Comparison of different techniques for purification of triamcinolone acetonide suspension for intravitreal use

J Garcia-Arumi, A Boixadera, J Giralt, V Martinez-Castillo, F Gomez-Ulla, B Corcostegui, E García-Arumi

MATERIAL AND METHODS

Different techniques applied to reduce the solvent agent benzyl alcohol (9.9 mg/ml) from a commercially prepared triamcinolone acetonide suspension (Trigon Depot; 40 mg/ml, Bristol-Myers Squibb SL, New York, NY, USA) were evaluated.

Non-filter techniques

Two different non-filter techniques were used: sedimentation and centrifugation. Commercially prepared triamcinolone acetonide suspension was allowed to sediment overnight and 0.9 ml of the supernatant were extracted with a tuberculin syringe. The pellet was resuspended with 0.9 or 0.5 ml of balanced salt solution (BSS) (Alcon Laboratories Inc, Texas, USA). In the other non-filter technique, the commercial suspension was centrifuged at 3000 x g for 5 minutes; 0.9 ml of the supernatant were extracted with a tuberculin syringe and the pellet resuspended with 1 ml of BSS.

Filter techniques

The triamcinolone had been prepared by taking 0.62 ml from the commercial triamcinolone acetonide ampule, following the technique described by Jonas. The extracted volume was placed in a tuberculin syringe (1 ml) filled with Ringer lactate solution (Laboratorios Grifols SA, Barcelona, Spain). Two different syringe driven filter units were evaluated: a Millipore filter (Millex-GS 0.22 μm pore size; Millipore Co, Cork, Ireland) and a Pall filter (Versapor Membrane 5 μm pore size, Pall Corporation, MI, USA). The selected filter was placed on the top of the syringe and most of the contents of the syringe were pressed through the filter, with the triamcinolone crystals remaining in the syringe. The syringe was refilled with Ringer lactate solution and the same procedure was repeated three times. At the end, 0.2 ml of the solution were left in the syringe.

Triamcinolone acetonide and benzyl alcohol quantification

Triamcinolone acetonide and benzyl alcohol quantification was performed using a modification of the Matysova method. Ten μl of the solution obtained by the different techniques and 10 μl of the commercial suspension were diluted with 3 ml of acetonitrile and water (40/60 v/v). Ten μl of the resulting dilution were injected into the HPLC system (Alliance 2695 Waters Corporation, Milford, MA, USA). Reversed phase HPLC was performed at room temperature. The mobile phase was a mixture of acetonitrile and water (40/60 v/v). The column used was a Symmetry C18 (5 μm) (150x4.6 mm ID) (Waters Corporation), and a guardcolumn Symmetry C4 (5 μm) (20x3.9 mm ID), (Waters Corporation). The flow rate was 0.8 ml/min and the eluate was monitored at a 240 nm with a Waters 2487 detector (Waters Corporation) and quantified based on comparison with a calibration curve of triamcinolone acetonide.
Techniques for purification of triamcinolone for intravitreal use

Identification of peaks was based on the comparison of retention times of compounds in standard solutions. Data were processed with Millenium software (Waters Corporation).

**Statistical analyses**

The non-parametric Kruskal-Wallis test and Mann-Whitney U test were performed for comparisons among the different techniques. Results are expressed as mean (standard deviation). Statistical significance was set at p<0.05. Statistics were performed using the Statgraphics software package (Manugistics Inc, Herndon, VA, USA).

**RESULTS**

The benzyl alcohol concentration obtained using the different methods is shown in figure 1. Benzyl alcohol concentration was decreased significantly in all the techniques used compared with the original commercial suspension (p<0.05). No statistically significant differences were found among the different methods used to reduce the solvent agent; the reduction was about one tenth of its original concentration.

We were also interested in studying the effect of these procedures on triamcinolone acetonide concentration. Triamcinolone acetonide concentration obtained with the different methods is shown in figure 2. The centrifugation method showed no differences versus the original commercial solution. However, two techniques (filter technique using a Millex-GS 0.22 µm pore size membrane and sedimentation technique with resuspension with 0.5 ml BBS (half of the original volume)) presented significantly higher triamcinolone acetonide concentration (an increase of about 1.5) than in the original commercial solution (p<0.05). Moreover, a significantly decreased triamcinolone acetonide concentration (around 75% of the original concentration) was obtained with the filter technique using a Versapor Membrane 5 µm pore size membrane and with the sedimentation technique and resuspension with 0.9 ml BBS (original volume) (p<0.05). The coefficient of variation was 7% for the commercial solution and lower than 15% for the different methods.

**DISCUSSION**

Intravitreal triamcinolone acetonide has increasingly been used in pilot studies for the treatment of oedematous, proliferative, and neovascular diseases. Some investigators have found that the vehicle, not the crystalline cortisone itself, could be toxic for intraocular tissue. Benzyl alcohol has been implicated in cases of sterile endophthalmitis after intravitreal injection, and in aseptic meningitis following intrathecal administration. As a result of the possible toxicity of benzyl alcohol, various techniques employed to reduce the solvent agent have been reported.

Recently, Rodriguez-Coleman et al., after using the different published techniques to reduce the solvent agent and quantifying triamcinolone and benzyl alcohol by HPLC, surprisingly observed that the mean level of benzyl alcohol, instead of being reduced, increased three or fourfold. They explained the higher level of benzyl alcohol by its affinity for a lipophilic environment being in the triamcinolone fraction when they attempted to concentrate the triamcinolone crystals. Our results are opposite to those of Rodriguez-Coleman et al., as the benzyl alcohol concentration was decreased significantly in all the techniques used compared with the original commercial suspension (p<0.05), with the reduction being about one tenth of its original concentration. No statistically significant differences were found among the different methods.

However, the final triamcinolone doses differed notably depending on the cleansing technique employed. With the filter technique published by Jonas et al using a Versapor 5 µm filter, the final mean dose of triamcinolone was 34 mg/ml (6.8 mg in 0.2 ml instead of the 20–25 mg in 0.2 ml reported). Our results are very similar to those observed by Rodriguez-Coleman et al (6.5 mg in 0.2 ml). Using a smaller pore size (0.22 µm), the final concentration increased (56 mg/ml), but was still 45% of the original concentration. The possible explanation for the drug loss is that part of the triamcinolone was entrapped in the filter and more easily when the pore was larger. These considerably lower than...
predicted doses of triamcinolone may lead to further evaluation of the published articles using this filter cleansing technique regarding the effectiveness, half life, and secondary effects of the intravitreally injected triamcinolone.

With the centrifugation and sedimentation techniques, the final dose was close to their target, with a 25% reduction using the latter technique. This loss of active drug may be corrected by resuspending the pellet with a lower BSS volume, as observed in our study with 0.5 ml of saline solution.

Standardisation of the aspirated volume of the vehicle from the commercially available suspension (we recommend 0.9 ml) would be interesting in sedimentation and centrifugation methods to obtain the lowest benzyl alcohol concentration, although it would influence little the final triamcinolone concentration.

In conclusion we recommend, as the best way of administering the drug, a non-filtering technique, concretely the centrifugation at 3000 x g for 5 minutes with extraction of 0.9 ml of the supernatant and the pellet resuspension with 0.9 ml of BSS, as the final triamcinolone concentration remains almost unchanged.

Authors’ affiliations
J García-Arumí, A Boixadera, J Giralt, V Martinez-Castillo, E García-Arumí, Vall d’Hebrón Hospital, Universidad Autónoma de Barcelona, Barcelona, Spain
J García-Arumí, B Corcostegui, Instituto de Microcirugía Ocular, Barcelona, Spain
F Gomez-Ulla, The Provincial de Conxo Hospital, Santiago de Compostela, Spain

Competing interests: none declared

Correspondence to: Dr J García Arumí, Instituto de Microcirugía Ocular, C/Munner n° 10, 08022, Barcelona, Spain; 17215jga@comb.es

Accepted for publication 1 April 2005

REFERENCES
Comparison of different techniques for purification of triamcinolone acetonide suspension for intravitreal use

J García-Arumí, A Boixadera, J Giralt, V Martinez-Castillo, F Gomez-Ulla, B Corcostegui and E García-Arumí

Br J Ophthalmol 2005 89: 1112-1114
doi: 10.1136/bjo.2005.067744

Updated information and services can be found at:
http://bjo.bmj.com/content/89/9/1112

These include:

References
This article cites 11 articles, 2 of which you can access for free at:
http://bjo.bmj.com/content/89/9/1112#BIBL

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/