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Research paper

Antimicrobial safety of a preservative-free nasal multiple-dose drug administration system

Norbert Klöcker[a], Axel Kramer[c,*], Thomas Verse[b], Claudia Sikora[c], Peter Rudolph[c], Georg Daeschlein[c]

[a]AUDIT Institute for Medical Services and Quality Assurance, Taunusstein, Germany
[b]University ENT Clinic, Mannheim, Germany
[c]Institute for Hygiene and Environmental Medicine, Ernst Moritz Arndt University, Greifswald, Germany

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Abstract

Recent technical developments in metered-dose pumps allow preservative-free nasal drug application with multiple-dose systems, avoiding the cytotoxic and allergic problems of preservatives. The use of the 3K System as a representative of those systems is demonstrated as microbiologically safe and without risk for the user and for the product during shelf life, under challenge up to 24 weeks, as well as under worst-case conditions with heavy bacterial contamination on the outlet surface. Therefore, the authors assess preservative-free pump systems as the new gold standard for mucosal drug application.

Keywords: Preservative-free; Nasal sprays; Multiple-use; Microbiology; Safety

1. Introduction

1.1. Hygienic requirements for nasal multiple-dose systems and toxicological risks of preserved formulations

Apart from antimicrobially effective or freshly prepared formulations, Ph. Eur. and other Pharmacopoeias require multiple-dose (MD) systems containing preservatives to guarantee no bacterial contamination above 100 CFU/ml. However, the Ph. Eur. provides no special quality control methods for extended testing. Additionally, these formulations must not be cytotoxic, nor alter local defense mechanisms—in particular phagocytosis, chemotaxis and mucociliar clearance—nor be sensitizing. In contrast, preservatives, especially benzalkonium chloride (BKC), which is used in the vast majority of nasal products, cause side effects such as allergic reactions, cell damage, and irritations of the nasal mucosa [1–5]. Despite these risks, the majority of nasal products still contain preservatives, although for eye-drops, the single-dose system (form-fill-seal) has been introduced to avoid preservatives [6].

1.2. Principle of the preservative-free 3K System for MD application

Recently, new nasal-spray metered-dose pumps for preservative-free (PF) nasal formulations for MD application have been developed. Since then, a number of preparations have been reformulated, and the positive impact on nasal formulations has been shown for the majority of leading products on the German market [7,8]. Consequently, the German regulatory authorities have drawn up a graduated plan regarding BKC in nasal sprays [9]. The 3K System (Fig. 1, see also 10) is currently used in the majority of PF products. From the same manufacturer (URSATEC, Homburg, Germany), a second system, called COMOD [11] is available for nose- and eye-drops. For other alternatives, the authors know of only one product on the market and another one under development which are contained in a PF system manufactured by Erich PFEIFFER GmbH (Radolfszell, Germany, Valois Group), and another existing but yet not marketed system from SOFAB

* Corresponding author. Institute for Hygiene and Environmental Medicine, Walter-Rathenau-Str. 49a, 17489 Greifswald, Germany. Tel.: +49-3834-515542; fax: +49-3834-515541.
E-mail address: kramer@uni-greifswald.de (A. Kramer).

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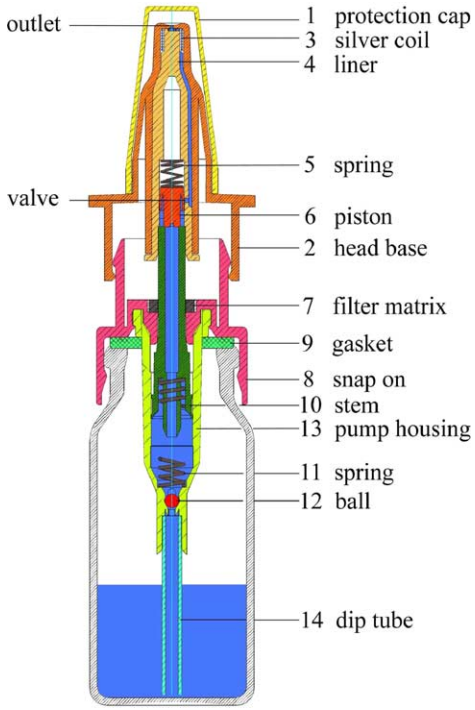

Fig. 1. Schematic drawing of the 3K System.

(Suresnes, France, REXAM Beauty Packaging Group). Neither system has yet been described in the literature.

The 3K System functions as an non-airless dosing pump with liquid compression in the storage chamber after each actuation. A ball-valve seals the dose to be administered from the remaining contents in the storage chamber. The spring of the delivery valve acts as a seal against the delivery opening of the applicator. The valve opens abruptly after the spring pressure of 3 bar is reached, and the product is delivered through a special duct. The valve immediately recloses hermetically, preventing contamination of the container. Any liquid in the outlet area will come into contact with the silver coil, causing an oligodynamic effect resulting in a germ reduction [12–14]. After actuation, the ball-valve opens and the next dose moves into the storage chamber. A subsequent negative pressure builds up in the product container which is compensated by the inflow of air through a filter matrix comprising a combination of filters, absorptive materials, and silver.

1.3. Requirements for microbiological safety and quality control of preservative-free MD systems

Three fundamental aspects must be considered [10]. Mechanical and physical sealing of the complete system has to be assured during the entire aseptic production process, shelf-life, and usage. The closure between pump and container must not only be microbiologically secure, it must also prevent the user from intentionally or unintentionally opening the system and re-using it. While in the past metered-pump systems for preserved nasal sprays were screwed or crimped onto the container, today the 'snap-on' closure is preferred for PF systems. This, together with other antimicrobial precautions (i.e. filter and silver-impregnated outlet), avoids contamination, especially of the first puff of spray.

Based on test procedures proposed by Wiedemann et al. [10,15], a valid test method for microbiological safety should simulate the following in vivo conditions:

- During the conventional use of nasal sprays, the outlet surfaces frequently contacts the nasal cavity and other areas such as skin or hands, as well as microbiologically polluted air, so that microorganisms from these areas may adhere to the delivery duct near the outlet, multiply, and invade the system.
- Traditional nasal MD systems, usually metered-dose pumps or even the squeeze bottles still on the market, cause negative pressure when the pump is actuated. Therefore, microbiologically burdened air or liquids stream into the container and a contamination of the system cannot be excluded.

Building on the first results on the microbial safety of the 3K System [10,15], more data with extended-use simulation under challenge and with 12 different products are presented. To exclude the influence of potential intrinsic antimicrobial activity, the products were tested for antimicrobial activity.

2. Materials and methods

2.1. Applied tests

2.1.1. Intrinsic antimicrobial activity

A precondition for the quantitative suspension test is to determine the suitable neutralizer in a dilution assay with *Pseudomonas aeruginosa* ATCC 15442, *Staphylococcus aureus* ATCC 6538 and *Candida albicans* ATCC 10231 (according to DIN EN 1040 resp DIN EN 1275). This testing simultaneously allows sensitive determination of microbiostatic activity. Subsequently, the quantitative suspension test was performed with the identified neutralizer 3%, tween 80 + 0.3% lecithin + 0.1% cysteine.

2.1.2. Challenge test with worst-case simulation after 4-day excessive contamination

The test procedure follows the propositions by Bagel and Wiedemann [10,15]. After actuating the pump by five sprays into the air, the test containers were dipped with the tip of the outlet into the germ suspension. Thereafter, one dose was sprayed into the air to guarantee a freshly and fully filled dip-tube, valve and outlet. Then the outlet was dipped again into the germ suspension at a low angle, and one dose was ejected into the germ suspension. Afterwards, the container was stored in an upright position without cleaning

the outlet or protecting it with a cap. This procedure was repeated three times a day for 4 days. Then the containers were stored for 3 days at room temperature to allow microbial growth. Two puffs were subsequently sprayed first onto blood agar and then onto Tryptone soya agar plates (CSA, Oxoid, Wesel, Germany) from a distance of 5 cm. After aseptic mechanical opening of the container, 0.5 ml of the contents were pipetted into 4.5 ml liquid Tryptone soya broth (CSL, Oxoid, Wesel, Germany) and 0.1 ml directly onto blood agar and CSA for plating analysis (spiral plater). Thereafter, the media were incubated at 36.5 ± 2.5 °C for 48 h (solid media) or 14 days (CSL).

For the test, an inoculum of a 12–15 h CSL culture was diluted 1:1000 in physiological saline. In addition to *P. aeruginosa* (ATCC 6538P, $2.3–2.8 \times 10^7$ CFU/ml)—the most frequent contaminant of wet areas and products—as used in the previous study [10,15], we also conducted the test with *Bacillus subtilis* (ATC 663, $2.1–4.1 \times 10^6$-CFU/ml), *S. aureus* (ATCC 9027, $3.9–6.7 \times 10^6$ CFU/ml), and *C. albicans* (ATCC 10231, $3.7–8.5 \times 10^6$ CFU/ml), because *S. aureus* and *C. albicans* are more common members of the normal mucosal nasal flora, and *Bacillus subtilis* is a typical representative of ubiquitous environmental flora. Furthermore, in the dilution inhibition tests, *P. aeruginosa* growth was marginally inhibited by some products in the presence of the neutralizer. Therefore, the test germs which were not inhibited—*S. aureus*, *B. subtilis* and *C. albicans*—were selected to better demonstrate test stringency.

2.1.3. Challenge test with long time simulation of use up to 24 weeks

The first spray dose and the contents were microbiologically tested for 6, 12, and 24 weeks of simulated use with defined intervals (Table 1), according to the modified methods of Wiedemann et al. [10,15]. Spraying was performed 10 times a week, divided into a morning and an evening series (5 days, two times per day). Each spray bottle was actuated for use in the first and second test period (6 and 12 weeks) a total of 60 times and 120 times in the third period (24 weeks). After spraying each time, the outlet was touched with a finger of the unwashed hand on the sprayer tip and the protection cap was replaced onto the actuator.

After finishing the in-use tests, the products were sprayed at a 90° angle onto blood agar from a distance of 5 cm. Forty-eight hours later, CFU were counted on the agar after incubation at 36.5 ± 2.5 °C. The contents of the bottles were tested for sterility in accordance with Ph. Eur.

2.1.4. Shelf-life safety

Microbiological safety must of course be assessed during the entire shelf-life. The tested samples are stored unprotected under normal room conditions to simulate real life and under standard ICH storing conditions according to Ph. Eur.

2.2. Tested products

All tested products were PF formulations Commercially available products were: Ems Brine Spray (HEXAL), Dexpanthenol 5% (Otriven Care, NOVARTIS; Panthenol Nasal Spray, ratiopharm), Dexpanthenol 5% + Xylometazoline 0.05% (Nasic for children, Cassella med), Dexpanthenol 5% + Xylometazoline 0.1% (Nasic for Adults, Cassella med), Xylometazoline 0.05% (Olynth for Children, Pfizer), Xylometazoline 0.1% (Olynth for Adults, Pfizer) and Chromoglycic acid 2% (Chromohexal sanft, HEXAL).

Four other products, all still under development and all containing Dexpanthenol 5%, were also used for testing.

3. Results

3.1. Intrinsic antimicrobial activity

After 24 h exposure time, a few products (Table 2) exhibited marginal inhibitory activity against the test germ *P. aeruginosa* only in combination with neutralizer. Corresponding to these results, the undiluted products show no microbicidal efficacy within 5 or 15 min. Therefore, the in-use tests are valid.

3.2. Challenge test with worst-case simulation after 4-day excessive contamination or up to 24 weeks of simulated use

Neither the first sprays nor the contents of any analyzed bottles revealed bacterial growth of the test germ or of other contaminants.

Table 1
Test scheme for the interval simulation (IS) over 6, 12 and 24 weeks (C, challenge test)

Week	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
n = 20 *spray bottles for each product*																											
		10	10	10	10	10	10		C																		
n = 20 *spray bottles for each product*																											
IS 12W		10	10	10		C					10	10	10		C												
n = 20 *spray bottles for each product*																											
IS 24W		10	10	10	10	10	10		C											10	10	10	10	10	10		C

Table 2
Microbiostatic activity of different products (undiluted) tested against *Pseudomonas aeruginosa*

Active agent	Number of different products	Neutralizer			
		Without	I	II	III
Saline solution	1	Growth	Growth	Growth	Growth
Xylometazoline 0.05%	1	Growth	No growth	Growth	No growth
Xylometazoline 0.1%	1	Growth	No growth	Growth	No growth
Dexpanthenol 5% + Xylometazoline 0.05.%	1	Growth	No growth	No growth	Growth
Dexpanthenol 5% + Xylometazoline 0.1%	1	Growth	No growth	No growth	Growth
Dexpanthenol 5%	6	Growth	No growth	Growth	Growth
Chromoglycic acid 2%	1	Growth	Growth	Growth	Growth

I, 3% tween 80 + 0.3% lecithin + 0.1% cysteine; II, 3% tween 80 + 3% saponin + 0.1% histidine + 0.1% cysteine; III, 3% tween 80 + 0.3% lecithin + 0.1% histidine + 0.5% sodium thiosulfate.

3.3. Shelf-life safety

None of the tested products showed contamination during up to 3 years of unprotected storage under normal room conditions and standard ICH storage conditions.

4. Discussion

The Ph. Eur. limits the acceptable number of microorganisms in nasal products to not more than 10^2 CFU/ml, regardless of whether the product contains preservatives or not. In contrast to the 3K System, conventional systems theoretically have a high risk of contamination by incoming air to compensate the vacuum generated during operation, because of the inevitable contact between the outlet and the nasal mucosa and other possibly contaminating parts of the body, as well as from germs in the air.

Our results did not reveal any detectable germs in the first spray or in the contents. Thus, the antimicrobial barriers of the 3K System (micropore filters for the incoming air, oligodynamically active silver at the outlet) seem to guarantee microbiological safety under varying risk situations. Therefore, the requirements of the Ph. Eur. are fulfilled.

In terms of pharmaceutical production and analytic quality, the use of BKC in liquid products should always be viewed critically. One of the major problems with BKC during the production process is filter saturation, which requires that additional BKC be added to the bulk in order to obtain the specified concentration in the finished product; the consequence would be intensive process monitoring.

Our results demonstrate the superfluous of preservatives in nasal sprays other than non-filterable formulations (e.g. suspensions). Omitting them will help to overcome the main risks, such as cytotoxicity [16] and bacterial contamination, which are evident in preserved products and even antiseptics [17,18].

It has been mentioned that the reformulation from a nasal spray containing preservatives to a PF nasal spray will usually require that production be transferred to specialized filling facilities, as the enormous investment in an aseptic filling line will be economically justified only for the internationally operating market leaders. Because at least the formulation and packaging system will be changed, an entirely new registration dossier is necessary. However, as the market leaders increasingly switch to PF products, the rest of the market will likely follow, and the demand for these products will increase accordingly. The current graduated plan of the German health authorities sends a clear message about this trend.

It is foreseeable that PF technology will be used for other indications and applications, e.g. wound healing, artificial saliva, or cosmetics.

5. Conclusion

Preservatives have a considerable and avoidable cytotoxic potential on the nasal mucosa. Thus, preservative-free nasal drug application is beneficial for patients requiring nasally applied drugs, either of topical or systemic action. Newly engineered nasal pump systems are microbiologically safe and guarantee longer in-use stability than conventional systems, even if the latter contain preservatives. Therefore, it can be concluded that PF nasal drug application is the new gold standard for this application form and that preservatives in nasal sprays are obsolete whenever PF alternatives are available. In the future development of new nasally applied drugs, PF systems are a prerequisite wherever technically possible.

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