ABSTRACT

Purpose: Concern has been raised about the retinal toxicity of vital dyes. We designed a prospective study to determine the possible toxicity of trypan-blue (TB) in macular hole surgery with TB-assisted internal limiting membrane (ILM) peeling through the performance of electroretinograms (ERGs).

Methods: Patients diagnosed with a macular hole underwent ophthalmological evaluation prior to surgery and at 6 months follow-up. All patients underwent vitrectomy and ILM-staining under air. All phakic patients underwent phacoemulsification and IOL implantation simultaneously. There were two study groups: In group 1, ILM-staining was performed with 0.06% TB, while in group 2 the procedure was performed with 0.15% TB. Preoperative ERG recordings were measured in the week prior to surgery. Postoperative ERGs were measured 3 to 6 months after surgery. The ERG data between eyes with macular hole and fellow eyes were compared in the pre- and post-operative stages. Visual acuity (VA) changes in both groups were evaluated.

Results: Nine patients were recruited in each group. VA improved significantly in both groups.

RESUMEN

Objetivo: Existen dudas sobre la posible toxicidad retiniana de los colorantes vitales. Se diseñó un estudio prospectivo para determinar la posible toxicidad del azul tripán (AT) en la cirugía del agujero macular (AM) con peleado de la membrana limitante interna (MLI) tras su tinción con AT mediante la realización de electrorretinogramas (ERG).

Métodos: Pacientes con AM fueron evaluados antes y 6 meses después de la cirugía. A todos se les realizó una vitrectomía con tinción de la MLI bajo aire. Los pacientes fáquicos fueron sometidos simultáneamente a una facoemulsificación con implante de lente intraocular. Se crearon dos grupos de estudio: en el grupo 1, la tinción de la MLI se realizó con AT al 0,06% y en el grupo 2 con AT al 0,15%. El ERG preoperatorio se realizó en la semana previa a la cirugía, el postoperatorio entre 3 y 6 meses después. Se procedió a la comparación de los datos del ERG entre el ojo con afecto y el adelfo en las fases pre- y postoperatoria. También se evaluaron los cambios en agudeza visual (AV) en ambos grupos.

Resultados: Se reclutaron nueve pacientes en cada uno de los grupos. La AV mejoró de forma signifi-
with more than 65% of patients improving more than 2 lines. There were no statistical differences in VA gain between groups or in ERG values between affected and fellow eyes.

**Conclusion:** No significant retinal toxicity of TB staining could be clinically detected (Arch Soc Esp Oftalmol 2008; 83: 659-664).

**Key words:** Electroretinography, macular hole surgery, side-effects, trypan-blue toxicity, vital dyes.

**INTRODUCTION**

A number of studies have described the usefulness of peeling the internal limiting membrane (ILM) in macular hole surgery (1-3). Due to the technical difficulty of this intervention, dyes have been developed to assist the surgeon. Indocyanine Green (IG) dyes ILM *in vivo* and facilitates its extraction. High rates of anatomic closure of the macular hole have been obtained in this manner (4, 5). However, the literature also describes cases of functional and anatomic damage after dyeing the ILM with IG (6-10).

Trypan blue (TB) is a vital dye which produces a softer coloring than that obtained with IG, sufficient to allow its identification during the peeling maneuver. Several groups have obtained good functional results after vitrectomy and peeling of ILM with TB dye, including significant visual acuity improvements without detectable perimetric defects (11,12).

After the publication of several studies which gave rise to doubts about the possible toxicity of TB (13,14), we decided to carry out a prospective study performing electro-retinograms (ERG) in macular hole patients to determine whether TB could be toxic for the retina. Preliminary studies performed with TB at 0.06% did not exhibit significant changes in ERG vis-à-vis the contralateral eye (15). As at present higher concentrations of TB are being utilized, the study was expanded to include the safety of ILM peeling after dyeing with TB at 0.15 % in macular hole treatments.

**SUBJECTS, MATERIAL AND METHODS**

A non-randomized, interventional and prospective study was designed with patients diagnosed with macular hole in stages 2-4 of Gass classification with less than one year of development. The exclusion criteria were in the presence of other ocular pathologies which could produce alterations in the ERG, excepting lens opacity both in the affected eye as well as in the contralateral one.

All the patients underwent a complete ophthalmological exploration prior to surgery, including visual acuity (VA) and biomicroscopy of the anterior and posterior segments.

A pars plana vitrectomy was performed on all patients under retrobulbar anesthesia. After removing the posterior hyaloid, a fluid-air exchange was carried out. The internal limiting membrane was dyed exceeding the temporal arcades with 0.1 ml of TB for two minutes. Asymmetric Tano pincers were used for peeling the ILM. At the end of the surgery C3F8 at 8% was introduced in the vitreous cavity, asking the patient to maintain a head downwards position for two weeks. The study was made in two stages. In the first, TB at 0.06% was utilized for dyeing the ILM (Group 1) and in the second stage, TB at 0.15% was utilized (Group 2). In all the phakic patients, a phacoemulsification procedure was performed with intra-ocular lens implant in the same operation as the vitrectomy.

The patients were assessed at day 1 and 7 and month 1, 3 and 6 after surgery. The macular hole was deemed to be closed if it had disappeared com-

**Conclusión:** No se detectaron signos de toxicidad retiniana tras la tinción con AT.

**Palabras clave:** Electroretinografía, cirugía del agujero macular, colorantes vitales, efectos secundarios, toxicidad por azul tripán.
pletely in the slit lamp exploration and the Watzke-Allen test gave negative results. Otherwise, it was classified as open in accordance with the criteria of the Moorfields macular hole study group (16).

The preop ERG was made the week before the surgery. The post-op ERG was made between three and six months after the vitrectomy. The complete field ERG (registering rods, combination of rods and cones, calls, oscillatory potentials and flicker at 30 Hz) was made according to the criteria of the International Society for Clinical Electrophysiology of Vision (ISCEV) utilizing a computer controlled PRIMUS 2.5 electric diagnostic system (17). The ERG data of the eye with the macular hole were compared with the other eye before and after surgery.

Non-parametric tests were utilized for statistical analysis. The ratio between pre-and post-stop VA was analyzed with the Wilcoxon test for paired samples. The ERG values for the affected eye and the other one were compared with the Mann-Whitney test.

The statistical analysis was made with the SPSS 8.0 software (SPSS Inc., Chicago, Illinois, USA).

RESULTS

Nine patients were included in each group. The patient data are seen in table I. And the macular hole was closed after surgery in eight patients of group 1 (88.9%) and in all the patients of group 2, without this difference being statistically significant. The mean pre-op VA was of 0.08 in Group 1 (standard deviation [SD] 0.06) and 0.09 in Group 2 (SD 0.07). In both groups the VA improved significantly post-op, to 0.38 in Group 1 (SD 0.23) and 0.35 in Group 2 (SD 0.15), p=0.012 for both groups. No statistically significant differences were found in the pre- or post-op VA between both groups. VA improved in two or more lines in seven of nine patients in group 1 (77.8%) and in six of nine patients in Group 2 (66.7%).

The ERG values are shown in tables II and III. No significant differences existed between the eyes with macular hole and the contralateral eyes before or after the surgery.

DISCUSSION

Vital dyes have been utilized in macular hole surgery to dye the ILM due to the difficulty involved in its identification and complete removal. Indocyanine Green was initially the most widely utilized dye, with positive anatomic and functional results published (4,5). However, several authors have described the appearance of adverse effects possibly related to the use of IG which gave rise to unfavor-
able visual results (7,8). Permanent campimetric defects have been documented, even with a concentration of 0.5% and exposure times under one minute (8-10) as well as the development of optic nerve atrophy (9,10). Also, the atrophy of the pigmentary epithelium of the retina has been related to the absence of visual improvement after surgery (7).

Trypan blue is a vital dye with lower diffusion than IG because it produces only a weak dye of the ILM at the available concentration (Vision Blue®, 0.06%). However, if injected under air, the ILM dyeing improves. The new commercial presentation, Membrane Blue® at 0.15%, allows for a better dye. This study was made in two stages in order to compare the risk/benefit ratio of the various TB concentrations. In the first, TB was utilized at 0.06% to facilitate the elimination of the ILM, and in the second the concentration was of 0.15%.

Experimental studies have not detected damages in the RPE after the utilization of TB, in contrast with the time- and dosage-dependent toxicity of IG (18). Positive visual results have been communicat-
ed after macular hole surgery with ILM peeling with TB, with visual acuity improvement and the absence of campimetric defects. No changes have been described in the funduscopic area (11,12) although some studies have suggested possible toxicity in the neurosensory retina (13,14).

It has been proved that vitrectomy with C3F8 accelerates the formation of cataracts (1,2). In addition, the opacification of the lens reduces the amplitude of ERG waves a and B and extends implicit times as a consequence of the reduction of light reaching photoreceptors. Accordingly, it was decided to perform phacoemulsification together with vitrectomy with IOL implant in all phakic patients.

The ERG was utilized in animal studies to detect possible toxic effects of vital dyes. Thus, Veckeneer et al did not detect changes in the main ERG values in rabbit eyes after exposure to TB (19).

In the ERG pattern, the P50 component which is utilized to assess the macular function evolves in parallel to VA after surgery. This could be an obstacle to the detection of macular damage. For this rea-

| Table II. Pre-and Post-op ERG values in group 1 (Trypan blue at 0.06%) in eyes with macular hole (MH) and contralateral eyes (control) |
|---|---|---|---|---|---|---|---|---|---|
|   | Pre-op |   | Post-op |   |
|   | Amplitude | Implicit time (ms) | Amplitude | Implicit time (ms) |
| Group 1 (0.06%) | MH | Control | p | MH | Control | p | MH | Control | p |
| Rods | 154.83 | 177.9 | 0.400 | 103.21 | 101.2 | 0.628 | 174.81 | 175.26 | 0.989 | 98.26 | 97.82 | 0.942 |
| Combined cones-rods Wave a | 238.9 | 249.37 | 0.674 | 24.15 | 23.77 | 0.542 | 209.36 | 225.53 | 0.501 | 24.76 | 23.60 | 0.271 |
| Wave b | 490.52 | 496.25 | 0.850 | 47.46 | 53.71 | 0.274 | 488.41 | 524.00 | 0.435 | 47.92 | 46.96 | 0.796 |
| OpsPS | 68.82 | 79.87 | 0.253 | 26.20 | 25.94 | 0.658 | 73.47 | 83.15 | 0.652 | 26.70 | 26.05 | 0.431 |
| Cones | 129.30 | 134.63 | 0.811 | 30.67 | 29.58 | 0.147 | 151.83 | 130.71 | 0.435 | 30.84 | 30.62 | 0.773 |
| 30 Hz flicker | 101.75 | 118.70 | 0.266 | 27.43 | 28.72 | 0.352 | 104.00 | 104.00 | 0.980 | 28.67 | 26.50 | 0.239 |

| Table III. Pre-and Post-op ERG values in group 1 (Trypan blue at 0.25%) in eyes with macular hole (MH) and contralateral eyes (control) |
|---|---|---|---|---|---|---|---|---|---|
|   | Pre-op |   | Post-op |   |
|   | Amplitude | Implicit time (ms) | Amplitude | Implicit time (ms) |
| Group 1 (0.06%) | MH | Control | p | MH | Control | p | MH | Control | p |
| Rods | 152.20 | 137.3 | 0.500 | 88.30 | 90.4 | 0.50 | 126.3 | 118.4 | 0.84 | 92.26 | 97.4 | 0.63 |
| Combined cones-rods Wave a | 182.90 | 143.7 | 0.14 | 24.16 | 25.5 | 0.91 | 191.2 | 163.3 | 0.48 | 25.20 | 25.3 | 1 |
| Wave b | 436.11 | 395.9 | 0.46 | 46.68 | 47.5 | 0.67 | 445.8 | 415.7 | 0.84 | 50.60 | 49.6 | 0.52 |
| OpsPS | 51.34 | 51.7 | 0.67 | 27.70 | 28.7 | 0.49 | 60.9 | 54.6 | 0.52 | 27.80 | 28.4 | 0.91 |
| Cones | 101.49 | 91.2 | 0.69 | 30.40 | 31.6 | 0.33 | 112.9 | 91.8 | 0.27 | 32.50 | 31.9 | 0.56 |
| 30 Hz flicker | 73.10 | 68.6 | 0.86 | 28.14 | 26.6 | 0.48 | 82.8 | 73.5 | 0.52 | 27.90 | 28.4 | 0.74 |
son, it was decided to carry out this study with a full field ERG. This type of ERG does not exhibit alterations in the presence of focal macular lesions because the macula represents only 10% of the responses, which account for the absence of differences in the pre-op ERG between the eyes with MH and the contralateral ones. However, if during surgery the dye is extended beyond the macula, the full field ERG could be useful to assess TB toxicity. In this study, the ERG post-op values did not exhibit differences between the eye with MH and the control eye, which seems to indicate that the TB is well tolerated and safe for dyeing the ILM.

We are aware that the multifocal ERG could be more appropriate for assessing and quantifying focal macular toxicity. However, multifocal ERG also evolves in parallel with visual acuity. A recent article which evaluated the toxicity of TB at 0.15% in patients with epiretinal membranes did not detect any sign of damage after vitrectomy (20).

In our study, the anatomic and visual results were favorable. The percentage of anatomic closure (89 and 100%) is similar to that obtained by other groups. Even though a vital dye was utilized, the VA improved significantly in both. Differences were not detected in the visual results on the basis of the TB concentration. The intensity of the TB dye can be increased by dyeing under air or diluting TB in glucose at 5%. At present we are investigating the usefulness of this method for reducing surgical maneuvers. Recently, positive results have been published for ILM dyeing in macular hole surgery with another vital dye, Brilliant Blue (21-23) which in addition seems to have advantages over TB, it would remain as a valid alternative for dyeing the ILM.

In summary, the results of our series suggest that dyeing with TB at concentrations of up to 0.15% are safe for dyeing the ILM because no functional damages were detected by means of ERG. In addition, significant visual acuity improvements are obtained at high as well as low concentrations.

REFERENCES