INTRAVITREAL MICRONIZED TRIAMCINOLONE VERSUS TRIAMCINOLONE ACETONIDE: A CLINICAL AND MORPHOLOGICAL COMPARATIVE STUDY

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SUMMARY

Nowadays many authors suggest the use of intravitreal triamcinolone acetonide (TA) for the treatment of vitreoretinal diseases, although it can be associated with a high risk of local toxicity.

In order to develop a safier injection for clinical use, the purpose of our study was to evaluate the *in situ* safety of two different triamcinolone preparations, a commercially available TA and a micronized triamcinolone.

The experiments were performed on 18 adult male age-matched New Zealand rabbits.
The clinical examination included funduscopy with an indirect ophthalmoscope and intraocular pressure (IOP) measurement.

At the end of the clinical observations, the animals were sacrified and the eyes enucleated and processed for the morphological evaluation.

In our study the main side effect observed has been the IOP elevation in the group injected with triamcinolone acetonide. In addition, in the TA-injected group, one eye has been enucleated following an endophthalmitis.

Our study evidences that doses as low as 4 mg of triamcinolone acetonide injected in the rabbit vitreous may have a local toxic effect, in terms of IOP elevation, endophthalmitis occurrence and changes in the retinal morphology. In contrast, the micronized triamcinolone injection shows a less toxic effect *in situ*, thus suggesting the alternative use of this more reliable preparation which seems to be safier for a clinical use.

INTRODUCTION

The pathological proliferation of intraocular tissue such as vascular cells in eyes with ischaemic retinopathies has remained an important problem in clinical ophthalmology. This abnormal proliferation is often accompanied and stimulated by intraocular inflammation, which complicates the pathology course. Moreover, defects in the blood-retina barrier due to capillary leakage, with accumulation of fluid in the intraretinal and subretinal spaces of the macula, are other major causes of impaired vision. The reasons for such conditions are various and include diabetic rethinopathy, retinal vein occlusions and uveitis, to mention only a few.

The corticosteroids are known to reduce intraocular inflammation and tighten the capillary walls, and, depending on the concentration, to suppress cell proliferation. For these reasons steroids have been widely used in the treatment of many ocular diseases, applied topically as drops, given sistemically or injected into the subconjunctival or sub-Tenon space. Often, however, the intraocular concentration of corticosteroids is too low or the systemic side effects are too pronounced to allow an extended treatment.

In order to overcome these limitations, Machemer et al. have suggested the intravitreal application of a crystalline form of steroid that provides intraocularly available drug for a considerably longer period (1).

In particular, the intravitreal injection of triamcinolone acetonide (TA) (9α-fluoro-16α-hydroxyprednisolone) was first proposed in an experimental study by Tano et al. (2). Today, many authors suggest the use of triamcinolone for the treatment of vitreoretinal diseases (3-5).

Although TA is only intermediate in its anti-inflammatory action compared with other corticosteroids, it has the advantage of a longer absorption time than soluble steroids (6-8).
Drug delivery into the posterior segment of the eye alleviates some issues, such as bioavailability, and can lead to the improvement of its therapeutic effects by increasing the intraocular drug concentration (9,10). However, it can be associated with a high risk of local toxicity. The side-effects of ocular corticosteroid therapy are well known and include elevation of intraocular pressure, caractogenesis, and infectious or sterile ophthalmitis (11-14).

It may be significant that triamcinolone acetonide is usually not found in the serum shortly after its intravitreal application, suggesting that major systemic side-effects are not very probable (15).

As concerns the IOP elevation, previous studies have suggested that the TA may obstruct the trabecular meshwork of the anterior chamber, thus blocking the aqueous humor outflow (16,17).

Regarding the risk of an infectious endophtalmitis, it may partially depend on the setting of the intravitreal injection itself. In fact previous studies suggest that if the injection is performed under sterile conditions, the risk may be less (18).

A sterile endophtalmitis has been reported to occur after an intravitreal TA injection (19,20). It is possible that the solvent agent of TA is responsible for the sterile intraocular inflammation after the injection. In fact the recent cases of sterile endophthalmitis using Kenalog® (i.e. the most commonly used formulation for intravitreal use) have thought to be related to the additives, because pure TA have been shown to be safe in animal studies (14,21).

Regarding the potential retinal toxicity of intravitreal TA, some studies have shown the safety of TA for intreavitreal injection (21,22), while others demonstrated that TA may have toxic effects to retinal pigmented epithelium and glial cells (23,24).

Since the intravitreal TA is increasingly being used, it's important to be aware of the risks associated with this procedure. In order to develop a more reliable, stable and safe injection for clinical use, the aim of the present study was to evaluate the *in situ* safety of the intravitreal injection of two different triamcinolone preparations, a commercially available TA and a micronized triamcinolone.

MATERIALS AND METHODS

The experiments were performed on 18 adult male age-matched albino rabbits of the New Zealand strain (purchased from Charles River Laboratories Italia, Calco, Lecco, Italy), weighing 1.5 ± 0.2 kg. They were housed two per cage under standard conditions (21 °C, 12 h light/12 h dark cycle, food and water *ad libitum*) for an adaptation period of 1 week after which they were admitted to the experimental procedures. Four rabbits served as controls and were injected with saline solution.

Since ocular pigmentation may indirectly protect against the toxic effects of the drug, albino rabbits were chosen for our study (25).

All animals were handled in accordance with ARVO Statement for the use of Animals in Ophtalmic and Vision Research and examined with this clinical schedule: baseline, 15 days , 30 days, 45 days, because the triamcinolone acetonide it has been shown to remain in the vitreous for an average time of 41 days after the intravitreal injection (26). This clinical examination included: funduscopy with an indirect ophthalmoscope and intraocular pressure measurement.

At the end of the clinical observations, the animals were sacrified with an intravenous injection of pentobarbitone sodium. Immediately after death, eyes were enucleated and processed for the histologic evaluation.

Fundus examination

Phenylephrine 10% eye drops combined with tropicamide 1% were used to dilate the pupil. Fundus examination was made in all animals by indirect binocular ophthalmoscopy, using a 20 D Goldman lens.

The evaluation of the vitreous opacity were carried out using the binocular indirect ophthalmoscopy score (BIO score), following the International Uveitis Study Group (IUSG) guide lines (27) and the standardization of uveitis nomenclature (SUN) working group (28).

Surgical procedure

Rabbits were prepared to the intravitreal injection with ofloxacine eye drops (Exocin® eye drops, Allergan Ltd, Marlow, UK) for 6 times/day for 3 days before the surgical procedure, and 4 days after.

The triamcinolone acetonide (Kenalog-40®, Bristol-Myers-Squibb Company, Princeton, NJ) was prepared in a syringe with a 27 Gauge (G) needle. A volume of 0.1 ml was injected in the eyes (4 mg dose).

The micronized triamcinolone (TM) (IVT®, SOOFT Italia s.r.l., Montegiorgio-AP, Italy) was supplied in disposable preservative-free syringes with 27 G needle. A volume of 0.1 ml was injected in the eyes (4 mg dose).

Before the injection, the eyes received a single use topical oxybuprocaine eye drops (OXB) (Novesine®, Novartis Pharma, Switzerland). The surgeon washed the hands thoroughly and weared sterile gloves. Povidone iodide at 5% was instilled on the ocular surface 3 minutes prior to injection. Periocular skin, eyelid margins, and eyelashes were cleaned with 10% povidone iodine. An eyelid speculum was inserted, ensuring that it was well positioned underneath the eyelids to direct the eyelashes away from the field.

The injection was performed infero-temporally at 3mm from the limbus.

Intraocular Pressure (IOP) Measurement

IOP was checked using applanation method by tonopen (Tono-Pen XL, Applanation tonometer, Medtronic Ophthalmics); rabbits were topically anesthetized using OXB.

Scanning electron microscopy (SEM)

The enucleated eyes were cut in half at the equator, fixed in 2% glutaraldeyde in 0.1 M cacodylate buffer (pH 7.4), post-fixed in 1% osmium tetroxide, dehydrated in increasing ethanol concentrations and then CPD-dried. They were then mounted on stubs and gold-sputtered. The observations were carried out with a Philips 505 scanning electron microscope.

Statistical analysis

The statistical analysis of the results was carried out in a blind method by a person not involved in the study, using the software SAS (version 8.1). The type of intravitreal injection in the eyes has been randomized for each rabbit using the program in the SPSS database (SPSS, Chicago, Illinois, USA). Student's t test, Mann-Whitney U test, and χ^2 test were used as appropriate. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Clinical findings

A marked progression of cataract and corneal abnormalities were not observed in rabbits that received both acetonide and micronized triamcinolone.

In the group that received the triamcinolone acetonide, a rabbit had the eye enucleated following an endophthalmitis after 4 days.

Regarding the IOP (**Fig.1**), there were no significant differences at any follow-up time in the group treated with micronized triamcinolone with respect to the baseline, whereas in the group injected with triamcinolone acetonide the IOP showed a statistically significant increase at 15 days ($p < 0.05$), 30 days ($p < 0.001$) and 45 days ($p < 0.001$) (**Fig. 1**).

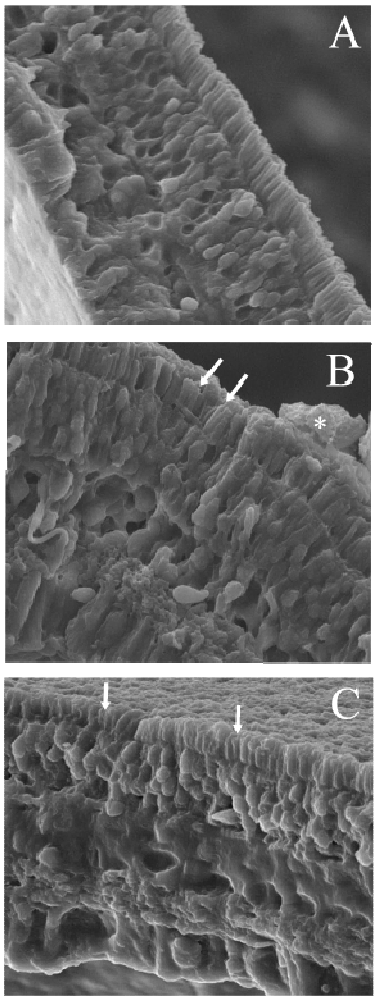

Fig. 1. Intraocular pressure (IOP) measurement in the triamcinolone acetonide (TA) treated-eyes and in the micronized triamcinolone (TM) injected-eyes. Means ± S.D. are shown at baseline and at 15, 30 and 45 days of follow-up.

Immediately after the injection (at “day 0”), the BIO score was 0+ in 83.3% (n=15) and 1+ in 16.7% (n=3) of the eyes treated with TM. On the contrary, in the group that received the TA there were 22.2% (n=4) of the eyes with 4+, 38.9% (n=7) with 3+, 33.3% (n=6) with 2+ and 5.6% (n=1) with 1+ (**Fig. 2**). At 45 days of follow-up, the BIO score was 0+ in all the eyes treated with TM, while in the group injected with TA the BIO score was: 5,9% (n=1) of the eyes with 4+, 17,6% (n=3) with 3+, 23,5% (n=4) with 2+, 35,4% (n=6) with 1+, 17,6% (n=3) with 0+.

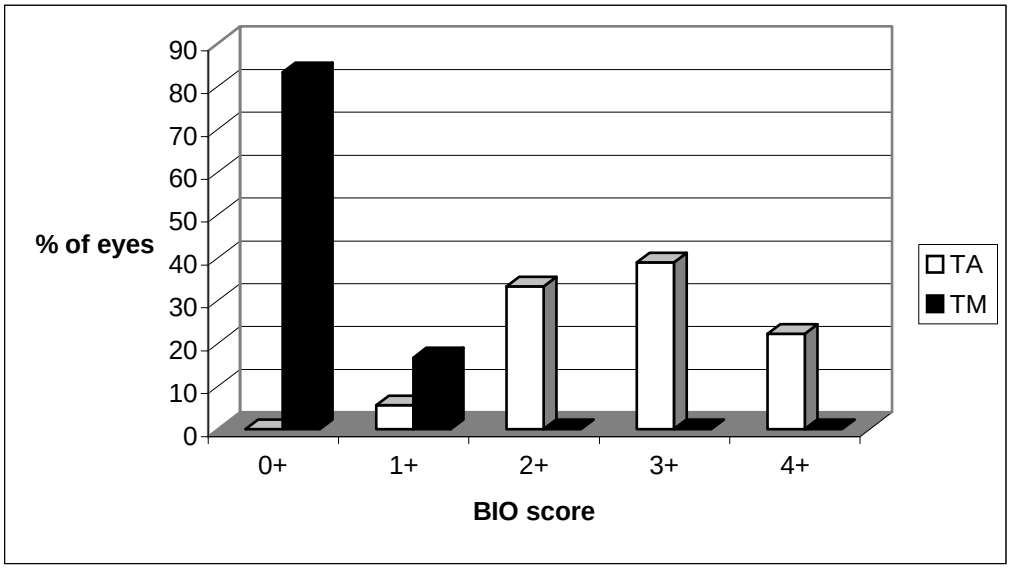

Fig. 2. BIO score values in the eyes treated with triamcinolone acetonide (TA) and in the micronized triamcinolone (TM) injected-eyes at “day 0”.

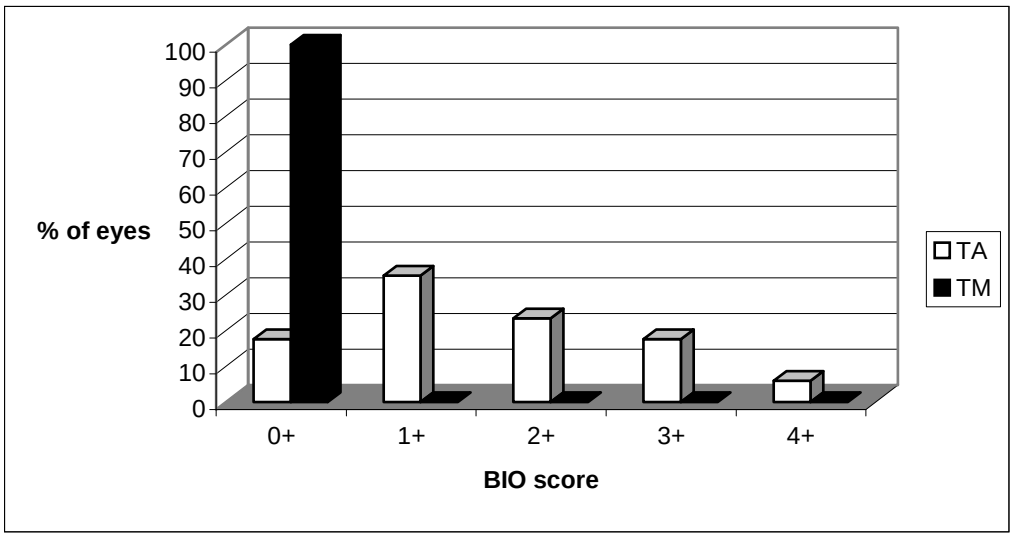

Fig. 3. BIO score values in the eyes treated with triamcinolone acetonide (TA) and in the micronized triamcinolone (TM) injected-eyes at “day 45” of follow-up. One eye was enucleated and not considered in the TA group.

Morphological findings

Morphological analysis of the rabbit eyes, evaluated by scanning electron microscopy, is reported in **figures 4, 5, and 6.**

In particular, **figure 4 and 5** show the morphology of the retinal vessels. In the eyes treated with TA it is possible to notice the presence of caliber irregularity and narrowing, together with multifocal areas of haemorrhage. In addition we can observe the deposition of drug particles on the retinal surface (**Fig 4, sections A and B**). On the contrary, the retinal vessels of the eyes injected with the micronized triamcinolone have a more regular caliber, without haemorrhages. We can also notice the absence of triamcinolone deposits (**Fig. 5, sections A and B**).

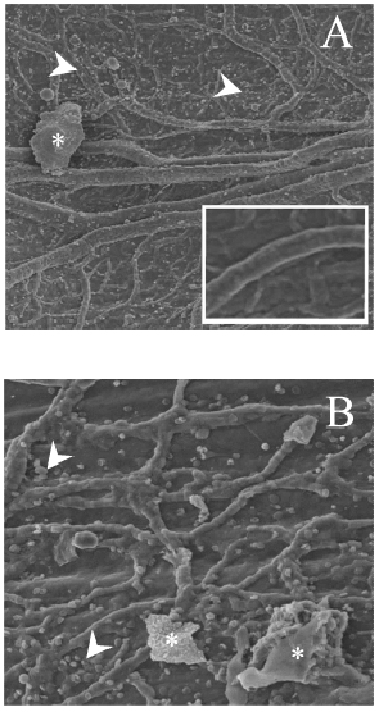

Fig. 4. Scanning electron micrographs of the TA-treated rabbit eyes, showing the morphology of the retinal vessels. Caliber irregularity and an apparent reduction in vessel diameter are present, together with areas of haemorrhage (arrow heads) more evident at higher magnification (section B). Several TA particles are noticeable on the retinal surface (*). The inset shows the morphological feature of control retinal vessels (Magnifications: section A = 100 x; section B = 250 x; inset = 200 x)

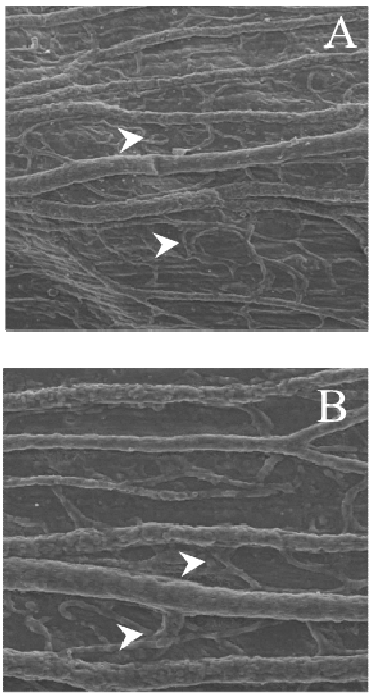

Fig. 5. Scanning electron micrographs of the eyes injected with the micronized triamcinolone, showing the morphological feature of the retinal vessels. Some loops and anastomosis are evident (arrow heads), while haemorrages are undetectable. (Magnifications: section A = 100 x; section B = 250 x).

Regarding the lens, none of the samples seems to have any abnormalities demonstrated by scanning electron microscopy (data not shown).

As concerns the retinal layers the SEM examination shows an apparent shortening of the photoreceptor outer segments both in the group treated with TA and in the eyes injected with the micronized triamcinolone with respect to the controls. Moreover, in the TA-treated eyes we can observe the presence of amorphous cellular debris on the outer retina surface (**Figure 6, sections A, B, and C**).

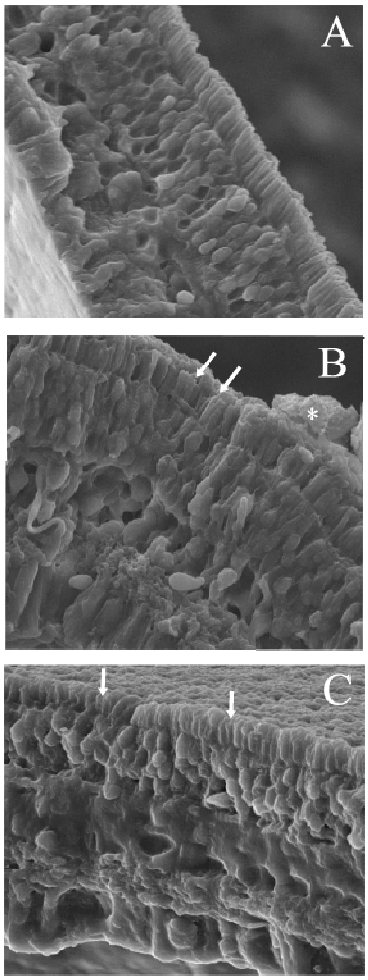

Fig. 6. Scanning electron micrographs of the retinal layers, showing an apparent shortening of the photoreceptor outer segments (arrows) both in the group treated with TA (section B) and in the eyes injected with the micronized triamcinolone (section C) with respect to the controls (section A). In the group treated with TA we can also observe the presence of cellular debris on the outer retina surface (*) (section B) (Magnifications: sections A, B, and C = 800 x).

DISCUSSION

Intravitreal corticosteroid injection is a recent therapeutic modality that is increasingly being used for the treatment of various edematous and neovascular intraocular conditions. These include the more common forms of edema, such as macular edema secondary to diabetes, central retinal vein occlusion and uveitis.

Previous experimental investigations and clinical studies on patients with exudative age-related macular degeneration as well as on subjects affected by other edematous and neovascular retinal diseases have suggested that the antiangiogenic and antiedematous effect of intravitreal triamcinolone acetonide may be used to treat such patients. (1, 29-32).
However, like other corticosteroids, TA can elicit adverse events or toxic reactions within the eye, such as elevation of IOP, progression of cataract, endophthalmitis and possibly retinal toxicity (13,33,16,34). The transient nature of intravitreal TA injection positive effects, together with the frequency of complications may limit the clinical utility of triamcinolone treatment.

Recent reports have shown a widespread use of intravitreal triamcinolone acetonide injections despite lack of convincing safety data. Altough it is generally believed that TA is relatively safe and non-toxic when administered intraocularly or in *in vitro* conditions (21), the cytotoxicity should be characterized if TA is used with confidence in the clinical practice.

For these reasons we have conducted an investigation on rabbits to determine whether there are differences in the clinical and morphological responses to the intravitreal injections of two triamcinolone preparations, a commercial triamcinolone acetonide and a micronized triamcinolone which have the advantage of stability and sterility. The micronized triamcinolone was in fact provided in single-use preservative-free syringes.

Toxic preservatives in the vehicles of commercially available corticosteorids have been suggested as possible contributors to proliferative vitreoretinopathy (35). Sterile endophthalmitis too has been reported to occur after an intravitreal injection of a corticosteroid, and the toxic effects of commercial vehicle preservatives has been proposed as a possible cause (14).

In our study the main side effect observed has been the IOP elevation in the group injected with triamcinolone acetonide, in agreement with previous studies (18). Moreover in the group treated with triamcinolone acetonide, one eye has been enucleated following an endophthalmitis.

Despite the disinfection of the vials before use and although the injection is performed under sterile conditions, utilizing multiuse vials may increase the risk of endophthalmitis.

Another important issue is the BIO score. Our BIO score analysis at “day 0” has evidenced that the eyes injected with TA had a more significant vitreous torbidity, while the eyes treated with TM showed a better distribution of the drug in the vitreous cavity. In addiction, this difference

has remained stable at 45 days of follow-up. These data could represent a basis for a future application in the clinical practice because the patients may have less complaints about vitreous opacities.

Concerning our morphological observations, we have evidenced, in the eyes treated with TA, the presence of drug deposits (i.e. triamcinolone crystals) on the retinal surface, in agreement with previous findings (17).

Regarding the retinal vessels, our SEM analysis have shown the presence of narrowing and haemorrhages in the eyes injected with triamcinolone acetonide, whereas in the micronized triamcinolone-treated eyes the vessels seemed to have a more regular caliber and we have evidenced the absence of haemorrhages.

Previous animal studies have shown that glucocorticoids reduce blood-retinal barrier permeability and that this effect is accompained by an apparent reduction in retinal vessel diameter (36).

As concerns the retinal haemorrage, it has been previously suggested that it may be caused by the vehicle used in the preparation of intravitreal TA (37).

Finally, regarding the retinal layers, our results have suggested the presence of a damage to the outer retina after a single intravitreal injection of triamcinolone acetonide, in terms of photoreceptor injury leading to an accumulation of cellular debris, in agreement with previous studies (38). In the eyes treated with the micronized triamcinolone our observations have evidenced a slight reduction in the outer segment length, without the accumulation of amorphous cellular particles.

In conclusion, this study has demonstrated that doses as low as 4 mg of triamcinolone acetonide injected in the rabbit vitreous may have a local toxic effect, in terms of IOP elevation, endophthalmitis occurrence and changes in the retinal morphology. In contrast, the micronized triamcinolone injection has shown a less toxic effect *in situ* and, in addition, have the quality of stability and sterility.

For these reasons we suggest the alternative use of this more reliable preparation which seems to be safier for a clinical use.

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The authors have no financial interest in any drugs mentioned in this study.

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