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The Tolerability of Nasal Drugs With Special Regard to Preservatives and Physico-chemical Parameters

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Abstract

Background: Recent technical developments allow preservative-free nasal drug application in multi-dose systems. New pharmaceutical formulations for better tolerable nasal sprays are now possible and consequently reformulations introduced to the market. Therefore, a representative and systematic overview on comparable products is mandatory.
Methods: Marketed nasal products in the indication groups: decongestants, antiallergics, care and wound-healing, hormones and saline solutions were tested for their cytotoxic properties according to DIN EN 30993 – 5, pH, and osmolality.
Results: In all indication groups reformulation to preservative-free application resulted in significant increase of cell growth and reduction of cytotoxicity. Physico-chemical galenic properties are of considerable importance too. With decongestants tolerability is dependant on the concentration of the active compound.
Conclusions: Our data lead to the conclusion that preserved nasal sprays are obsolete, when preservative-free alternatives are available. Attention should be paid to galenic properties and dosage of the active.

Key words

Nasal sprays · preservatives · benzalkonium chloride · tolerability · cytotoxicity

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Bibliography
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Introduction

The European Pharmacopoeia (Ph. Eur.) stipulates the addition of suitable preservatives at an appropriate concentration for preparations for nasal application (nasal drugs) in multidose containers. The only exception are established and approved exemptions such as, e.g., oils, auto-microbicidal solutions or preparations for immediate and short-term application prepared by the pharmacist. Most of the nasal drugs on the market are preserved with benzalkonium chloride (BZC) at concentrations of 0.005% to 0.02%. The pharmacopoeia furthermore stipulates for preparations for nasal application that they are not irritating and do not have any adverse effects on the function of mucociliary clearance. This is a conflict in itself since the allergenic and cytotoxic potential of preservatives has been known for some time and the negative effect of preservatives on ciliary function has been described in many ways [1-6]. For this reason, the Federal Institute for Drugs and Medical Devices (FIDMD) has since then initiated a step-by-step plan combating BZC in nasal drugs [7].

Knowledge of the harmful effect of preservatives [8] first led to the development of preservative-free (psf) alternatives in ophthalmology, initially in form of single-dose containers (SDC), then also multidose containers (COMOD system). Psf systems have also been available for nasal drugs for some time. A considerable amount of preparations have been changed or are currently being changed. The microbiological safety of the new psf systems has been proven without a doubt in extensive testing and they are thus the state of the art [9].

To date, there were no extensive practical comparative studies that would allow for a justified statement on the comparison of the cytotoxic properties of market-based preparations and provide the physician or pharmacist with a rational selection. The data available to date on the toxicity of preservatives in nasal drugs is mostly based on animal experiments ex vivo, in vivo examinations on the cilia or rather in vitro examinations of the cell culture. In vivo studies were barely possible to date due to lack of comparative preparations free of preservatives.

For this reason we conducted an extensive cytotoxic examination of almost all available preparations on the market. The selection was based on the respective market share so that the most important preparations were included. We also determined the pH and osmolality in all cases since a direct statement on the prefabricated compound is only possible if the main properties of the matrix are known. Most of the examinations were performed as part of a dissertation. The results presented here represent an excerpt that is relevant for the medical practice.

Methodology

Cytotoxicity testing was performed according to DIN EN 30993-5. The growth values indicate the mean value from 19 individual tests per preparation, compared to the respective control. These are thus relative values.

The exact course of the testing was already described in an earlier work [10]. FL cells, a cell line of the human amnion, was used for cell cultivation. The inventory was kept in 250 ml tissue culture bottles (Greiner GmbH, Solingen). The cells were passaged every 4 hours. The inventory was cultivated again from the cell culture after the 100th passage. For this, the medium was decanted, the cell layer rinsed with 20 ml PBS (phosphate buffered saline), uniformly moistened with 20 ml of the enzyme solution (0.05% 1:250 trypsin + 0.02% EDTA in Ca^{2+} and Mg^{2+} free PBS) and the solution decanted again. After incubation of the bottles at 37ºC for about 10 minutes, the separated cells were suspended in 40 ml MEM (minimum essential medium) + 8% serum and the cell count determined by means of the universal counter. 4 x 10^6 cells were sowed in 75 ml growth medium per subculture bottle.

For testing the cells were sowed in culture tubes (ca. 200,000 cells/1.5 ml growth medium per tube) after previous separation. The growth medium consists of 70% lactalbumin hydrolysate and 30% MEM with additives of 1% antibiotic solution (final concentration: 100 IU Penicillin G and 100 µg Streptomycin sulfate/ml) and 8% bovine calf serum. The pH was adjusted to 7.2 with 1 M NaOH solution. Then, the medium was heated in a water bath at 37ºC.

The cells were available as monolayer after 72 hours of cultivation. Afterwards, a medium change with incubation medium is performed. It consists of MEM + 1% antibiotic solution + 1% bovine calf serum (control). The incubation medium additionally contains the trial substance.

The substances to be tested were initially dissolved in water. Medium was used for the two last dilution steps and the pH was adjusted to 7.2. The incubation medium was heated to 37ºC in a water bath. The solutions are prepared anew for each test. The incubation medium is made about 30 minutes before the medium change and stored in a refrigerator after cooling. 1 ml of the antibiotic solution of Penicillin and Streptomycin is applied to 100 ml medium. The inventory solutions of antibiotics and serum are stored in a freezer at -18ºC. The cell were incubated in the medium with the trial substance for 24 hours.

The medium is decanted, the cell layer uniformly moistened with 0.3 ml enzyme solution (0.05% 1:250 trypsin and 0.02% EDTA in Ca^{2+} and Mg^{2+} free PBS) and the solution decanted. After the tubes are incubated at 37ºC for about 10 minutes, the separated cells are suspended in 1 ml MEM and 1% serum each and the cell count is determined by means of the universal counter.

Tab. **1** Decongestants, Xylometazoline

Trade name	*Manufacturer*	*Form*	*Active agent*	*Conc. (%)*	*Additives*	*Preservation*	*pH*	*Osmolality*	*Growth (%)*
Xylometazoline + Dexpanthenol	New development	NS	Xylometazoline	0.1	Dexpanthenol 5%	Ø	5.58	422	84
Xylometazoline + Hyaluronic acid	New development	NS	Xylometazoline	0.1	Hyaluronic acid	Ø	5.90	286	41.8
Nasic	Casella med	NS	Xylometazoline	0.1	Dexpanthenol 5%	BZC	6.01	419	38
Otriven OK	Novartis	NS	Xylometazoline	0.1	Ø	Ø	5.79	274	20
Olynth OK	Pfizer	NS	Xylometazoline	0.1	Ø	Ø	5.86	295	17
Nasan	Hexal	NS	Xylometazoline	0.1	Ø	BZC	5.91	297	13
Schnupfen Endrine	Asche	NS	Xylometazoline	0.1	Ø	BZC	6.51	292	8
Nasenspray E	ratiopharm	NS	Xylometazoline	0.1	Ø	Ø	5.75	274	6
Otriven	Novartis	NS	Xylometazoline	0.1	Ø	BZC	6.35	289	4
Olynth	Pfizer	NS	Xylometazoline	0.1	Ø	BZC	6.24	279	2
Xylometazoline	Bundeswehr	NS	Xylometazoline	0.1	Ø	BZC	5.92	289	1
Nasic for children	Cassella med	NS	Xylometazoline	0.05	Dexpanthenol 5%	BZC	5.95	407	46
Otriven 0.05% OK	Novartis	NS	Xylometazoline	0.05	Ø	Ø	5.72	270	21
Olynth 0.05% OK	Pfizer	NS	Xylometazoline	0.05	Ø	Ø	5.86	295	15
Nasenspray K	ratiopharm	NS	Xylometazoline	0.05	Ø	Ø	6.00	300	11
Otriven 0.05%	Novartis	NS	Xylometazoline	0.05	Ø	BZC	6.35	282	7

Tonicity and pH were always determined by the same person using the same device and conventional standard procedures.

A statistical analysis was not sensible due to the large number of tests performed. However, differences in growth of >10% are always of statistical significance ($p < 0.05$, α=10, Wilcoxon-Mann-Whitney U test) and the significances are of descriptive nature.

Results

The results are shown in seven groups with respective tables and short comments on each. The highest concentration of a substance class is always listed first in the presentation. The substance tolerated best is shown first, measured by relative cell growth, and the comparable preparations follow based on their tolerance in descending order. Providing the absolute values with standard deviation is not helpful since the values are based on many different test series with different values for the respective controls. For this reason, the growth percentage in relation to the control is indicated. Anomalous matrix properties are highlighted; these mostly relate to osmolality since the pH range of almost all examined preparations was comparable.

We following common abbreviations were used: WP = without preservatives, A = adult preparation, C = child preparation, NS = nasal spray, ND = nasal drops, ED = eye drops, MD = medical device.

1. Decongestants, Xylometazoline (tab. **1**)
This market-dominating group, like the two next groups, that also include decongestant α sympathomimetics are mostly purchased directly from the pharmacy over-the-counter. For this reason, controlled and limiting ingestion cannot be achieved and the medicinally induced side effects caused by "too often, too much, too long" such as mucosal atrophy, rhinitis medicamentosa and allergic reactions must thus be taken particularly seriously. Thus, any improvement in tolerance is welcome.

Firstly, we generally noticed that the psf products are better tolerated than those preserved (fig. **1**) at each concentration level and, secondly, that the tolerance was dose-dependent. These differences are particularly visible in the example of the market-leading products Otriven® and Olynth®, both of which are available preserved and unpreserved at two different concentrations each. The excellent tolerance of

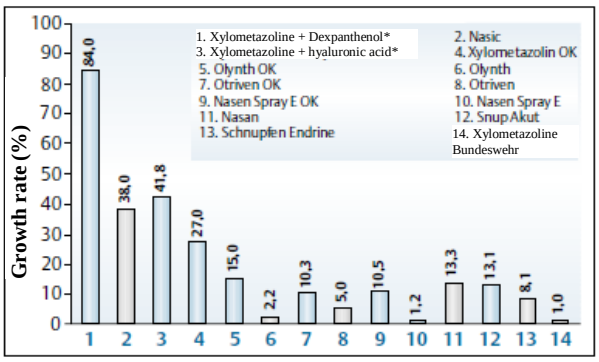

Fig. **1** Cell growth using the example of Xylometazoline (0.1 %, *new developments).

Tab. **2** Decongestants, Oxymetazoline

Trade name	*Manufacturer*	*Form*	*Active agent*	*Conc. (%)*	*Additives*	*Preservation*	*pH*	*Osmolality*	*Growth (%)*
Nasivin-Sanft	Merck	NS	Oxymetazoline	0.05	Ø	Ø	6.85	297	23
Nasivin	Merck	NS	Oxymetazoline	0.05	Ø	BZC	6.85	294	20
Sinex Schnupfen-Spray	Wick	NS	Oxymetazoline	0.05	Camphor, cineol, levomenthol	Chlorhexidine digluconate	5.53	213	3
Nasivin-Sanft	Merck	NS	Oxymetazoline	0.025	Ø	Ø	6.86	292	30
Nasivin	Merck	ND	Oxymetazoline	0.025	Ø	BZC	6.86	297	27
Nasivin-Sanft for babies	Merck	NS	Oxymetazoline	0.01	Ø	Ø	6.85	301	28

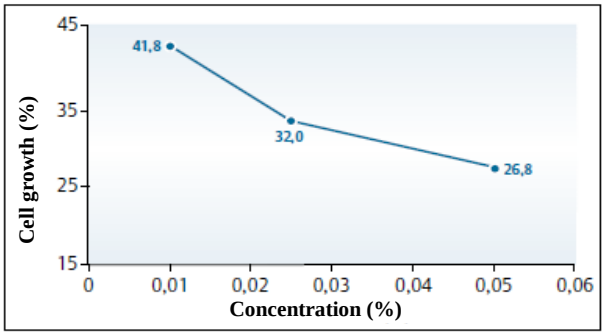

Fig. **2** Dependence of tolerance on active agent concentration using the example of Oxymetazoline (unpreserved).

Nasic® at both concentrations must be highlighted, even though the product is still preserved. This is achieved by the combination with Dexpanthenol. Our own examinations, in the meantime confirmed by clinical data, showed that Dexpanthenol is able to significantly lower the toxic potentials of Xylometazoline and BZC [10-12]. The preparation by the Bundeswehr is at the bottom of the rankings regarding tolerance. This should be emphasized since it is based on the standard approval by the FIDMD.

2. Decongestants, Oxymetazoline (tab. **2**)
The statements in the previous chapter regarding better tolerance when no preservative is used and the worse tolerance the higher the dose were also confirmed in the Oxymetazoline preparation (fig. **2**). The assertion sometimes made that tolerance is better due to half of the dose burden is no longer comprehensible in comparison to modern preservation-free Xylometazoline preparations.

The preparation Sinex® with the combination of camphor, cineol and levomenthol is not very adequate in comparison. Interestingly, it is the only one of the agents shown here that is not preserved with BZC. Chlorhexidine gluconate used instead is questionable from today's point of view [8]. The significantly hypo-osmolar galenic must also be considered an additional source of damage.

3. Decongestants, Tetryzoline, Tramazoline, Naphazoline, Dimetin (tab. **3**)
The remarks made for Xylometazoline and Oxymetazoline regarding the effect of preservation and concentration on tolerance also apply to this group. The combination with other substances, as in Rhinospray plus® and Dexa-Rhinospray® also seems questionable. Furthermore, the latter preparation has very hypertonic galenic with a very low pH.

Tab. **3** Decongestants, Tetryzoline, Tramazoline, Naphazoline, Dimetindene

Trade name	*Manufacturer*	*Form*	*Active agent*	*Conc. (%)*	*Additives*	*Preservation*	*pH*	*Osmolality*	*Growth (%)*
Tetrilin E	MIP	NS	Tetryzoline	0.1	Ø	Ø	6.03	310	60
Tetrilin K	MIP	NS	Tetryzoline	0.05	Ø	Ø	6.06	314	73
Yxin	Pfizer	ED	Tetryzoline	0.05	Ø	BZC	6.36	292	2
Rhinospray-sensitive	Boehringer Ing.	NS	Tramazoline	0.1	Ø	Ø	6.1	303	16
Dexa-Rhinospray	Mann	ND	Tramazoline	0.1	Dexamethasone 0.02%	BZC	4.62	760	2
Rhinospray plus	Boehringer Ing.	NS	Tramazoline	0.1	Cineol menthol Camphor	BZC	6.17	297	3
Privin	Novartis	NS	Naphazoline	0.1	Ø	BZC	5.1	310	18
Rhinex "S" for infants	Wernigerode	ND	Naphazoline	0.02	Ø	BZC	5.17	307	10
Fenistil nasal	Novartis	ND	Dimetindene mal.	0.1	Phenylephrine	BZC	5.09	345	72
Vibrocil	Novartis	NS	Dimetindene mal.	0.025	Phenylephrine 2.50 mg/g	BZC	6.43	306	9

Tab. **4** Antiallergics

Trade name	*Manufacturer*	*Form*	*Active agent*	*Conc. (%)*	*Additives*	*Preservation*	*pH*	*Osmolality*	*Growth (%)*
Allergo-COMOD	Ursapharm	NS	Cromoglycin	2	Ø	Ø	6.01	313	53
Cromohexal sanft	Hexal	NS	Cromoglycin	2	Ø	Ø	6.81	286	17
Cromohexal	Hexal	NS	Cromoglycin	2	Ø	BZC	6.72	287	4
DNCG nasal spray	STADA	NS	Cromoglycin	2	Ø	BZC	6.73	293	4
Allergocrom	Ursapharm	NS	Cromoglycin	2	Ø	BZC	5.45	290	3
Crom-Ophtal	Dr. Winzer	NS	Cromoglycin	2	Ø	BZC	5.40	291	3
Cromoglicin Heumann	Heumann	NS	Cromoglycin	2	Ø	BZC	6.77	287	2
Lomupren comp.	FISONS	NS	Cromoglycin	2	Xylo 0.025%	BZC	5.48	72	2
LOMUPREN	FISONS	NS	Cromoglycin	2	Ø	BZC	5.52	70	2
Cromo-ratiopharm	ratiopharm	NS	Cromoglycin	2	Ø	Ø	5.54	70	1
Nasacort	Aventis	NS	Triamcinolone	0.9	Ø	BZC	5.02	326	3
Livocab	Janssen	NS	Levocabastine	0.5	Ø	BZC	7.01	976	3
Irtan	Aventis	NS	Nedocromil	1	Ø	BZC	4.95	288	2
Nasonex	Essex	NS	Mometasone	0.5	Ø	BZC	4.60	316	1
Pulmicort Topinasal	Astra-Zeneca	NS	Budesonide	0.1	Ø	BZC	4.34	317	4
Beconase	Glaxo	NS	Beclomethasone	0.05	Ø	BZC	6.34	301	4
Flutide Nasal	Glaxo	NS	Fluticasone	0.05	Ø	BZC	6.41	334	2
Beclomet	Orion	NS	Beclomethasone	0.05	Ø	BZC	6.30	304	2

4. Antiallergics (tab. **4**)

Most of the preparations have Cromoglycin as the active ingredient. The tolerance of the substance as well as of the steroids can generally be described as unsatisfactory. It is worth mentioning that there were significant improvements in cell growth in the psf formulations in the comparisons between Allergocrom® and Allergo-COMOD® and between Cromohexal® and Cromohexal-sanft®. This is even more remarkable considering that Cromo-ratiopharm® (the market leader, by the way), which is also unpreserved, had the worst tolerance of all nasal sprays containing Cromoglycin. One can plainly see the reason for this when looking at the matrix properties (fig. **3**). The effect of pH and tonicity has been known since 1965 and this is outstandingly documented [13-14]. A tonicity of only 70 (!) mOsm/kg as in Cromo-ratiopharm® is simply not consistent with the physiology of an intact nasal mucosa. It is therefore surprising that this preparation was approved. The similar applies to the preparations Lomupren® and Lomupren comp®. The extremely high tonicity of Livocab® should give cause for caution during longer-term use.

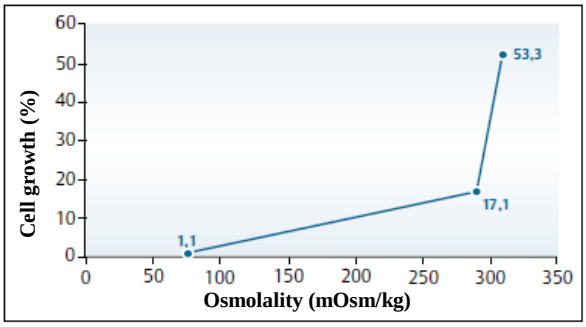

Fig. **3** Influence of osmolality on tolerance using the example of nasal sprays containing Cromoglycin.

Particularly the comparison between the two unpreserved preparations Cromohexal-sanft® and Cromo-ratiopharm® which are interchangeable due to the same indication, application, the same active ingredient, potency and the same dose according to the aut-idem rule shows the dangers of the aut-idem rule.

5. Medicines and care products with pharmaceutical additives, oils (tab. **5**)

Dexpanthenol is in the foreground in this group; we already alluded to its positive characteristics. In total, this group is well tolerated. Even the preserved Nasicur® shows good cell growth. It is remarkable that the attempt to sneak Dexpanthenol past the Medicinal Products Act as a medical device containing a pharmacologically ineffective concentration (for example Mar plus®) achieved neither the tolerance of saline solutions nor that of the pharmacologically effective Dexpanthenol preparations.

The example Kamillan supra® has tolerance that is low for medicines and care products. The application of alcohol extracts on the nose does not seem to make much sense due to a high danger of allergies, particularly for chamomile.

Emser Nasenspray® was the only saline solution added to this group since it is approved as a pharmaceutical and has shown a pharmacological effect.

We would like to expressly point out the three oils with their excellent tolerance which make them appear perfectly suitable for nasal tamponades and preparing prescriptions at the pharmacy.

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