Preservative-free Triamcinolone Acetonide Suspension Developed for Intravitreal Injection

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ABSTRACT

Objectives: All commercially available triamcinolone acetonide (TACA) suspensions, used for intravitreal treatment, contain retinal toxic vehicles (e.g., benzyl alcohol, solubilizer). Our aim was to find a convenient and reproducible method to compound a completely preservative-free TACA suspension, adapted to the intraocular physiology, with consistent quality (i.e., proven sterility and stability, constant content and dose uniformity, defined particle size, and 1 year shelf life).

Methods: We evaluated two published (Membrane-filter, Centrifugation) and a newly developed method (Direct Suspending) to compound TACA suspensions for intravitreal injection. Parameters as TACA content (HPLC), particle size (microscopy and laser spectrometry), sterility, and bacterial endotoxins were assessed. Stability testing (at room temperature and 40°C) was performed: color and homogeneity (visually), particle size (microscopically), TACA content and dose uniformity (HPLC) were analyzed according to International Conference on Harmonisation guidelines.

Results: Contrary to the known methods, the direct suspending method is convenient, provides a TACA suspension, which fulfills all compendial requirements, and has a 2-year shelf life.

Conclusions: We developed a simple, reproducible method to compound stable, completely preservative-free TACA suspensions with a reasonable shelf-life, which enables to study the effect of intravitreal TACA—not biased by varying doses and toxic compounds or their residues.

INTRODUCTION

Although evidence for safety and efficacy have not been provided,[1,2] intravitreal injection of triamcinolone acetonide (TACA) is a common off-label therapy for various retinal diseases,[3] such as diffuse diabetic macular edema,[4,5] exudative age-related macular degeneration,[6–9] uveitis,[10] and macular edema from central retinal vein occlusion.[11,12]

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Despite a promising therapeutic outcome, pseudoendophthalmitis (incidence between 0.1% and 6.7%)[13–19] and endophthalmitis (incidence between 0.38% and 1.7%)[16,20] are unsolved problems related to injection procedure, the drug, or the vehicle. Retinal toxicity of TACA has been discussed in several experimental studies.[21–23]. McCuen[24] and Hida[25] were the first to associate ingredients of the vehicle with retinal toxicity, for example, benzyl alcohol (BA), a preservative in all commercially available TACA suspensions.

Recently, vehicle toxicity has been proven by Macky,[26] corresponding to the findings of Morrison[27] and Kai.[28] Various techniques[29–31] have been proposed to replace additives (BA, polysorbat 80) of commercially available TACA suspensions by a compatible vehicle. However, there are two problems: first, TACA suspensions produced from commercial products are never completely free of BA[32]; and second, the TACA content of such preparations is variable, resulting in inconsistent TACA dosing.[32–34]

Due to incomplete information about the different TACA products administered intravitreally (see Table 1) and connected parameters (i.e., adverse events, injection procedure, and needle size) results from clinical investigations are difficult to compare. Standardized samples of completely preservative-free TACA suspensions are urgently needed for clinical trials.[18,33,35]

Our aim was to find a technique to compound a completely preservative-free TACA suspension, adapted to the intraocular physiology, and with consistent quality. Proven sterility, content, and dose uniformity, defined particle size, and a 1-year shelf-life are preconditions to study safety and efficacy of intravitreal TACA therapy, not biased by toxic vehicle compounds or their residues.

METHODS

Three different methods to compound TACA suspensions (40 mg/mL) were evaluated: (1) the membrane-filter method, (2) the centrifugation method, and (3) direct suspending.

Membrane-filter method[30] (aseptic conditions)

The vehicle of Kenacort® A 40 syringes (Dermapharm AG, Hünenberg, Switzerland), which is equivalent to Volon A® and Kenalog®, was removed through a 5-μm membrane-filter (Acrodisc® 5 μm Supor® membrane; PALL Corporation, Newquay, UK). The TACA particles were washed three times with Ringer's solution Hartmann (B. Braun Medical AG, Emmenbrücke, Switzerland) by adding and discarding the washing solution through a three-way cock (Discofix®; B. Braun Medical AG). The washed particles were resuspended in the syringe in a sterile eye gel consisting of 2.5 mg/mL carmellose-sodium (BUFA b.v., Uitgeest, The Netherlands) in Ringer's solution Hartmann.

Centrifugation method[31] (aseptic conditions)

Kenacort A 40 (Dermapharm AG, Hünenberg, Switzerland) was centrifugated 2 min at 4000 rpm (Rotofix 32; Hettich Zentrifugen, Bäch, Switzerland) in sterile centrifuge tubes and the supernatant vehicle was rejected. TACA particles were washed two times with aliquots of Ringer's solution Hartmann (B. Braun Medical AG) by vortexing, centrifugation, and rejection of supernatant. The washed TACA particles were resuspended in a mixture of 25% Celluvisc® Unit Dose (Allergan AG, Lachen, Switzerland) and 75% Ringer's solution Hartmann and bottled in 5-mL injection vials. BA residues were analyzed in the second washing solution.

Direct suspending

Direct suspending is a new method that has been recently developed by our research group: micronized (95% of TACA particles <15 μm) TACA (Fagron GmbH & Co. KG, Barsbüttel, Germany) was directly suspended in Balanced Salt Solution (BSS; Cytosol Ophthalmics, medilas ag, Geroldswil, Switzerland) in a 250-mL injection vial containing a magnetic stir bar. The entire contents were autoclaved for 20 min (121°C, 2 bar). To adjust viscosity, a hyaluronic acid product (Vitrax® II; AMO Switzerland GmbH, Lachen, Switzerland) was mixed in under aseptic conditions. The suspension was aliquoted into 5-mL injection vials, for example, for 100 units of 1 mL TACA suspension (40 mg/mL) 5.2 g TACA, 120.25 mL BSS, and 9.75 mL Vitrax® II were used.

Analytics

We measured TACA content and dose uniformity of injection volumes (0.1 mL) of suspensions produced by the centrifugation and direct sus-

TABLE 1. PRESENTED INFORMATION ABOUT INTRAVITREAL INJECTED TRIAMCINOLONE ACETONIDE PRODUCTS IN RECENT CLINICAL INVESTIGATIONS

Author (year)	Commercial product with BA	Purification technique	Analytics of BA	Injection dose/vehicle	Analytics of content dose uniformity
Jonas et al.[38] (2001)	Volon A	Sedimentation	No	"Approximately 20 mg/ 0.2 mL Ringer's solution"	No
Beer et al.[37] (2003)	Kenalog 40	No	NA	4 mg/0.1 mL original vehicle	No
Gillies et al.[9] (2003)	Kenacort 40	No	NA	4 mg/0.1 mL original vehicle	No
Jonas et al.[38] (2003)	Yes	"Removing the solvent agent"	No	25 mg/0.2 mL Ringer's solution"	No
Jonas et al.[39] (2003)	Yes	"Most of the vehicle removed"	No	25 mg/0.2 mL Ringer's solution"	No
Jonas et al.[40] (2003)	Yes	Membrane-filter (not specified)	No	25 mg/0.2 mL Ringer's solution"	No
Moshfeghi et al.[20] (2003)	Kenalog	No	NA	4 mg/0.1 mL original vehicle	No
Nelson et al.[16] (2003)	Kenalog 40	No	NA	4 mg/0.1 mL original vehicle	No
Roth et al.[17] (2003)	Kenalog	No	NA	1 or 4 mg/0.1 mL original vehicle	No
Sutter and Gilles[14] (2003)	Kenalog A 40	No	NA	4 mg/0.1 mL original vehicle	No
Jonas et al.[41] (2004)	Volon A	Membrane-filter (5 μm)	No	25 mg/0.2 mL Ringer lactate solution	No
Jonas et al.[42] (2004)	Not specified	Not specified	Not specified	"Approximately 20 to 25 mg"/ vehicle and volume not specified	No
Klais and Spaide[43] (2004)	Not specified	Not specified	Not specified	4 mg/vehicle and volume not specified	No
Massin et al.[44] (2004)	Kenacort	No	NA	4 mg/0.1 mL original vehicle	No
Moshfeghi et al.[45] (2004)	Yes	No	NA	4 mg/0.1 mL original vehicle	No
Sutter et al.[35] (2004)	Kenacort 40	No	NA	4 mg/0.1 mL original vehicle	No
Chieh et al.[46] (2004)	Kenalog	No	NA	1 or 4 mg/volume original vehicle not specified	No
Jonas et al.[47] (2005)	Yes	"Most of the vehicle removed"	No	"About 20–25 mg/0.2 mL Ringer's solution"	No
Westfall et al.[13] (2005)	Kenalog	Sedimentation	No	Approximately 20 mg/0.1 original vehicle	No
Jonas et al.[48] (2006)	Yes	Membrane-filter (not specified)	0.0013 ± 0.0001 mg/0.1 mL	"Approximately 20 mg triamcinolone" / vehicle and volume not specified	No "The dosage eventually injected was approximately 23.8 ± 0.6 mg triamcinolone"
Quiram et al.[49] (2006)	Not specified	Not specified	Not specified	4 mg/0.1 mL vehicle not specified	Not specified
Thompson et al.[19] (2006)	Not specified	Not specified	Not specified	4 mg/0.1 mL vehicle not specified	Not specified

BA, benzyl alcohol.
NA, not applicable.

pending methods with HPLC (Hitachi LaChrome, Tokyo, Japan) Elite system with autosampler L-2200, samples dissolved in 60% methanol, pump L-2130, flow 1.0 mL/min isocratic, mobile phase acetonitrile/water 40%–60%, injection volume 10 μL, Waters XTerra (Waters Chromatography Ireland Ltd., Dublin) RP18 3.5 μm, 3.9 × 100 mm, column temperature 30°C, DAD L-2450 by 240 nm, EZ-Chrome Elite software, Scientific Software Inc., Pleasanton, CA). BA concentration was measured in the second washing water (centrifugation method) with HPLC (same parameters as for TACA, samples without dilution, injection volume 15 μL, DAD L-2450 by 258 nm, limit of quantification (LOQ) 6 μg/mL, limit of detection (LOD) 2 μg/mL). For the centrifugation and direct suspending methods, particle size was determined by microscopy (Olympus BX 50, Olympus Optical Co., Tokyo, Japan), according to British Pharmacopoeia 2007 (BP 2007) and particle-size distribution was determined by laser spectrometry (polydisperse model 2PAD; MastersizerX, Malvern Instruments Ltd., Malvern, UK). Particle size was assessed before and after autoclaving in suspensions compounded by the Direct Suspension method. Sterility and bacterial endotoxins were assessed in suspensions compounded by the Centrifugation and Direct Suspending methods, according to Pharmacopoeia Europea 5 (Ph. Eur. 5).

For TACA suspension compounded by the direct suspending method, samples stored at room temperature (0, 3, 6, 9, and 12 months) and 40°C (1, 2, 3, 6, and 12 months) were tested for stability: color and homogeneity (visually), particle size (microscopically), TACA content and purity (HPLC) were analyzed according to International Conference on Harmonisation (ICH) guidelines.

RESULTS

The membrane-filter method is an ad hoc production technique only suitable for sporadic preparation, but not to satisfy a great demand of TACA suspensions. Standardization of the multiple-step procedure is not possible. Therefore, this production method does not comply with quality requirements based on the guidelines for Good Manufacturing Practice (GMP).

The suspension compounded with the centrifugation method had contents of 3.7–4.5 mg (range, 93%–112%) in 0.1-mL injection doses. BA

concentration in the second washing solution was detectable (LOD; 2 μg/mL) but below LOQ (6 μg/mL). The median TACA particle size was 28 μm. Some particles were as large as 100 μm, which does not comply with the requirements of BP 2007 (Fig. 1). According to Ph. Eur. 5, the suspension was sterile and bacterial endotoxins were <5 EU/mL.

The simple method of direct suspending has a low risk for microbiologic contamination and is practical for batch production. The TACA dose uniformity in 0.1-mL injection doses were between 3.8 and 4.0 mg (range, 95%–100%). Autoclaving did not change the particle size of TACA crystals. The median particle size was 11 μm and no particle was larger than 38 μm, corresponding to BP 2007 (Fig. 1). According to Ph. Eur. 5, the
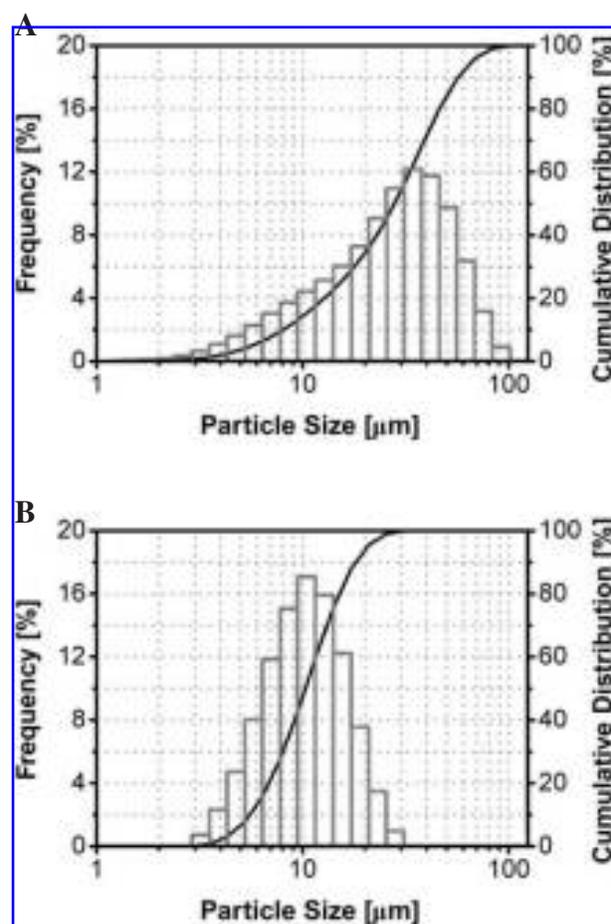
FIG. 1. Particle size of triamcinolone acetonide (TACA) suspensions. Diagrams show particle-size distribution analysis of TACA suspensions, compounded by the centrifugation (**A**) and direct suspending methods (**B**). Columns represent frequency of particle classes [%] (left ordinate), and the sigmoid curve represents cumulative distribution [%] (right ordinate) of particle size.

suspension was sterile and bacterial endotoxins were <5 EU/mL.

TACA content of suspensions, stored at room temperature and 40°C for 12 months, fulfilled the requirements of ICH guidelines for stability testing. TACA particles were homogenously resuspendable. Color and particle size were unchanged.

DISCUSSION

We evaluated two published[30,31] and a newly developed method (direct suspending) to compound TACA suspensions for intravitreal injection. Our aim was to find a convenient compounding method for TACA suspensions, completely preservative-free, with defined particle size, proven sterility and stability, constant injection doses, and a 1-year shelf life.

To withdraw constant doses of a suspended compound, and to satisfy the requirements of Ph. Eur. 5 for suspension stability, TACA particles have to be embedded in a viscous vehicle. Preparations for intravitreal administration must also be adapted to the intraocular physiology. TACA doses are most constant when the ophthalmologist withdraws the TACA suspension directly before the injection from a shaken injection vial with a defined TACA content.

Prefilled syringes are not suitable for long-term storage and, therefore, ad hoc prepared ready to use syringes have a short shelf-life (<24 h). Despite using extra viscous vehicles, sedimentation of TACA particles in ready-to-use syringes cannot be completely prevented. Furthermore, sedimented TACA particles in the syringe conus are difficult to resuspend. For exact dosing, an additional amount of TACA suspension (as large as the volume of the needle) is required. These factors lead to varying doses of TACA, not corresponding with the required range of 90%–110% (BP 2007).

The membrane-filter method was not practical at all from a pharmaceutical point of view. TACA particles, which are smaller than the pore size of the filter, get lost. The product is not completely preservative free and cannot be analyzed before application. The membrane-filter method failed our aims.

The centrifugation method provided a TACA suspension with constant doses of TACA due to the adjusted viscosity. The major part of the vehicle could be removed. Preliminary investigations of all washing waters (to determine the optimal numbers of washing cycles by summarizing removed BA and lost TACA) showed that it is adequate to use the last washing water for BA analytics. Our results correspond with those of Garcia-Arumi et al.[32] Most of BA, which is soluble in the original vehicle, could be removed, but there is still BA left. The presence of large TACA particles (up to 100 μm) is not a result of the removal technique. We also detected particles of this size in commercially available TACA suspensions. Such large particles did not satisfy the requirements of BP 2007 for the Triamcinolone Acetonide injection (rarely exceed 40 μm in diameter) and could be the reason for clogging of 30-gauge needles[13,14] that have a minimal inner diameter of 125 μm.

TABLE 2.   EVALUATION AND COMPARISON OF THREE COMPOUNDING METHODS AND THE RESULTING PRODUCT CHARACTERISTICS OF TRIAMCINOLONE ACETONIDE SUSPENSIONS

Characteristics	Membrane-filter method	Centrifugation method	Direct suspending method
Adapted to intraocular physiology	n.a.	n.a.	High
Total absence of preservatives	No	No	Yes
Practicability of technique	n.a.	Medium	High
Few numbers of production steps	n.a.	Medium	High
Minimized risk of microbiologic contamination	n.a.	Medium	High
Batch production	n.a.	Medium	High
Possibility of sterile testing	No	Yes	Yes
Shelf life of product	24 h	Not assessed	2 years[a]
Low operating expenditure	n.a.	Medium	High
Meets pharmacopoeial requirements	No	No	Yes

n.a., not applicable.
[a]Extrapolated shelf life.

Production with the centrifugation method is possible, but the many production steps add a high risk of microbiologic contamination. In addition, particle size is not sufficient for BP 2007 (Fig. 1). Despite a large input of material, time, and analytics, no completely preservative-free product, fulfilling all pharmacopoeial requirements and our aims, resulted.

The new direct suspending method enables hospital pharmacies to compound a sterile, completely preservative-free TACA suspension with a defined particle size and constant dose uniformity. The suspension is developed for intravitreal use and is composed of constituents used in ophthalmic surgery. BSS is added for suspending the TACA particles and hyaluronic acid (component of the vitreous body) to adjust viscosity. We used a nonantigenic and nonpyrogenic hyaluronic acid with an average molecular weight of 550,000–800,000 Daltons, storable at room temperature.

Solid TACA is very stable. The melting point is above 274°C. Only 0.004% ± 0.002% (40 μg/mL) is soluble in isotonic saline (determined at 23 and 37°C)[50] and available for possible degradation. Particle size remained unchanged after autoclaving. Additionally, we confirmed stability of autoclaved TACA by a stability indicating HPLC method following the ICH guideline for Q3B (specification of the guideline) impurities in new drug products.[51] Furthermore, no decrease in content was detected during stability testing. Stability testing, according to ICH guidelines, at accelerated conditions (40°C, 1 year) and storage conditions (room temperature, 1 year) resulted in an extrapolated 2-year shelf-life and 1-year shelf life, respectively. An adequate shelf-life provides the possibility for quality testing and permanent availability of the suspension for the ophthalmologist.

The direct suspending method allows pharmacists to compound suspensions of different TACA contents (e.g., 4, 8, or 20 mg/0.1 mL) and viscosities, according to individual prescriptions.

Robinson[52] showed, in a pharmacokinetic investigation in rabbits, that vitreous half-life of TACA does not depend on particle size. Therefore, to prevent the clogging of needles, we used micronized TACA (95% of TACA particles <15 μm). Furthermore, thin needles cause minimal lesions in contrast to surgically implantation of drug delivery systems by 20-gauge needles where sutures to close the wounds are needed.[53]

The proposed method is simple and saves time, work, and costs, compared to the other techniques. The microbiologic contamination risk is minimal, and the detection of residues of additives is no longer necessary.

Table 2 summarizes the most important characteristics to select a production technique for a TACA suspension for intravitreal use, based on our experiences with the membrane-filter, centrifugation, and direct suspending methods.

To compare the data of different clinical studies, detailed information is needed not only about the indication, adverse effects, and injection procedure, but also about pharmaceutical parameters of the intravitreal injected TACA suspension (i.e., commercial product, removed additives and amount of residues, or complete preservative free).

We developed the new compounding method in collaboration with the Department of Ophthalmology of the University Hospital Basel (Basel, Switzerland). Since January 2006, the newly developed TACA suspension has been used for more than 150 intravitreal injections. The convenient handling of the presented TACA suspension for intravitreal injection meets the clinical needs.

CONCLUSIONS

Hospital pharmacists, ophthalmologists, and patients benefit from a proven sterile TACA suspension, adapted to the intraocular physiology, with constant dose uniformity. By describing the technique of direct suspending to compound a TACA suspension, we provide the basis to study the safety and efficacy of intravitreal TACA therapy, one that is not biased by varying doses and toxic vehicle compounds or their residues.

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