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Benzalkonium chloride used as an excipient

Report published in support of the 'Questions and answers on benzalkonium chloride used as an excipient in medicinal products for human use' (EMA/CHMP/495737/2013)

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Executive summary

This document and the related questions and answers [29] have been written in the context of the revision of the Annex of the European Commission Guideline on 'Excipients in the labelling and package leaflet of medicinal products for human use' [2, 16].

Benzalkonium chloride is a quaternary ammonium compound used in pharmaceutical formulations as an antimicrobial preservative in applications similar to other cationic surfactants. Solutions containing benzalkonium chloride are active against wide range of bacteria, yeasts and fungi. Activity is more marked against Gram-positive than Gram-negative bacteria and minimal against bacterial endospores and acid-fast bacteria [31]. Benzalkonium chloride is usually non-irritating, non-sensitising and is well tolerated in the dilutions normally employed on the skin and mucous membranes. However benzalkonium chloride has been associated with adverse effects when used in some pharmaceutical formulations [32].

Introduction

Benzalkonium chloride appears to be the main preservative in ophthalmic preparations on the EU market. Approximately 74% of ophthalmic preparations have benzalkonium chloride as a preservative [28]. It is used as an antimicrobial preservative in numerous medicinal products for nasal route of administration and in many preparations for inhalation use authorised on EU markets. Only in limited cases the medicinal products that contain benzalkonium chloride are intended for cutaneous, oral, oromucosal, rectal, vaginal, auricular, intravenous/ subcutaneous and intramuscular/intralesional/intraarticular use.

Benzalkonium chloride has three main categories of use: as a biocide, a cationic surfactant, and phase transfer agent in the chemical industry. It is widely used in cosmetics, wet wipes, hand and surface sanitisers. Benzalkonium chloride was found to be an effective method of contraception [24]. Lozenges containing benzalkonium chloride are used for the treatment of superficial infections of the mouth and throat [23].

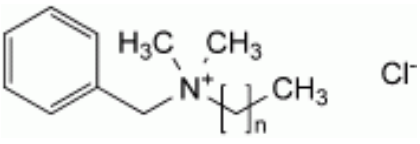
Scientific discussion

1. Characteristics

1.1. Category (function)

Benzalkonium chloride is a quaternary ammonium antiseptic and disinfectant with actions and uses similar to those of other cationic surfactants. It is also used as an antimicrobial preservative for pharmaceutical products [23].

1.2. Physico-chemical Properties



Benzalkonium chloride is a mixture of alkylbenzyldimethylammonium chlorides, the alkyl groups mainly having chain lengths of C12, C14 and C16. It is presented as a white or yellowish-white powder or

gelatinous, yellowish-white fragments. Benzalkonium chloride is hygroscopic. On heating it forms a clear molten mass [11].

1.3. Use in medicinal products

For most multidose aqueous nasal, ophthalmic and otic products, benzalkonium chloride is the preservative of choice [22]. Benzalkonium chloride is a preservative that is commonly used in ophthalmic preparations. It has been used in eye drops as a preservative since the 1950s and in 2011 it was still the most common preservative used in ophthalmic solutions. It is an effective bactericidal and fungicidal agent that helps to minimise the growth of organisms in multidose containers [26].

Only a few medicinal products containing benzalkonium chloride are intended for other routes of administrations i.e. cutaneous, oral, oromucosal, rectal, vaginal, auricular and parenteral use. Therefore the warnings will be developed only for the most commonly used routes of administration. For other routes not mentioned in the proposal for update, the information included in the package leaflet should follow the same principles.

2. Pharmaco-toxicological data

2.1. *Toxicity after single administration of benzalkonium chloride*

The acute toxicity (LD_{50}) of benzalkonium chloride after oral administration was approximately 344 mg/kg while LD_{50} after dermal administration was 3.56 mL/kg (80% ethanol/water solution) [10].

2.2. *Toxicity after repeated administration of benzalkonium chloride*

A number of oral repeated dose toxicity studies in rats (up to two years treatment), mice (up to 78 weeks treatment) and dogs (up to one year treatment) are available for benzalkonium chloride. At high doses (approximately 500 mg/kg/day) benzalkonium chloride was lethal to rats and mice due to local effects in the gastrointestinal tract. Below those high doses repeated-dose oral toxicity studies revealed no organ-specific toxicity. Responses in 90-day and chronic toxicity studies were limited to body weight changes and other general responses. The NOAELs from subchronic and chronic studies across species ranged from approximately 14 mg/kg/day in a chronic dog study to approximately 192 mg/kg/day in a subchronic mouse study [10].

LD_{50} of benzalkonium chloride in rats was reported as 14 mg/kg when administered intravenously [1, 8, 14].

In humans, an oral dose of 100–400 mg/kg [35] or a parenteral dose of 5–15 mg/kg [33, 39] is thought to be fatal.

2.3. *Local toxicity*

Ocular

Extensive literature is available examining the tolerability of benzalkonium chloride in ophthalmic medicinal products and most in vitro and in vivo studies suggest that benzalkonium chloride may induce ocular damage [36]. The in vivo studies have primarily been performed in rabbits, but several factors have to be taken into consideration when extrapolating from rabbits to humans. These include the presence of a nictitating membrane in the rabbit, less tear production, a thinner cornea, a larger conjunctival sac (resulting in greater drug accumulation and residence time) and a significantly lower blink rate in rabbits when compared to humans [36]. In a recent study in monkeys, ocular application

of 0.01% benzalkonium chloride twice daily for one year as well as 4 times per day for 39 weeks, did not give rise to changes in the ophthalmology and histopathology of the eyeball, eyelid and lacrimal gland [27]. Still, considering that the dilution of benzalkonium chloride is reduced in patients suffering from dry eyes, a risk for adverse ocular effects caused by benzalkonium chloride cannot be excluded in this patient group.

Nasal

In vitro studies in primary human nasal epithelial cell cultures suggest that benzalkonium chloride (0.001–0.05%) may cause ciliary beat stasis [21, 30, 18]. Intranasal administration of benzalkonium chloride (0.05 and 0.1%) 8 times during one day has been associated with nasal lesions in rats [20].

Auricular

Cochlear and middle ear toxicity of benzalkonium solution was evaluated in juvenile guinea pigs. Juvenile guinea pigs were instilled daily for 7 days with benzalkonium solution into the bullae and sacrificed on the 4th day. Tympanic membranes with 0.05% concentration of benzalkonium solution group were twice as thick compared to those of the 0.026% group (NS significant). Benzalkonium 0.026% showed no evidence of cochlear toxicity, mild middle ear mucosal thickening (similar to normal saline), and mild tympanic membrane thickening. The clinical significance of these changes remains unknown [4].

2.4. *Genotoxicity*

Benzalkonium chloride was non-mutagenic in *Salmonella typhimurium* strains TA1535, TA1537, TA102, TA98 and TA100 in the presence and absence of S9 [13, 10]. While benzalkonium chloride neither induced chromosomal aberrations in a Syrian hamster embryo cell assay [17], human lymphocytes in vitro [10], the unscheduled DNA synthesis assay in rat primary hepatocytes [10] nor in the mammalian cell forward mutation assay conducted in CHO cells [10], it was reported to be genotoxic in an *in vitro* micronucleus assay at concentration ≥ 1 mg/L [12]. Although erythropoiesis was affected, oral benzalkonium chloride (400 mg/kg) was non-genotoxic in an in vivo micronucleus assay performed in mice [10]. Hence, based on the weight of evidence, benzalkonium chloride is considered non-genotoxic.

2.5. *Carcinogenicity*

Benzalkonium chloride was non-carcinogenic in 78-week and 2-year dietary studies conducted in CD-1 mice and Sprague-Dawley rats, respectively [10]. In the mice study, doses up to approximately 259 mg/kg/day were administered, while the animals in the rat carcinogenicity received up to approximately 102 mg/kg/day.

In addition, it is reported that dermally applied benzalkonium chloride was non-carcinogenic in mice and rabbits treated twice weekly for 80 and 90 weeks, respectively [34, 13].

2.6. *Reproductive and developmental toxicity*

In a two-generation reproduction study conducted in Sprague-Dawley rats, 300, 1000 and 2000 ppm (approximately 145 mg/kg/day) benzalkonium chloride was administered via the diet (n=28/sex/group) [10]. Although parental toxicity was observed at 145 mg/kg/day, no effect on reproductive parameters was observed in the F0 and F1 generations. Moreover, perinatal toxicity in the form of reduced pup body weight was evident at the maximal dose of 145 mg/kg/day.

Oral (gavage) administration of 10, 30 and 100 mg/kg/day benzalkonium chloride to female Sprague-Dawley rats on gestation days 6 through 15 did not give rise to fetal visceral or skeletal malformations (25 pregnant females/group) [10]. In addition, no treatment-related effect on fetal body weight or incidences of external, visceral or skeletal malformations were observed following treatment of pregnant New Zealand White rabbits on gestation days 6 through 18 with 1, 3 and 9 mg/kg/day benzalkonium chloride (n=16 pregnant females/group) [10].

In a non-oral embryotoxicity study, Wistar rats received a single intravaginal administration of an aqueous solution of benzalkonium chloride 24 hours post-conception. Fetuses were obtained and examined for malformations on day 21 of gestation. At 100 mg/kg or more, abnormal bone development, increases in resorptions and reduced fetal growth were seen. At 50 mg/kg and above, a significant dose-related decrease in the number of live fetuses per litter and in litter size and weights was noted. In this study, NOELs of 50 and 25 mg/kg/day were retained for maternotoxicity and embryotoxicity, respectively [6].

Altogether, oral administration of benzalkonium chloride was not associated with reproductive or developmental toxicity in rats and rabbits. Embryotoxicity was observed when benzalkonium chloride was applied locally near the developing fetus (instillation into vagina). This could be due to a disturbance of the functional integrity of the placenta.

3. Pharmacokinetics

3.1. ADME (absorption, distribution, metabolism, elimination)

The distribution and disposition of benzalkonium chloride after a single oral dose of a benzalkonium chloride-containing product commercially available in Japan (Osvan 8) has been studied in rats [40, 38]. A dose of 2.5 ml/kg Osvan ® (250 mg/kg benzalkonium chloride) was administered to 30 fasted rats by stomach tube and blood samples were collected by cardiac puncture 1, 2, 4, 8 and 24 hours later (six rats/time point). The authors noted that concentrations of benzalkonium chloride in blood and tissues were substantially higher in animals that aspirated the benzalkonium chloride-containing product, suggesting that benzalkonium chloride is absorbed by the pulmonary blood vessels if inhaled. In animals that did not aspirate benzalkonium chloride, concentrations of benzalkonium chloride in blood and tissues were relatively low (0.01–1 µg/g), and did not increase over time, suggesting that only a small amount of orally administered benzalkonium chloride is absorbed through the gastrointestinal tract of rats. Because benzalkonium chloride is a large, positively charged molecule it is poorly absorbed and likely eliminated largely in faeces, similar to other quaternary ammonium compounds [3].

3.2. Interactions

Benzalkonium chloride is not suitable for use in eye drops containing local anaesthetics [23].

Incompatible with soaps and other anionic surfactants, citrates, iodides, nitrates, permanganates, salicylates, silver salts, tartrates, and zinc oxide and sulfate.

4. Clinical safety data

4.1. Ocular use

Average content of benzalkonium chloride used in medicinal products for ocular use is between 0.01 and 1 mg/ml.

Adverse effects

Benzalkonium chloride has been reported to cause punctate keratopathy and/or toxic ulcerative keratopathy. In addition, benzalkonium chloride may cause eye irritation and is known to discolour soft contact lenses.

Data on special populations

The children-specific data on effects of preservatives used in eye drops is scarce or completely missing. Where data is available, there is no difference in adverse event profile of benefit in children found compared to adults. Generally, however, eyes in children show a stronger reaction for a given stimulus than eyes of adults or elderly people. In addition, if eye drops cause stinging and pain (potentially due to preservatives) this may have an effect on the compliance in children [9].

4.2. Nasal use

Average content of benzalkonium chloride used in medicinal products for nasal use is between 0.02 and 0.33 mg/ml.

Adverse effects

The preclinical data show that benzalkonium chloride produces a concentration- and time-dependent toxic effect on cilia, including irreversible immobility, both in vitro and in vivo using rats in the animal model. The substance also induces histopathological changes in the nasal mucosa.

Data on special populations

Not available.

4.3. Auricular use

Average content of benzalkonium chloride used in medicinal products for auricular use is between 0.02 and 0.2 mg/ml.

Adverse effects

Ototoxicity can occur when benzalkonium chloride is applied to the ear [19].

Data on special populations

Not available.

4.4. Inhalation use

The content of benzalkonium chloride used in metered dose medicinal products for inhalation is about 2.21 µg/dose and in nebuliser solutions, suspensions or emulsions in concentrations between 0.1 and 0.2 mg/ml.

Adverse effects

Benzalkonium chloride used as a preservative in nebulised solutions of anti-asthma drugs has been reported to cause dose-related bronchoconstriction especially in asthmatic patients [7] and has been associated with the precipitation of respiratory arrest [5].

Data on special populations

Not available.

4.5. Cutaneous use

Average content of benzalkonium chloride used in medicinal products for cutaneous use is between 0.05 and 1mg/g.

Given the widespread exposure to the chemical, results of animal studies and limited human evidence of sensitisation only in relatively small proportions of individuals, benzalkonium chloride is not considered a sensitizer [25].

Data on special populations

There is no concern for developmental neurotoxicity resulting from exposure to Benzalkonium chloride because there is no evidence it will induce neurotoxic effects.

There is no evidence of increased susceptibility to the foetus following in utero exposure in the prenatal developmental toxicity studies or to the offspring when adults are exposed in the two-generation reproductive study; and the risk assessment does not underestimate the potential exposure for infants and children [37].

Animal studies do not indicate direct or indirect harmful effects with respect to pregnancy, embryonal/foetal development, parturition or postnatal development. The potential risk for humans is unknown.

4.6. Other routes of administration where very limited data is available

- Oral use
- Oromucosal use
- Rectal use
- Vaginal use
- Intravenous use/Subcutaneous use (NeoRecormon)
- Intramuscular use/intralesional use/intraarticular use (Celeston)

The inclusion of Benzalkonium chloride in the composition of the medicinal product is important information for patients, hence the quantity of preservative used has to be reflected in product information. However, it is incorporated in very few products intended for mentioned routes of administration and due to the limited data availability the warning ‘May cause local irritation’ was considered sufficient for oromucosal, rectal and vaginal routes.

5. Risk assessment and thresholds

Although some reports indicate an increased incidence of adverse effects after long term use of products containing benzalkonium chloride as a preservative it is not possible to recommend any safety limit for the general population of patients.

The children specific data on effects of preservatives used in medicinal products is scarce or missing. Where data is available, there is no difference in adverse event profile of benefit in children found compared to adults.

As most of the safety data come from toxicity studies in animals, human safety is limited by the need for extrapolation. Therefore, a clinical 'safe threshold' cannot be established.

The benefit-risk balance has to be established individually for each medicinal product during the marketing authorisation procedure, in line with the guideline on 'Excipients in the dossier for an application for marketing authorisation of a medicinal product' where it is stated that for each antimicrobial preservative, the application should contain a reason for inclusion and justification of the level of inclusion. The concentration of benzalkonium chloride in medicinal products should be optimised so that the minimum sufficient amount is present to achieve compliance with the Ph. Eur. test for Efficacy of Antimicrobial Preservation [15].

6. Updated information for the package leaflet

Name	Route of Administration	Threshold	Information for the Package Leaflet	Comments
Benzalkonium chloride	All routes of administration	Zero	This medicine contains x mg benzalkonium chloride in each <dosage unit><unit volume> <which is equivalent to x mg/<weight><volume>>.	
	Ocular	Zero	Benzalkonium chloride may be absorbed by soft contact lenses and may change the colour of the contact lenses. You should remove contact lenses before using this medicine and put them back 15 minutes afterwards. Benzalkonium chloride may also cause eye irritation, especially if you have dry eyes or disorders of the cornea (the clear layer at the front of the eye). If you feel abnormal eye sensation, stinging or pain in the eye after using this medicine, talk to your doctor.	From the limited data available, there is no difference in the adverse event profile in children compared to adults. Generally, however, eyes in children show a stronger reaction for a given stimulus than the adult eye. Irritation may have an effect on treatment adherence in children. Benzalkonium chloride has been reported to cause eye irritation, symptoms of dry eyes and may affect the tear film and corneal surface. Should be used with caution in dry eye patients and in patients where the cornea may be compromised. Patients should be monitored in case of prolonged use.
	Nasal	Zero	Benzalkonium chloride may cause irritation or swelling inside the nose, especially if used for a long time.	Long-term use may cause oedema of the nasal mucosa.

Name	Route of Administration	Threshold	Information for the Package Leaflet	Comments
	Inhalation	Zero	Benzalkonium chloride may cause wheezing and breathing difficulties (bronchospasm), especially if you have asthma.	
	Cutaneous	Zero	Benzalkonium chloride may irritate the skin. You should not apply this medicine to the breasts if you are breast feeding because the baby may take it in with your milk.	Use during pregnancy and lactation is not expected to be associated with harmful effects to the mother as cutaneous absorption of benzalkonium chloride is minimal. Not for application to mucosa.
	Oromucosal, rectal and vaginal	Zero	Benzalkonium chloride may cause local irritation.	

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