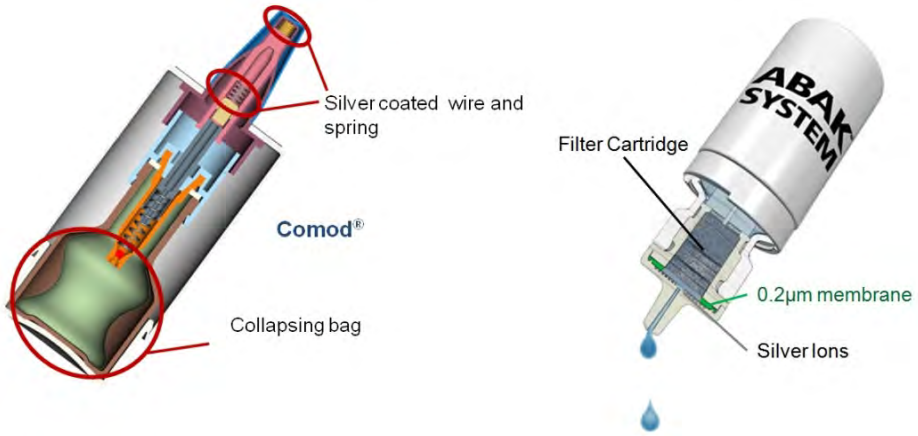
When using a preserved or unpreserved multi-dose product, there are three ways for microorganisms to enter the system:

- via the orifice and
- via the venting air which is replacing the dispensed liquid or
- insufficient container/dispenser fit.

In preserved formulations (conventional system), the added preservative controls microbial growth and no additional measures need to be taken to prevent microbial occupation via the orifice or venting air. If the formulation does not contain preservatives, the device must be able to keep microorganisms out off the system.

Today a range of technical solutions is available to overcome this issue. The highest risk of contamination obviously comes from the orifice, because it may come in contact with skin and mucosa as well as with infected body fluids. Some marketed systems use the oligodynamic activity of a silver wire in the tip of the actuator, a silver coated spring and ball (Groß, 2000). These components release silver ions into the formulation, which is a time dependent process. The system is able to keep microorganisms down between long dosing intervals, even when the tip is immersed into bacterial contaminated fluid (Bagel and Wiedemann, 2004). Silver ions are widely used for their antiseptic properties and even when used for wound dressings, it is safe and no adverse effects are attributed to this treatment. One general limitation of course must be considered: the silver ions may react with the

formulation, e.g. chloride ions and form micro-precipitations. This effect may be overlooked because it is most relevant for spans of 6-12 hours between individual actuations which are not routinely tested during development.



Picture 4. Examples for ophthalmic devices using the oligodynamic effects of silver in combination with other measures like collapsing bags or sterile filtration of the venting air.

Consequently, the most recent preservative free systems follow a purely mechanical approach to minimize interactions between parts of the device and the formulation. One technical solution to prevent contamination via the orifice is named "tip seal technology" which may be utilized for both nasal spray pumps and ophthalmic droppers. A spring loaded valve is located directly below the opening of the tip orifice and does not allow any microbes to migrate from any surfaces or contacted liquids into the system, the orifice is "sealed" under resting conditions. The tip seal keeps the system closed until a defined pressure (for nasal sprays it is more than 3 bar) is reached by actuating the system. Then the system will open and the formulation is forced through the orifice with a higher pressure than needed to open the valve. When the pressure drops at the end of the actuation the tip seal will immediately close the orifice with an outward movement. So no backflow of potentially contaminated medication or other liquid is possible. Depending on the pump system, the fluid path may even be "metal-free", which means the springs needed for the device operation do not come in contact with the formulation.

To avoid contamination of the formulation via venting air different technical solutions are used. The simplest way is sterile filtration of the venting air using separate filters or filter gaskets. For oxygen-sensitive formulations, so called collapsing bags or depressed systems are used. The formulation is filled in a special, microbial tight bag which is protected by a surrounding bottle. When dispensing the product, the bag collapses with the content not coming in contact with the ambient air. Some pumps are constructed in such a way, that the whole system is air-thigh and during use a certain vacuum (up to -300 mbar) is generated within the bottle. Those systems allow even a purging with inert gases to reduce oxygen content in the container head space.

These approaches to avoid the use of preservatives for multi-dose devices sound complicated but are well established and mature technologies. Preservative-free single dose

containers, most often presented as blow-fill-seal (BFS) containers can be an alternative for short term treatments. Disadvantages of these systems are linked to the quite complicated filling technology, the need to overfill and amount of material needed for each dose. Any efforts to provide reliable preservative free multi-dose systems are certainly appreciated by the growing number of patients who experienced discomfort with preserved formulations.

Prevent contamination via orifice	
Oligodynamic components in the actuator or inside the pump	Open orifice, but silver-ions are released into the formulation which kills germs but may interact with the formulation
"tip-seal" technology	Mechanical barrier system to prevent bacterial contamination
Prevent contamination via venting air	
Sterile filter incorporated into delivery system	The venting air in pressure balanced systems are forced though sterile filters with pore sizes less than 0.2 µm, the filter membranes are normally hydrophobic which prevents leakage from liquids out of the container via the venting system
Collapsing primary container	Best suited for oxygen-sensitive formulations, because the venting air does not come in contact with it, this technology also enables spraying at different angles
Unvented container/pump system	Tight fit of bottle and pump design prevent venting of the bottle, vacuum up to -300 mbar are reached

Table 4. Measures/technical solutions for preservative free multi-dose devices

5.1 Preservative free ophthalmic devices

With aging populations, conditions which require chronic treatment like dry eye syndrome or glaucoma are on the rise (WHO fact sheet, 2009) and preservative free multi-dose devices come into play. Chronic use of preserved formulation is not always tolerated by some patients. That is why the EMA encourages the development of such devices (EMEA public statement, 2009). This is not an easy task and poses some risks for the manufacturer. The standards for ophthalmic medications are set high to make sure that no patient or consumer 'risks an eye'. Agencies enforce guidelines on sterility of the product, absence of particles and microbial stability. Typically, the observation of microbial growth in a product leads to rejection of the entire batch, which will impact the manufacturer significantly.

Production and filling of ophthalmic medications are highly sophisticated, so pharmaceutical manufacturers are reluctant to alter established processes or to invest substantially in new filling technologies that obviously require further process qualification.

Because of these difficulties, only a very few multi-dose devices are available on the market yet, most of them relying on the oligodynamic effects of silver ions.

A patient-friendly, multi-dose system for unpreserved liquids is what patients and consumers are looking for, regardless whether one considers "simple" products like artificial tears or more complex pharmaceutical products such as prostaglandins or the like for treatment of serious diseases such as glaucoma.

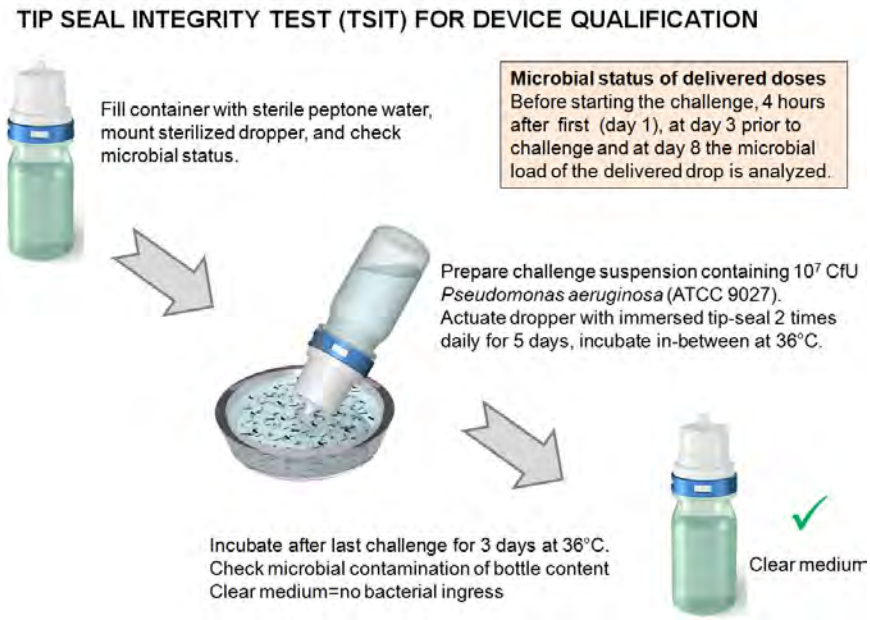
Picture 5. On the left: Rendered picture of the Ophthalmic Squeeze Dispenser (OSD) showing the tip seal and filter for the venting air in blue. Right: the complete system after filling and with removed protection cap.

A break through approach may be the Ophthalmic Squeeze Dispenser (OSD) which uses the mechanical tip-seal technology combined with sterile filtration of the venting air known from preservative free nasal spray pumps. The OSD is simply actuated by squeezing a LDPE (low density polyethylene) bottle and will deliver a drop in the range of 35 µl. The device can easily be adapted for a certain range of viscosity to keep forces within a rational limit and to deliver a consistent drop. Not only is the dropper highly sophisticated but the bottle too. The thickness of the bottle wall must be a compromise between minimizing water vapor loss and providing acceptable actuation forces. Of course the fit between bottle and dropper must be perfect to avoid contamination via this way. As some ophthalmic formulations have compatibility issues with metal, the OSD features a metal-free fluid path.

6. Microbial device integrity tests

To introduce a preservative-free multi-dose device successfully on the market, the microbial integrity must be clearly demonstrated to customers and authorities. Although there are guidelines in place for preserved multi-dose and preservative-free single dose containers, there is no guideline on microbial testing of preservative-free multi-dose devices. So the industry has to develop convincing test methods on their own. A well known procedure is the so called Wiedemann test, which was developed to characterize the 3K/Comod-system (Bagel and Wiedemann, 2004). This test for nasal sprays focuses on the microbial load of the next sprayed dose and was developed according to the needs of the system which releases silver ions into the formulation. The acceptance criteria are less than 100 CfU/ml delivered dose (according the EU Pharmacopeia) and no contamination of the bottle content. Similar tests were developed using a very agile germ (*Pseudomonas aeruginosa*) for the tip seal test and the tiny and robust spores from *Baccilus subtilis* for the whole package integrity test. These tests may be used for the characterization of preservative-free multi-dose nasal spray

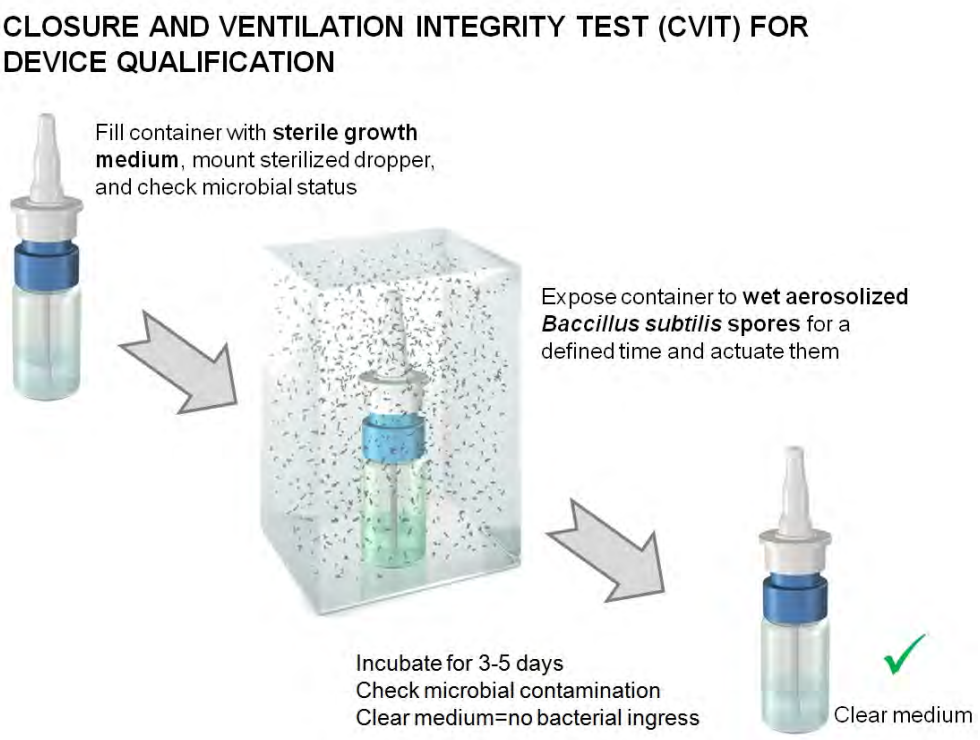
devices (Bommer et al., 2004) as well as for ophthalmic devices (Marx et al., 2010). For such device qualification tests most often the devices are filled with broth medium provide optimum conditions for the germs to growth in the case of any contamination. The final product will promote bacterial growth much less which provides more confidence.



Picture 6. Microbial test to challenge the tip seal function and contamination of delivered doses with a preservative-free ophthalmic dropper.

7. Summary

The intranasal route can be used for a wide range of applications and administration procedure is safe and easy. On the other side, development of a nasal formulation and to identify the right device is a complex process. Cooperation with an experienced pump manufacturer and specialized test laboratories on the selection of the delivery system and setting the right specifications can save resources and increase probability of success. Selecting the wrong spray pump or bottle can jeopardize the entire project so a range of pumps should be tested early in the development process. In this chapter we have focused on multi-dose devices for liquids because they clearly dominate the market. In the future we will see more of so called unit-dose devices which deliver only one dose for one or both nostrils and are discarded then. Such devices can be used for the administration of controlled substances (like fentanyl) to reduce the risk of misuse or for vaccinations (Marx et al., 2011). There are also some propellant-driven devices on the market (e.g. for nasal steroids) but discussion about the impact on the environment may limit their success. The future will also see powders for intranasal administration but all known projects are still in various stages of clinical trials. Last not least electronic add-ons, such as counters or locking features, may bring advantages and opportunities in nasal drug delivery, but will obviously need to comply with cost challenges.



Picture 7. Microbial test to challenge the venting system and closure tightness for preservative free multi-dose systems. During the aerosol-exposure the systems are actuated to generate an under pressure within the system.

Non-invasive administration of eye care products is still by far the most used way to reach therapeutic targets, let it be in diseases such as glaucoma or in self-medicated conditions such as dry-eye syndrome. Alternative methods such as implants or intravitreal injections are getting more attention lately, but given the implications associated with these methods it is fair to conclude that the multidose eye dropper still has a promising future. Avoiding or at least reducing the use of preserving agents in ophthalmic formulations is one way to maintain or extend life cycles of existing products and to ease acceptance of new entities. However, in particular in eye care habits of patients and consumers need to be taken into account carefully.

To answer the question from the beginning: will drug formulations for multiple use, presented in multi-dose containers for nasal or ophthalmic drugs, become preservative free? The answer is not as easy to give as it seems, because cultural aspects are no less important than regulatory aspects, safety concerns or clinical findings. However, as seen today in Europe, a trend begins to establish itself towards preservative-free multi-dose formulations and, consequently, delivery systems, in order to meet patient's expectations. Any development of novel drugs or research on life cycle management should take this into serious consideration.

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ORIGINAL ARTICLE *free on line*

Prevalence and risk factors for ocular surface disease among patients treated over the long term for glaucoma or ocular hypertension

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Purpose. *To determine the prevalence of ocular surface diseases and identify risk factors in a population of patients receiving antiglaucomatous eyedrops over the long term.*
Methods. *An observational cross-sectional study was designed to investigate ocular surface signs and symptoms using simple clinical tools. An ocular surface disease intensity score was calculated based on 10 questions regarding ocular surface symptoms and signs with a 4-grade scale. Patients were classified into 3 groups (A, B, and C) according to this total score. A multinomial logistic regression was performed in order to identify risk factors for surface disease.*
Results. *In an overall population of 516 patients, 49% belonged to group A, 30% to group B, and 21% to group C. The multivariate analysis showed that the following factors were correlated with the severity of ocular surface disease: patient age, number of daily eyedrops, past topical treatment changes for ocular intolerance (found in the history of 40% of the patients), intraocular pressure (found to be significantly higher in patients with more severe ocular surface disease), and glaucoma severity.*
Conclusions. *Patients treated for primary open-angle glaucoma or ocular hypertension often have ocular surface diseases, more often and more severely in older patients receiving more drugs and presenting with more severe glaucoma. These high prevalence values might therefore have consequences on the burden of the disease in terms of adherence to treatment and quality of life.*

Key words. *Benzalkonium chloride, Dry eye, Glaucoma, Ocular surface disease, Preservatives*

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INTRODUCTION

For the vast majority of primary open-angle glaucoma (POAG) patients, first-line therapy consists of medical management. Most patients therefore receive long-term medical treatment, often for several decades and some for the greater part of their lifetimes, since even surgical patients often require adjunctive medical therapy. There has recently been mounting evidence from both basic science and clinical research demonstrating that long-term eyedrop use may induce frequent and significant ocular surface changes (1). In patients treated with long-term topi-

cal glaucoma medications, numerous signs of low-grade, chronic inflammation have been described. These medications, which are often used for decades in clinical practice, may alone cause chronic inflammation and/or may exacerbate preexisting ocular surface disease, such as dry eye, Meibomian gland dysfunction, or chronic allergy. Many patients may eventually be placed on 2 or more glaucoma drops. The safety profiles of topical medications, which are determined by brief clinical trials as monotherapy in otherwise healthy eyes, may therefore not correlate satisfactorily with the safety of these formulations as used in actual clinical practice.

Although there have been few observational studies of ocular surface disease in glaucoma, those that do exist have all demonstrated that the prevalence of ocular surface disease in glaucoma is much higher than that found in randomized preapproval clinical trials or that found in the general population (2-5). Signs and symptoms of ocular surface disease are observed in 15%-50% of glaucoma patients, which is substantially more common than in normal controls (6). A number of studies have documented a correlation of these signs and symptoms with the presence of benzalkonium chloride (BAK) (2, 3) and the number of concomitantly used eyedrops containing BAK (5). In addition, these signs and symptoms significantly improved upon discontinuation of the BAK-preserved drops and substitution of nonpreserved drops (2, 7). Various histologic and impression cytology techniques have been reported to document an underlying inflammatory etiology of dry eye in glaucoma patients (8-13). The technique of flow cytometry in impression cytology specimens has repeatedly shown that even asymptomatic glaucoma patients significantly overexpress HLA-DR class II antigens, ICAM-1, interleukins IL-6, IL-8, and IL-10, and CCR4 or CCR5 in their ocular surface epithelium. Patients treated with polytherapy and/or preserved eyedrops consistently exhibit higher levels of inflammatory markers or cytokines than age-matched, normal controls receiving nonpreserved drops (14-16).

From these data, it is becoming evident that glaucoma patients exhibit a predictable, chronic ocular surface disease, the incidence and severity of which is most likely underappreciated. When faced with the medical necessity of topical treatment for a potentially blinding disease, the ocular surface becomes only a secondary concern for most clinicians. However, since ocular surface disease can dramatically impact patients' quality of life, and subsequently their glaucoma outcomes, by influencing therapeutic compliance and adherence and possibly surgical results (9, 10, 17), glaucoma treatment in general might be significantly improved by developing a better understanding of ocular surface disease in glaucoma and by performing a few simple diagnostic tests in the clinic. In order to further elucidate this disease association, we designed an observational study documenting signs and symptoms in a large POAG/ocular hypertension (OHT) population. The goals of the study were 1) to estimate the prevalence of ocular surface disease, stratified by severity, in patients treated for POAG/OHT and 2) to identify risk factors for development of ocular surface disease in glaucoma patients through statistical analysis.

MATERIALS AND METHODS

This study was designed as an observational, cross-sectional study to describe ocular surface disease in patients treated for POAG/OHT, with a 1-month inclusion period. Eligible patients were 18 years or older presenting with POAG or OHT and concurrently being treated with at least one topical pharmacologic preparation at the time of the examination.

Patients were recruited by a group of 60 ophthalmologists specializing in POAG/OHT management, who were instructed to collect data from 10 consecutive patients meeting the inclusion criteria, without any attempt to select cases, especially patients particularly motivated by ocular surface complaints.

Data were collected through anonymous questionnaires via a standardized Internet-based case reporting form. The information recorded included the following:

Sociodemographic data: age and gender.

Clinical data: POAG severity, as rated by the investigator (ocular hypertension, mild, moderate, or severe, according to the Hodapp-Parrish-Anderson classification), intraocular pressure (IOP), ocular surface symptoms (burning, itching, dryness, foreign body sensation, and tearing) and signs (conjunctival hyperemia, lid margin erythema, corneal and conjunctival fluorescein staining, lid edema), graded on a scale from 1 to 4: 1) no symptoms/signs, 2) mild, 3) moderate, 4) severe.

Current medical treatment of POAG/OHT: drug class, time since initial POAG/OHT treatment, prior medical management of POAG/OHT, and prior treatment modification due to ocular intolerance.

Tolerability of current treatment: the patient's rating of eyedrop tolerance, the ophthalmologist's rating of eyedrop tolerance prior to clinical examination, and the ophthalmologist's rating of eyedrop tolerance after clinical examination, all on a scale ranging from 0 to 10.

In an observational study, the target sample size depends upon the precision, quantified as a confidence interval (CI), desired to measure the primary outcome criterion. The primary objective of this study was to quantitatively estimate the proportion of patients in 3 groups—A, B, and C—according to their sign and symptom scores. At the onset of the study, these proportions were unknown. The CI of a proportion reaches its maximum for a 50% proportion, and we chose this worst-case scenario for the calculation of the required sample size. A study population of 600 patients should allow the frequency of dry eye to be estimated with a CI of 95%±4%, regardless of the proportion of the 3 groups defined above. Based on prior experience, it was assumed that 60 investigators should be sufficient to enroll the 600 patients required for the study.

Group assignment was based on the ocular surface symptom and sign scores, rated from 0 to 3, and the sign scores rated from 1 to 3. The totals for combined symptom and sign severity ranged between 1 and 30, and the patients were then classified into 3 groups, according to their total scores:

Group A: score = 1-4

Group B: score = 4-10

Group C: score = 10-30

Statistical analysis was performed with SAS software, version 9.1 (Cary, NC, USA). Qualitative and ordinal variables were described by frequency counts and percentages (of total responses). Quantitative variables were described by the number of responses, mean values, standard deviations, and median, maximum, and minimum values for all patients providing data. Statistical testing for qualitative variables was performed using Pearson chi^2 test. For quantitative variables, Student *t*-test or analysis of variance was performed. Where data did not fit a normal distribution, nonparametric tests were used.

Risk factors for ocular surface disease were identified in 2 steps:

1. Bivariate analyses were performed in order to identify factors significantly associated with ocular surface disease severity, defined by the proportions of patients in each of the 3 groups.
2. The factors significantly associated with ocular surface disease severity (defined as p value <0.2) were input into a multivariate multinomial nominal logic model, in which the dependent variable was the group to which the patient had been assigned.

The independent variables were age, sex, time since POAG/OHT diagnosis, number of daily eyedrops, number of daily eye medications (since several active compounds may be combined in one eyedrop), prior modification of eyedrop regimen, prior treatment modification due to ocular surface intolerance, IOP, POAG severity (as defined by the ophthalmologist), and presence of concomitant ocular disease that could also lead to ocular surface disease. These data were collected and input into the multivariate model as independent variables, in order to separate out the results for the condition in question.

This study was observational and did not alter the medical care of, nor physically or psychologically influence, the patients included, nor did it require participating patients to attend a dedicated study visit. However, information regarding the study goal was fully disclosed to all patients, and all data were collected anonymously. All subjects gave informed consent, and this observational study followed the guidelines of the Declaration of Helsinki with regard to clinical trials.

RESULTS

A total of 516 patients were included in this study. The characteristics of these patients are reported in Table I: 45.9% were female; 71.1% were over 60 years of age and 41.3% over 70 years. The time elapsed since the first POAG/OHT treatment was over 5 years for 64.5% and over 10 years for 36.8% of the 516 patients. Of the 513 patients (for 3 patients, these data were not available):

224 (43.7%) were treated with only one topical drug for their POAG/OHT, 184 (35.8%) with 2 drugs, and 105 (20.5%) with 3 drugs.

227 (44.2%) were treated with only one daily drop for their POAG/OHT, 111 (21.6%) with 2 drops, and 175 (34.2%) with 3 drops or more.

The differences were explained by the use of fixed combinations in which 2 drugs were delivered through 1 (81 patients) or 2 drops (31 patients).

The POAG/OHT topical treatment had been modified at least once in the past for 387 patients (75%), for any reason, including 208 patients (40.3%) who had at least one

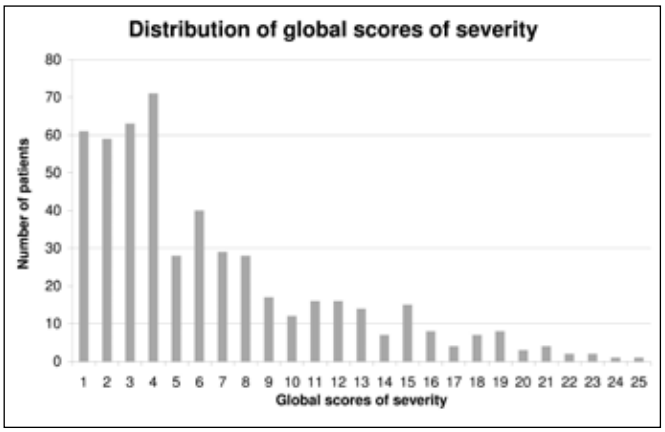

Fig. 1 - *Distribution of ocular surface disease scores.*

modification of their POAG/OHT treatment due to ocular surface–related intolerance (topical drug change, laser trabeculoplasty, surgery, or addition of a treatment for alleviating ocular surface symptoms or signs) (Tab. I). As considered by the investigator, 15.7% of the patients had ocular hypertension, 30.6% mild glaucoma, 26.8% moderate glaucoma, and 26.9% severe glaucoma.

The frequencies of the symptoms were burning (47.3%), eye dryness (44.0%), foreign body sensation (39.9%), itching (39.1%), and tearing (31.6%). The frequencies of the signs were conjunctival hyperemia (60.3%), eyelid margin redness (46.7%), positive corneal fluorescein staining (34.7%), positive conjunctival staining (28.3%), and eyelid swelling (23.6%). In addition, a severely decreased break-up time (<5 s) was reported for 20.9% of patients.

Among the 516 patients included, 254 (49%) were assigned to group A as defined by our scoring method, 154 (30%) were assigned to group B, and 108 (21%) to group C (Tab. I). The number of cases for each individual score are given in Figure 1.

Ocular surface disease prevalence increased with age (p=0.007) (Tab. I): 59% of patients aged over 69 years belonged to group B or C (28% to group C), 51% of patients aged between 60 and 69 years (18% for group C), and 39% of patients aged less than 60 years (15% for group C). We did not find any difference in the prevalence of ocular surface disease according to patient gender (Tab. I).

Ocular surface disease prevalence increased with time since the first POAG/OHT treatment (p=0.002): 57% of patients whose first treatment was prescribed more than 10 years ago belonged to group B or C (26% to group C), 55% of patients whose first treatment was prescribed 5-10 years ago (16% for group C), and 41% of patients whose first treatment was prescribed less than 5 years ago (20% for group C).

Ocular surface disease prevalence increased with the number of different topical drugs taken for POAG/OHT treatment (p<0.0001) (Tab. I): 71% of patients treated with 3 drugs or more belonged to group B or C (33% to group C alone), 54% of patients treated with 2 drugs (23% for group C), and 38% of patients treated with monotherapy (13% for group C).

Similar results were obtained when considering the number of drops instilled each day. Ocular surface disease prevalence increased with the number of drops taken for POAG/OHT treatment (Tab. I): 46% of patients treated with only 1 drop belonged to group B or C (16% to group C alone), 41% of patients treated with 2 drops (18% for group C), and 63% of patients treated with more than 2 drops (28% for group C).

There was also a relationship between the ocular surface score and the history of treatment changes. Ocular surface disease prevalence increased in patients whose antiglaucoma treatment had been modified in the past due to ocular surface intolerance (p<0.0001) (Tab. I): 71% of patients whose antiglaucoma treatment had been modified due to an ocular surface disease belonged to group B or C (37% to group C alone) and 37% of patients whose antiglaucoma treatment had not been altered due to an ocular surface disease (10% for group C). Conversely, past modification of antiglaucoma treatment was more frequently reported for patients with more severe ocular surface disease (Tab. I): for 24% of patients belonging to group A, 46% of patients belonging to group B, and 70% of patients belonging to group C (p<0.0001). Changes in therapy for ocular surface issues were found in 38% of patients on monotherapy, rising to 44% of the patients with 2 or more drugs.

Ocular surface disease prevalence increased with glaucoma severity (p<0.0001) (Tab. I): 63% who had severe glaucoma belonged to group B or C (34% to group C alone), compared to 61% of those with moderate glaucoma (25% in group C), 41% of the patients with mild glaucoma (12% in group C), and 31% of those with ocular hypertension (10% in group C). Moreover, IOP increased with the severity of ocular surface disease: right and left eye IOP values were higher for patients belonging to group C (17.0 and 17.4 mmHg) as compared to group B (15.8 and 16.5

TABLE I - IDENTIFICATION OF RISK FACTORS: BIVARIATE ANALYSIS (PERCENTAGES ARE CALCULATED IN LINE WHEN COMPARING THE 3 GROUPS A, B, AND C AND IN COLUMN FOR THE LAST TOTAL COLUMN)

	Group A, 254 (49%)	Group B, 154 (30%)	Group C, 108 (21%)	Total, 516 (100%)	p value
Gender, n (%)					0.11
Male	123 (52)	74 (31)	40 (17)	237 (45.9)	
Female	131 (47)	80 (29)	68 (24)	279 (54.1)	
Age category, y, n (%)					0.007
50-59	91 (61)	36 (24)	22 (15)	149 (28.9)	
60-69	76 (49)	51 (33)	27 (18)	154 (29.8)	
70+	87 (41)	67 (31)	59 (28)	213 (41.3)	
Time since first POAG/OHT treatment, y, n (%)					0.002
≤5	118 (59)	39 (21)	36 (20)	183 (35.5)	
5-10	64 (45)	56 (39)	23 (16)	143 (27.7)	
>10	82 (43)	59 (31)	49 (26)	190 (36.8)	
Daily eye active compound, n (%)					<0.0001
NR	1	—	2	3	
Monotherapy	138 (62)	57 (25)	29 (13)	224 (43.7)	
Bitherapy	85 (46)	57 (31)	42 (23)	184 (35.8)	
Tritherapy	30 (29)	40 (38)	35 (33)	105 (20.5)	
Daily eyedrops, n (%)					0.0008
NR	1	—	2	3	
1	122 (54)	68 (30)	37 (16)	227 (44.2)	
2	66 (59)	25 (23)	20 (18)	111 (21.6)	
≥3	65 (37)	61 (35)	49 (28)	175 (34.2)	
Modification of the eyedrop in the past (any reason), n (%)					<0.0001
Yes	160 (41)	129 (33)	98 (25)	387 (75.0)	
No	94 (73)	25 (19)	10 (8)	129 (25.0)	
Modification of the treatment in the past due to ocular surface intolerance, n (%)					<0.0001
Yes	61 (29)	71 (34)	76 (37)	208 (40.3)	
No	193 (63)	83 (27)	32 (10)	308 (59.7)	
IOP right eye, mmHg, mean (SD)	15.7 (3.6)	15.8 (3.6)	17.0 (4.2)	16.0 (3.7)	0.01
IOP left eye, mmHg, mean (SD)	15.6 (3.4)	16.5 (4.3)	17.4 (4.2)	16.2 (3.9)	0.001
Glaucoma severity, n (%)[a]					<0.0001
Ocular hypertension	56 (69)	17 (21)	8 (10)	81 (15.8)	
Mild glaucoma	93 (59)	46 (29)	19 (12)	158 (30.6)	
Moderate glaucoma	54 (39)	50 (36)	34 (25)	138 (26.7)	
Severe glaucoma	51 (37)	41 (29)	47 (34)	139 (26.9)	
Known concomitant ocular surface disease, n (%)[b]					<0.0001
Yes	9 (12)	27 (36)	40 (53)	76 (14.7)	
No	245 (56)	127 (29)	68 (16)	440 (85.3)	

NR = not reported.
[a] If both eyes of a patient had different severity stages, then the worst stage was considered.
[b] Excluding blepharitis and dryness as they could not been discriminated between a concomitant state or that induced by the treatment.

mmHg) and group A (15.7 and 15.6 mmHg) (p=0.01 for the right eye and 0.001 for the left eye) (Tab. I).

Finally, we also considered the existence of a concomitant ocular surface disease, such as Sjögren syndrome, rosacea, or atopy, in order to take this confounding factor into account when performing the multivariate analysis. A dry eye condition and blepharitis were not considered independent concomitant pathologies as they could not be discriminated from those related to treatment, thus possibly leading to an underestimation of the frequency of associated disorders underlying topical drug intolerance. Nevertheless, as could be expected, the prevalence and the se-

verity of ocular surface disease were highly correlated with a concomitant ocular surface disease (p<0.0001) (Tab. I).
According to these bivariate comparative analyses, ocular surface disease severity was positively correlated with the following factors: patient age, time since the topical treatment was initiated, number of topical drugs, number of daily drops, past changes in topical treatment, IOP, and severity of the POAG (as defined by the ophthalmologist).
Based on the multivariate analysis, the following factors were still correlated with the severity of ocular surface disease: patient age, number of topical drugs, past changes of topical treatment for ocular intolerance, concomitant ocular surface disease, IOP. and glaucoma severity (Tab. II). As our scoring method defined 3 groups (A, B, and C), a multinomial logistic regression was conducted. This led to 2 odds ratios (OR) for each class considered. For example, the OR of being aged 60-70 years as compared to being aged less than 60 was 1.78 (95% CI [1.00-3.17]) for group B and 1.76 (95% CI 0.86-3.81) for group C.

DISCUSSION

Randomized clinical trials (RCTs) are conducted prior to approval of topical glaucoma medications, and these RCTs generally demonstrate satisfactory tolerability, with only a small minority of patients having to discontinue the medication due to local intolerance or allergy. However, it is important to note several major differences between clinical trials of glaucoma medications and actual clinical practice, such as short duration and exclusion of patients most at risk of intolerance, whereas real life may expose sensitive patients to drug toxicity and often require long-life exposure to multiple therapies (18).
In fact, several observational studies (2-6) conducted in large series have reported a much higher percentage of patients with signs and symptoms of ocular surface disease than that found in prospective clinical trials and than expected from the known prevalence of such diseases (19).
The present observational study confirms the high rate of ocular surface involvement in patients treated for POAG/OHT. A large group of general ophthalmologists enrolled the first 10 patients consecutively examined, so as to limit selection bias. This could have overrepresented severe cases who probably consult more often. This is a classical bias in observational studies but should have a limited impact in a population mainly followed for glaucoma and usually consulting every 6 months. On the whole, our results are in line with similar previous studies, with 51% of the 516 patients exhibiting ocular surface disease, including 21% rated moderate to severe. No specific symptom stood out, and, as expected, dryness, burning, and foreign

TABLE II - IDENTIFICATION OF RISK FACTORS: MULTIVARIATE ANALYSIS USING A MULTINOMIAL LOGISTIC REGRESSION MODEL

Patients with an ocular surface score from 1 to 4 (group A) are the reference group	Patients with an ocular surface score from 5 to 10 (group B)		Patients with an ocular surface score from 11 to 30 (group C)		p
	OR	95% CI	OR	95% CI	
Age, y (reference: <60 years)					
60-70	1.78	1.00-3.17	1.76	0.81-3.81	0.001
>70	1.93	1.11-3.36	3.13	1.54-6.37	
Number of active drugs (reference: monotherapy)					
Bitherapy	1.09	0.65-1.84	1.28	0.65-2.51	0.003
Tritherapy	2.43	1.25-4.70	3.29	1.46-7.39	
IOP >16 mmHg (reference: ≤16 mmHg)	1.23	0.78-1.94	2.64	1.5-4.65	0.002
Glaucoma severity moderate/severe (reference: ocular hypertension)	1.44	0.88-2.34	3.20	1.68-6.08	0.002
Concomitant ocular surface disease[a] (reference: no)	5.76	2.52-13.17	13.80	5.83-32.68	<0.001
Modification of the treatment in the past due to ocular surface intolerance (reference: no)	2.85	1.78-4.57	7.40	4.12-13.30	<0.001

CI = confidence interval; IOP = intraocular pressure; OR = odds ratio.
A multinomial logistic regression model can be interpreted like a logistic model but applies to outcomes with more than 2 categories.
[a] Excluding blepharitis and eye dryness as they could not been discriminated between a concomitant state or induced by the treatment.

body sensation were the most frequently reported symptoms. The most significantly affected ocular sign was decreased tear break-up time, indicating tear film instability. Ocular or periocular hyperemia was observed frequently, while corneal and conjunctival staining was a reliable indicator of severity. Nevertheless, these criteria illustrate that a simple oral history and examination techniques can be routinely and rapidly performed in the clinical setting without detracting from glaucoma assessment, since a few questions, brief observation, and fluorescein staining can easily be managed on a routine basis.

As expected, older patients, those with longer treatment duration, and those with more severe glaucoma were more likely to present with more severe ocular surface involvement. Indeed, age, duration of treatment, and severity of disease may be related, since older patients are more likely to have had the disease longer, to have undergone more treatment, and to have more severe glaucoma. Age may also be related to a higher prevalence of dry eye, along with glaucoma. However, disease severity proved to be an independent factor in multivariate analyses, as did higher IOP at the time of examination. These patients were most likely treated with more medications, and the severity of disease could have required lower target pressures, necessitating additional eyedrops. Indeed, a clear relationship was observed between ocular surface changes and number of medications, thus supporting previous data (2, 5, 20). However, it should be emphasized that, even with monotherapy, a high proportion of patients exhibited ocular surface changes, much higher than predicted by short-term randomized trials. In addition, the relationship between disease severity, ocular surface changes, and poor IOP control, as IOP was found in this study to be higher in severely affected and multitreated patients, raises interesting issues deserving additional studies.

Moreover, 40% of the study population had reported a history of treatment modification related to ocular surface issues. This could include discontinuing a poorly tolerated medication, switching to another drug, undergoing laser trabeculoplasty or surgery due to ocular surface intolerance, or adding a symptomatic treatment to improve ocular surface signs or symptoms, such as tear substitutes or anti-allergy drugs. The high rate of symptoms likely results in poor patient satisfaction and reduced adherence to therapy, leading to more frequent visits to the ophthalmologist (17). A US survey from 2001 to 2004, analyzing the refill rate of initial therapy in 300 patients, revealed adverse effects as the second most common reason for physicians to switch the patients' medication (21). Taken together, these data show that tolerance interferes with glaucoma management and outcome.

Besides the ocular surface changes related to dry eye or allergy, causing discomfort or compliance issues, there is a large body of evidence showing that the long-term use of antiglaucoma medications also leads to chronic, subclinical conjunctival inflammation, subconjunctival fibrosis, epithelial apoptosis, goblet cell loss, and potential failure of subsequent glaucoma surgery (8-15, 22, 23). However, the imputed mechanisms, be they allergic, toxic, or inflammatory, as well as the respective roles played by the active compounds and the preservatives present in ophthalmic solutions in inducing such toxic or proinflammatory effects, continue to be topics of debate. The most frequently used preservative, BAK, has consistently demonstrated toxic effects in laboratory, experimental, and clinical studies (1). As a quaternary ammonium, this compound has been shown to cause tear film instability, goblet cell loss, conjunctival squamous metaplasia and apoptosis, disruption of the corneal epithelial barrier, and damage to deeper ocular tissues, including to corneal nerves, causing corneal hypoesthesia (24-28).

Therefore, drug-induced adverse effects extend over a spectrum much broader than simple allergic reactions, and side effects are often very difficult to identify, since they usually occur in a delayed or nonspecific manner. Indeed, mild symptoms should not be underestimated or ignored, since they may represent the "tip of the iceberg," heralding subclinical yet more severe processes that may eventually cause major problems and threaten glaucoma outcomes.

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Allergy to Ophthalmic Preservatives

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Abstract and Introduction

Abstract

Purpose of review The purpose of the present review is to examine the hypersensitivity reactions to preservatives in topical ophthalmic therapies.

Recent findings Ocular hypersensitivity reactions to different types of preservatives in different chemical classes of topical ophthalmic treatments reviewed in the literature include IgE-mast cell mediated, cell mediated and toxic. Quaternary ammoniums (benzalkonium chloride) are most commonly (8% reported cases in *OVID* and *PubMED* based searches) associated with irritant toxic reactions whereas the organomercurials (thimerosal) and the alcohols (chlorobutanol) have the highest association (19% of *OVID* and 14% of *PubMED* based searches and 20% of *OVID* and 11% of *PubMED* searches), respectively, with allergic responses although the term allergy for the 'alcohols' appears to be actually an irritant effect whereas the organomercurials appear to truly interact with the immune system as neoantigens.

Summary A large number of clinical and experimental studies reveal that preservatives in topical ophthalmic medications have been demonstrated to produce effects from inflammation/hypersensitivity to permanent cytotoxic effects involving all structures of the eye.

Introduction

The use of preservatives in topical ophthalmic treatments is ubiquitous as they allow their use in compromised eyes with a poor defense against infection. However, although providing effective biocidal properties with well tolerated short-term use at low concentrations, preservatives can cause serious inflammatory effects on the eye with long-term use in chronic conditions, such as glaucoma or potentially ocular allergies. This study reviews the reactions associated with the most commonly used ophthalmic preservatives in animal and human participants.

Preservatives and Hypersensitivity Reactions in the Eye

There are many adverse reactions associated with topical ophthalmic medications. Most of these reactions are toxic and result from chemical irritation. Only about 10% of all adverse reactions to topical ophthalmic drugs are truly allergic. Furthermore, allergies (IgE and cell mediated) are more commonly caused by the active pharmaceutical agents, such as neomycin or sulfa-based agents and rarely by preservatives or other additives.[1,2] As the incorporation of preservatives in topical ophthalmic solutions becomes more common, sensitization toward preservatives is increasing. The salts of benzalkonium have been classified as being moderately allergenic (4–11% skin test positive) whereas mercurial products are strongly allergenic (13–37% of skin tests are positive). True allergic sensitization by other preservatives (chlorhexidine and chlorobutanol) is unusual.

The different types of hypersensitivity reactions can be separated into the following categories: allergic reactions (IgE-mast cell mediated hypersensitivity), cicatrizing allergic conjunctivitis (type II and III hypersensitivities) due to antibody localizing to ocular tissue or immune complexes deposition and allergic contact conjunctivitis, a type IV hypersensitivity reaction ().[3–5] The term allergy in the ophthalmological literature is commonly used interchangeably with immunological responses of any type and does not necessarily denote an IgE-mast cell mediated process.

Table 1. Hypersensitivity reactions in the eye and the associated preservatives

Type of reaction	Description	Ocular manifestations	Preservative association
Type I hypersensitivity	Triggered by the classical activation of the IgE-Mast Cell axis and its associated early phase and late phase responses with an inflammatory reaction characterized by infiltration of PMNs, eosinophils and mononuclear cells into the corneo-conjunctival tissues and are also known as anaphylactoid reactions.	Characterized by acute itching, conjunctival hyperemia and chemosis and by edema of the eyelids either as urticaria (hives or wheals) in the superficial layers of the skin (epidermis and dermis) or angioedema (in the deeper subcutaneous tissues) or both, as well as production of significant quantities of mucus, edema and neovascularization of the cornea, and inflammation of the iris and infiltration of the anterior chamber.[1] Histopathologically, these reactions show edema of the eyelids and conjunctiva, dilation of the venules and capillaries and infiltration of lymphocytes, eosinophils and neutrophils.	Chlorhexidine: a 58-year -old male patient developed anaphylactic shock, possibly due to the use of chlorhexidine as an ophthalmic wash solution. He was successfully resuscitated without any sequelae. The patient had increased levels of both histamine and tryptase. The skin test for allergy resulted in strong positive to chlorhexidine. There have been many reports regarding severe adverse reactions associated with use of chlorhexidine.[3]
Type II–III hypersensitivity	Antibody-mediated hypersensitivity reactions are also known as localized antibody-specific disease or immune complex mediated reactions.	Cicatrizing allergic conjunctivitis (pseudopemphigoid) reaction is a response to topical medication that results in cicatrizing conjunctivitis resembling ocular cicatricial pemphigoid (OCP). It is characterized by scarring in the bulbar, forniceal, and palpebral conjunctiva that is worse inferiorly along with conjunctival keratinization and punctual occlusion. The progression of symptoms cease once the offending medication is discontinued.[1]	BAC, Kilp[4] report a case of a woman instilling artificial tear solution containing benzalkonium for treatment of dry eye syndrome, who developed a superficial keratitis which regressed after substitution with a preservative-free treatment.[5]
Type IV hypersensitivity	Drug induced ocular allergies are most often the result of type IV hypersensitivity. Type IV hypersensitivity is cell mediated and also known as delayed-hypersensitivity reactions. (contact conjunctivitis)	Many of the type IV hypersensitivity reactions occur at the eyelid level that often makes it difficult to differentiate from other causes of eyelid inflammation or contact dermatitis. These types of allergic reactions can be detected by skin tests.	Thimerosal: the manifestations of the ocular delayed hypersensitivity reactions include conjunctival hyperemia, corneal infiltrates, and intolerance to lens wear with the use of soft contact lens solutions or other topical ophthalmic medications containing thimerosal. Delayed

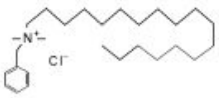
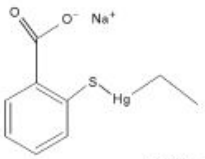
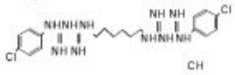
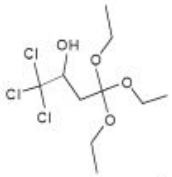
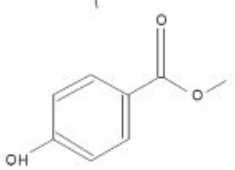
			hypersensitivity to thimerosal can be demonstrated by an occlusive patch test or intradermal injection.[5]
	Allergic contact lens keratoconjunctivitis (CLK) reaction is a type IV delayed hypersensitivity reaction secondary to use of contact lens solution.	The patient must be exposed to the preservative for several years before sensitization occurs. It is characterized by progressively increasing intolerance of contact lenses, punctuate staining along the limbus for 360 degrees and above the superior limbus, and a whorl-like staining over much of the cornea. There is also an associated fine papillary conjunctival reaction.[1]	The classic cause of CLK is thimerosal, although it can also be attributable to chlorhexidine gluconate or EDTA.

PMNs, polymorphonuclear leukocytes.

Preservatives

Nature and properties of the various preservatives: the different chemical classes ().

Table 2. Preservatives commonly used in topical ophthalmic agents

Chemical class	Most commonly used preservative	Structure	Properties of class	OVID search (and 'preservatives' and 'ophthalmology')[a]	'Irritant', 'inflammatory', 'allergic'[b] %	PubMED sear 'preservatives' 'ophthalmolog
Quaternary ammonium	BAC		Act mainly via detergent activities by dissolving the bacterial walls and membranes and destroys the semi-permeable cytoplasmic layer	593 (25)	Irritant 40 (8%); inflammatory 114 (21%); allergic 99 (19%)	438 (27)
Organo-mercurial derivative	Thimerosal		Act as a result of the sulfur-removing properties of the mercuric ion by combining with the sulfhydril groups of proteins to precipitate bacterial proteins by forming proteinates of mercury	500 (7)	Irritant 19 (4%); inflammatory 43 (9%); allergic 96 (19%)	309 (1)
Amidine	Chlorhexidine		Acts by destroying the semi-permeable layer of the cytoplasmic membrane and has antimicrobial activity mainly against cocci and gram positive bacteria and some gram negative; Also has fungistatic activity	2685 (3)	Irritant 65 (2%); inflammatory 305 (11%); allergic 124 (5%)	1499 (0)
Alcohol	Chlorobutanol and phenylethanol		Chlorobutanol: increases lipid solubility and can cross the bacterial lipid layer; Phenylethanol: exhibits synergistic activity when combined with other preservatives (chlorobutanol, BAC, chlorhexidine)	Chlorobutanol 46 (3); phenylethanol 221 (1)	Irritant 3 (7%); inflammatory 8 (17%); allergic 9 (20%); irritant 0; inflammatory 3 (1%); allergic 1 (0.5%)	Chlorobutanol 27 (3); phenylethar 640 (0)
Parabens	Esters of parahydroxy-benzoic acid		Activity targets mold and fungi rather than bacteria	381 (0)	Irritant 9 (2%); inflammatory 17 (4%); allergic 40 (10%)	339 (0)

All search terms in the year range 2004 to current.
[a] The number of results from an OVID or PubMED search for the specific type of preservative (e.g., BAC, chlorhexidine, chlorobutanol, etc.) is shown in the table. The number in parenthesis represe result from combining the specific preservative with the terms 'ophthalmology' and 'preservative'. (e.g. chlorhexidine and preservative and ophthalmology).
[b] The number of results when combining the preservative with the search terms "irritant", "inflammatory", or 'allergic' is shown in the table. The number of parenthesis is the percentage found by divid search category with the number of total search results for that preservative. (e.g., searching chlorobutanol and irritant yields 3 results, 3 is then divided by 46, the total number of results for th

Benzalkonium Chloride

Benzalkonium chloride, also known as BAC, is a quaternary ammonium, which is a highly hydrosoluble bipolar compound with surfactant properties. Their mechanism of action primarily involves its intrinsic detergent activities depending on its concentration (ranges from between 0.004 and 0.02% in most topical products) leading to dissolution of bacterial cell walls and membranes. The spectrum of activity is mainly focused on Gram -positive bacteria. BAC is used in a wide range of commonly used products, such as soaps, cosmetics, cleaning products, ophthalmic preparations, disinfectants, and spermicides. BAC is known to cause damage/toxicity in almost all ocular structures.

Animal Studies

Although the use of BAC does not appear to interfere with the absorption of the therapeutic agent in animal models,[6•] Becquet *et al*.[7] performed a study using rats to demonstrate the toxic and immunoallergic reactions that take place in the corneo-conjunctival surface after subjecting the eyes to the application of various preservatives. They found that even at low concentrations of a single instillation of BAC, toxic effects on the corneo-conjunctival surface were noted most likely due to its intrinsic detergent properties that can alter tear fluid stability, particularly in its lipid phase. In rats treated with various other preservative solutions (BAC 0.01%, methyl parahydroxybenzoate 0.05%, and thiomersal 0.004%) there was an infiltration of immunocompetent cells into the limbus and bulbar conjunctiva that expressed class II and CD11b membrane HLA antigens (leukocyte integrin). Similar results were reported later by Baudouin[8] in rats treated by timolol 0.5% containing BAC (0.01%) with abnormal expression of antigens human leukocyte antigen-DR and clusters of designation 23.

Human Studies

Human in-vitro studies performed by Becquet *et al*.[7] showed that unpreserved β-blockers showed no toxic effects on cultured human Tenon's capsule fibroblasts, whereas preserved β-blockers showed toxicity and inhibition of fibroblast proliferation. Mietz *et al*.[9] also demonstrated that instillation of another β-blocker, metipranolol 0.3% preserved in BAC, produced deterioration of the composition of the extracellular matrix and the organization of the conjunctival stroma, combined with an increase in the number of activated subepithelial fibroblasts, in the deposits of collagen and the thickening of the basal membrane of the endothelium. In a tissue culture model utilizing immortalized corneal and conjunctival epithelial cells, toxicity was observed with all preservatives, but dependent upon concentration with the order of decreasing toxicity observed for thimerosal (0.0025%) more than BAC (0.025%) more than chlorobutanol (0.25%) more than methylparaben (0.01%) more than sodium perborate (0.0025%).[10•] Goto *et al*.[11] performed a study in which human lens epithelial cells were cultured in medium containing different dilutions of latanoprost, timolol maleate, and BAC and then assessed using phase-contrast microscopy after 7 days' culture to determine the morphological changes that take place. The experiment showed that there is a dose-dependent toxic effect of BAC induced by the expression of prostaglandin E2 (PGE2), IL-1α and IL-6, resulting in the inhibition of the proliferation and elongation of the human lens cell and then to cell death.

The effects of preservatives on the eye are sometimes obscured by the chronic disease process, for which topical ophthalmic medications are used. A study performed by Hamard *et al*.[12] showed that BAC played a role in trabecular cell death in glaucoma patients from the chronic use of topical ophthalmic medications containing BAC. In the study, normal and glaucomatous trabecular cell lines were treated for 15 min with antiglaucoma drugs (1/100 and 1/10 dilutions): timolol BAC+ or BAC-, betaxolol BAC+ or BAC-, latanoprost BAC+ or pure BAC. Apoptotic marker (Apo2.7) expression, annexin V binding and DNA content were evaluated by flow cytometry and confocal microscopy. They found that benzalkonium-containing β-blockers and prostaglandin analogue triggered mild expression of one out of three apoptotic markers, whereas the proapoptotic effect observed with BAC appeared to be largely hindered by active compounds in the preserved eyedrops. The use of BAC may be worse in patients with more chronic ocular disorders as patients with atopic dermatitis had an increased sensitivity to preservatives, such as thimerosal, parabens, and BAC.[5] However, when actually trying to assess the impact of BAC in cell-mediated responses, a recent study[13] tested 42 898 patients with BAC 0.1% in petrolatum (topical drugs, ophthalmics, and disinfectants; http://www.ivdk.org) between 1996 and 2006 demonstrated 0.6–1.5% reactions with a total of 41 stronger positive reactions.

Although human in-vivo studies have generated rare reports of BAC induced IgE-mast cell type reactions, a recent study[14] demonstrated bronchoconstriction in asthmatics when challenged with BAC suggesting a nonspecific trigger. Specifically relating to the eye, a study by Ishibashi *et al*.[15] evaluated preserved and nonpreserved topical timolol and noted that the NIBUT (noninvasive breakup time) of the precorneal tear film was significantly shortened. They evaluated precorneal tear film stability without fluorescein instillation that facilitates the in-vivo noninvasive observation of precorneal tear film breakup and found that eye exposure to preserved timolol resulted in significant instability in the precorneal tear film at 30 min after instillation, whereas the preservative-free timolol had no such effect suggesting that even a single exposure to 0.005% BAC may produce precornealtear film instability.

Thimerosal

Thimerosal, in its usual concentrations range from 0.001 to 0.004%, is an organomercurial derivative that acts as a result of the sulfur-removing properties of the mercuric ion. They act by combining with the sulfhydryl groups of proteins to precipitate bacterial proteins by forming proteinates of mercury. The proteinates act as a neoantigen that causes the highest frequency of cell-mediated responses of the ophthalmic preservatives.[10•] It is most commonly found in soft contact lens solutions and may cause ocular delayed hypersensitivity.

Animal Studies

In 1991, a study[16] on ocular hypersensitivity to thimerosal in rabbits documented that the signs and symptoms observed included corneal edema, corneal infiltration and erosion, infiltration of the anterior chamber, iritis, conjunctival edema and hyperemia, and a significant increase in mucous production. They found that the IgG tear antibodies increased as a result of increased vascular permeability with the tear IgA titers increasing to a lesser extent than IgG during the ocular challenge. The major class of serum antibodies consisted of IgG, with IgA compromising approximately 5% of serum antibodies. Histologic analysis showed that the ocular inflammatory response was accompanied by both polymorphonuclear (PMN) and mononuclear cell infiltrates into the cornea and conjunctiva. Both serum and tear antibodies correlate with the severity of the ocular inflammatory response and support an immune complex mediated or Arthus type of ocular hypersensitivity to foreign antigens. In another animal study utilizing rat model performed by Becquet *et al*.,[7] thimerosal application to the eye resulted in hyperplastic changes to the corneo-conjunctival surface with increasing expression of Limbal class II antibody. In this study, anticlass II (RT1b) antibody was found to be the most reliable marker to locate and count inflammatory cells.

Human Studies

Thimerosal has demonstrated in a concentration-dependent manner on human dendritic cells, inhibition of lipopolysaccharide (LPS)-induced proinflammatory cytokines including TNFα, IL-6, and IL-12p70 while having no effect on IL-10. These thimerosal-exposed dendritic cells induced increased TH2 (IL-5 and IL-13) and decreased TH1 (IFNγ) cytokine secretion from the T cells in the absence of additional thimerosal added to the coculture.[17] In addition, there is a potential impact of thimerosal on limbal stem cells as documented in a recent case report.[18]

Tosti and Tosti[3] provides a case report of 36 patients with follicular allergic contact conjunctivitis induced by thimerosal. All of these patients report using eye drops containing thimerosal. Furthermore, 13 patients were soft contact lens wearers who became sensitized to their contact lens solution containing thimerosal. In the majority of these cases, the eyelids were spared. But in five patients, they also developed an allergic contact dermatitis of the eyelids. All of the 36 patients had a positive patch test reaction to thimerosal.

Chlorhexidine

Chlorhexidine is a cationic agent that belongs to the family of the bis-diguanides. It is used in the digluconate form, and acts by destroying the semi-permeable layer of the cytoplasmic membrane and produces its antimicrobial activity mainly against cocci and Gram-positive bacteria, Gram-negative bacteria as well as fungistatic activity.

Human Studies

Although chlorhexidine has been associated with IgE-mast cell mediated reactions, such as anaphylaxis, the evidence for localized ocular allergy is lacking.[19–22] Vaahtoranta-Lehtonen *et al*.[23] performed an experiment comparing ethyl-6-*O*-decanoyl-glucoside 0.005% (EDG) combined with 0.00025% chlorhexidine acetate (EDGC) to a commercial polyaminpropylbiguanide (PAPB) used daily as a cleaning and disinfectant agent for both ionic and nonionic contact lenses in 59 patients. The following symptoms were compared for each solution; blurred vision, dryness, foreign body sensation, redness, and dirty lenses. The following signs were also compared for each solution; conjunctival hyperemia, papillary hypertrophy, corneal deposits, purulence, limbal vascularization, subepithelial scarring, visual acuity, bulbar hyperemia, and tear breakup time. After 8 weeks, 52% of the participants in the EDGC group showed no evidence of corneal or conjunctival abnormalities. In contrast, only 19% of the participants in the PAPB group showed no abnormalities of the conjunctiva or cornea. After 8 weeks, 25% of the EDGC group showed evidence of papillary hypertrophy, whereas 50% of the PAPB group showed similar findings.[23]

In three consecutive cataract operations, chlorhexidine was inadvertently used as an intraocular irrigating solution as a result of inattentiveness of an assistant. In two of the three patients, corneal endothelium damage was so severe that penetrating keratoplasty had to be performed. Further effects included pronounced iris atrophy, anterior chamber applanation, and a retrocorneal membrane. In one case, an increase in intraocular pressure developed. No effects were observed in the retina or optic nerve.[24]

Chlorobutanol and Phenylethanol

Chlorobutanol is an alcohol that acts by increasing lipid solubility, and its antimicrobial activity is based on its ability to cross the bacterial lipid layer. Chlorobutanol is a widely used, very effective preservative in many pharmaceuticals and cosmetic products, for example, injections, ointments, products for eyes, ears and nose, dental preparations, etc. It has antibacterial and antifungal properties. Chlorobutanol is typically used at a concentration of 0.5% where it lends long-term stability to multi-ingredient formulations.

Phenylethanol is an antimicrobial, antiseptic, and disinfectant, which is used also as an aromatic essence and preservative in pharmaceutics and perfumery.

Animal Studies

Two drops of a chlorobutanol-containing or BAC-containing artificial tear were instilled into the right eye of six rabbits. At the same time six control animals received no eyedrops. The central region of the corneal epithelium was quantitatively assessed using a computer system. There were up to 5% exfoliating cells evident at the ocular surface in treated rabbits but with no difference between the two products. Controls had no cell exfoliation (<0.5%). The distribution of surface areas of the squamous cells in the treated eyes was shifted to slightly larger values than in the controls after use of the chlorobutanol-containing product but the number of epithelial cell craters/cell was unchanged from that of the controls. Cell surface areas were shifted to significantly smaller values than controls after use of the BAC-containing product and there were much fewer epithelial cell craters/cell. The results reveal differences in the effects of preservative-containing artificial tears on the squamous cells of the corneal epithelium in a clinically relevant situation.[25]

Human Studies

Human in-vitro studies have been reported by Tripathi and Tripathi[26] who evaluated the cytotoxicty of BAC and chlorobutanol by exposing primary cultures of human corneal epithelial cells to a single dose of each preservative. Control and experimental cultures were analyzed by continuous time-lapse videomicrographic recordings as well as by sequential phase-contrast microscopy. Both BAC at a concentration of 0.01% and chlorobutanol at 0.5% caused immediate cessation of normal cytokinesis and mitotic activity, and epithelial cells degenerated within 2 and 8 h, respectively. A more recent version of this experiment demonstrated that the survival of corneal and conjunctival epithelia in culture with preservatives polysorbate and benzalkonium were highly cytotoxic with cell survival decreasing to 20% at the concentration estimated in commercial ophthalmic solutions as compared with 80% survival of cells exposed to chlorobutanol.[27] Although there have been limited

human in-vivo studies that demonstrate any immediate reactivity through an IgE-mast cell mediated mechanism, Garcia-Medina *et al.* [28] describes a case of a woman who presented with intense ocular pruritus and conjunctival hyperemia with each instillation of Cloircusi fluotest (Alcon, El Masnou, Spain), containing chlorobutanol. Skin tests were all negative. Chlorobutanol was presumed as the reagent through elimination and positive conjunctival provocation test with purified chlorobutanol confirmed the hypothesis.[28]

Parabens

Parabens are esters of *p*-hydroxybenzoic acid. They act by targeting molds and fungi rather than bacteria. In an assessment of preservative sensitivity in the United Kingdom, the results of patch testing using the extended British Contact Dermatitis Society Standard series, they found that parabens mix has the highest irritancy rate.[29]

Animal Studies

Becquet *et al*.[7] showed alteration of the rat conjunctival surface after 1 month of treatment with instillation of eye drops containing 0.05% methyl parahydroxybenzoate with increased number of epithelial cell layers, loss of goblet cells, and appearance of keratinization of the most superficial cell layers.

Human Studies

There are few human studies performed recently because of their well established role in delayed hypersensitivity. Nagel *et al*.[30] presents a case report of bronchospasm and pruritus when a hydrocortisone preparation containing methylparaben and propylparaben is given intravenously to an asthmatic patient. Administration of the hydrocortisone preparation without the paraben preservative did not elicit the same response. Skin tests for immediate hypersensitivity to parabens were positive. They were able to conclude that parabens are capable of producing immunologically mediated, immediate systemic hypersensitivity reactions. Henry *et al*.[31] also describes the relationship of parabens and the development of contact urticaria.

Preservatives and Complications of Chronic Treatment

The toxicity of preservatives manifest with prolonged use in the chronic treatment of ocular diseases. The cytotoxic effects increase with the concentration of the preservative and the duration of the exposure. As the widespread use of preservatives in many commonly used products continues, more studies such as those with contact lens formulations and ocular allergy treatments (as the ocular allergy therapeutic market has continued to increase by 10–20% per annum) will have to be done to study its effects,[32•] especially those that are adverse and compromise the safety of the consumer in order to better define the toxic from the immunologic/allergic adverse effects.

Conclusion

Many topical ophthalmic agents use preservatives as they are designed for 'multiuse' treatment regimens. Many clinicians commonly refer to the induction of the signs and symptoms of itchy, burning, gritty (dolor), red (rubor), and swollen (tumor) as an 'allergy'. In actuality, it appears that many of these forms of 'allergic conjunctivitis' are associated with the continuous administration of the preservative in constant contact with the conjunctival surface and not a true allergy to the drug. Preservatives are present in almost all ophthalmic preparations and play an important role as they may cause adverse side effects, from mild irritation to severe toxic reactions. Continued study on the effects of these preservatives and other inactive ingredients is important to ensure that the therapeutic result of the ophthalmic preparations is not diminished by the adverse effects that preservatives may produce in the patient.

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Papers of particular interest, published within the annual period of review, have been highlighted as:

• of special interest

•• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 486–487).

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