Triamcinolone acetonide without solvents

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\textbf{Abstract}

Objective: The purification of commercially prepared triamcinolone acetonide is important in order to avoid the potential toxic side-effects of the solvent benzyl alcohol. We present a new technique for preparation of pure triamcinolone acetonide by dissolving the powder in sterile distilled water with no additional solvents. As the triamcinolone powder is relatively insoluble in water, we describe the sterile method used for the preparation and control of this suspension.

\textit{Materials and methods}: The triamcinolone acetonide is prepared in our hospital pharmacy, under optimum sterile conditions, and then packaged in a primary vial, sealed and sterilized in an autoclave at 121 °C. This vial contains an individual dose of 4 mg/0.1 ml.

\textit{Results}: A final dose for an intravitreal administration of 3.77 mg/0.1 ml triamcinolone acetonide was obtained using high pressure liquid chromatography (HPLC). The chemical, physical and microbiological stability allows the solution to be kept at a temperature of 2–8 °C for 6 months.

\textit{Conclusions}: A rapid method is presented for preparing triamcinolone acetonide in pure state without preservatives in a concentration near the standard dose and under optimum sterile conditions.

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Introduction

Numerous clinical studies demonstrate the usefulness of intravitreal triamcinolone for treating choroidal neovascularization, proliferative diabetic retinopathy, diabetic macular edema, central retinal vein occlusion, pseudophakic macular edema, macular edema in uveitis and for intra-surgery visualization of the posterior hyaloids.

Triamcinolone acetonide is not specifically available for intraocular use and ophthalmologists have to use commercial preparation (Trigon Depot 40 mg/ml, Bristol-Myers Squibb SL), which contains benzyl alcohol as excipients and carboxy methyl cellulose and polysorbate 80 as suspending agents. Several research papers have related benzyl and not corticoid crystals with toxicity in ocular tissues.

Various triamcinolone purification techniques have been described in order to reduce the benzyl alcohol present in the commercial preparation.

A new method for preparing pure acetonide triamcinolone is described. The preparation is formulated as a sterile suspension for intravitreal application and is solvent-free. Due to its low solubility in water, we describe how to prepare and control the quality of 4% triamcinolone suspension contained in a primary vial from which individual doses are obtained for each aseptic administration at a dose of 4 mg/0.1 ml.

Materials and methods

The Pharmacy Service of our hospital has pure acetonide triamcinolone (C24H21F06 Roig Farma batch 04K16FO). The method requires that all the material that enters in contact with the triamcinolone must be previously cleaned and sterile. Firstly, 400 mg of powdered triamcinolone is weighed in electronic precision scales (Sartorius), and pulverized for 5 min in a mortar. Subsequently it is deposited in a precipitate cup with 10 ml of pyrogen distilled water, heated at 40 °C for 10 min in a magnetic shaker to enhance the highest solubility. In a horizontal laminar flow box (Gelaire), a 15 ml Guinama sterile closed topaz glass vial is decapsulated and the previously prepared suspension is added without leaving any residue in the precipitate cup. The vial is closed with a sterile rubber top and encapsulated with a vial closing device (S. Doménech), for subsequent sterilization in autoclave at 121 °C. The vial is then submitted to quality control and adequately identified, applying physical, chemical and biological quality control assessing pH, state of the suspension, checking the absence of germs in culture and determination of the quality and stability of triamcinolone by means of high-resolution liquid chromatography (HPLC Hitachi). All the above tests are carried out on the same sample after 24 h and 6 months of the preparation. In this way, a sterile primary source of triamcinolone is obtained (Fig. 1), on the basis of which the dose of 0.1 ml/4 mg to be administered is obtained in maximum sterility conditions (horizontal laminar flow box), placed in a 1 mm syringe, covering the cone with a sterile rubber top. The maximum quantified use and preservation time of the triamcinolone vial is of 6 months and of each individual syringe of 24 h (Fig. 2).

Fig. 1 – Capsulated primary vial.

Fig. 2 – Individual triamcinolone dose.
Results

The results obtained in the physical, chemical and biological controls in the steam sterilization processes have been favorable, thus ensuring the vial sterilization process.

The physical quantity controls on the vial contents have proven the absence of caking, with the suspension adequately dispersing up to the 6-month limit. The pH remained at 6.

Microbiological cultures confirmed the absence of aerobic and anaerobic germs and fungi with staining and culture during the essay period.

The quantitative determination of purity of the triamcinolone in the primary vial, determined by means of HPLC, yields a value of 3.77 mg/0.1 ml corresponding to 94.5% 24 h after preparation and of 3.66 mg/0.1 ml at month 6 after the preparation, thus representing 91.5% of purity, in comparison to the value of 4 mg/0.1 ml of theorectical concentration which would represent 100% purity.

Intravitreal triamcinolone injections have been applied for 12 months for several retinal diseases including diabetic macular edema, exudative macular degeneration, macular edema secondary to central venous occlusion and pseudophakic macular edema, with efficacy results similar to those published in other series.

No cases of infectious endophthalmitis or sterile endophthalmitis have occurred, with ocular hypertension being the most frequent complication which responded in all cases with the administration of hypotenive topical treatment.

Discussion

The toxicity of the intravitreal triamcinolone vehicle has been assessed in various clinical and experimental papers.\textsuperscript{11-16} It was involved in a toxicity syndrome in premature newborn babies after being used in intravenous solutions.\textsuperscript{3} For this reason it was banned by the FDA in 1982.

In an experimental paper, Morrison et al. demonstrated that benzyl alcohol at a concentration 3.3 times higher to that present in a 4 mg/0.1 ml injection of intravitreal triamcinolone acetone obtained from the commercial preparation is toxic for rabbit eyes, producing changes in photoreceptors and pigmneary epithelium cells analyzed with electronic microscopy, as well as localized vitritis. Said authors recommend maximum clarification of the injection vehicle because toxic concentration is very close to that usually administered through the intravitreal pathway,\textsuperscript{15} above all if the volume to be injected exceeds 0.1 ml.

Various methods for purifying triamcinolone acetone have been published in endeavors to reduce or eliminate the concentration of benzyl alcohol present in the commercial preparation. Jonas et al. described a multiple step separation method requiring approximately 25 min.\textsuperscript{4} Hernaez-Ortega et al. described a gradient centrifugation method consisting in a centrifuge in the preparation at 3000 rpm during 5 min and extracting the supernatant and substituting it with 0.9 ml of BSS.\textsuperscript{16} García-Arumí et al.\textsuperscript{19} carried out a comparative study of the various preparation techniques, including filtering techniques with 5 μm and 0.22 μm filters as well as long filtering techniques (sedimentation and centrifugation), and demonstrated that all of these techniques produce a statistically significant reduction of the benzyl alcohol concentration of up to one tenth when compared against the original suspension. These results are opposed to those of Rodríguez-Coleman et al.,\textsuperscript{20} who found that the filtering techniques concentrate benzyl alcohol. However, the concentration of triamcinolone cannot be very precise and for this reason they recommend the centrifugation process, as the concentrations it achieves are similar to the expected ones.

The method for preparing triamcinolone acetone from the pure active principle for intravitreal injection described in this paper avoids the problems associated to benzyl alcohol. The preparation is made in maximum sterility conditions by the Hospital Pharmacy Service, diminishing the possibility of samples contamination due to excessive manipulation.

The final obtained concentrations of triamcinolone are very close to the expected concentration (94.5%), avoiding losses which occur with other described methods.

The preparation method is reliable and safe for intravitreal use. The physical, chemical and microbiological stability of the vial, which is kept at a temperature range of 2 °C and 8 °C, is maintained for 6 months.

The pharmacological and cost study derived from the raw materials and the technological manipulation thereof is as follows:

- 42.12 euros for a multidose vial: this includes 400 mg of triamcinolone acetone powder, one topaz glass vial with rubber top and metal capsule, pH assessment strip, solvent (10 ml API bleb), sterilization, microbiological culture and labor.
- The cost of each dose (in a 1 ml syringe) corresponding to the parts of raw material obtained from the multidose vial (0.1 ml) added to the cost of the 1 ml syringe and the sterile polymer top adds up to 0.63 euros.

Conflict of interests

No conflict of interest has been declared by the authors.

REFERENCES