

INTRAVITREAL TOXICITY OF DOXYCYCLINE. A PILOT STUDY

TOXICIDAD RETINIANA DE DOXICICLINA INTRAVÍTREA. ESTUDIO PILOTO

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ABSTRACT

Objective: To assess the retinal toxicity of varying concentrations of intravitreally administered doxycycline, a member of tetracycline family.

Methods: Fourteen New Zealand albino rabbits, divided into 5 groups, were used for this study. The initial concentration of doxycycline (100 mg) was titrated using 5% dextrose solution to the following concentrations in a volume of 0.1 ml: 2000 µg, 1000 µg, 500 µg, 250 µg, 125 µg, and 62.5 µg. Each concentration was injected into 2 rabbit eyes. Two control eyes received 0.1 ml of 5% dextrose solution. All animals were examined before and after injection using indirect ophthalmoscopy and slit-lamp biomicroscopy. Electroretinography (ERG) was performed on all animals prior to the intravitreal injection and 2 weeks post-injection. The animals were re-examined at this time by indirect ophthalmoscopy and slit-lamp biomicroscopy and were then subjected to euthanasia. Their eyes were enucleated and examined using light microscopy.

Results: The doxycycline injected group exhibited significant decreases in ERG of the eyes injected with 2000 µg, 1000 µg, 500 µg, and 250 µg/0.1 ml. No significant changes in the ERG were observed

RESUMEN

Objetivo: Valorar la toxicidad retiniana de la doxiciclina administrada en inyecciones intravítreas.

Método: Utilizamos catorce conejos albinos neozelandeses que dividimos en 5 grupos. Inyectamos en dos ojos cada una de las siguientes concentraciones de doxiciclina: 2.000 µg/0,1 ml; 500 µg/0,1 ml; 250 µg/0,1 ml; 125 µg/0,1 ml y 67,5 µg/0,1 ml. Dos ojos, usados como control, recibieron 0,1 ml de solución de dextrosa al 5%. Examinamos a todos los animales antes y después de la inyección con oftalmoscopia indirecta y biomicroscopia. Realizamos electroretinogramas en todos antes de la inyección intravítrea y dos semanas después; y posteriormente los animales fueron eutanizados. Se enuclearon los ojos y se prepararon para estudio histológico.

Resultados: Los grupos que mostraron una disminución significativa en los electroretinogramas fueron los inyectados con las siguientes concentraciones de doxiciclina: 2.000 µg/0,1 ml; 500 µg/0,1 ml y 250 µg/0,1 ml. No se observaron cambios significativos en los electroretinogramas de los inyectados con 125 y 67,5 µg/0,1 ml. No hubo signos de toxicidad retiniana en los exámenes de biomicroscopia, en oftalmoscopia indirecta e histología de los

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following the injection of lesser concentration levels. There were no signs of retinal toxicity on slit-lamp examination, indirect ophthalmoscopy, or light microscopy in all the eyes injected with doxycycline concentrations of 125 µg or lower.

Conclusions: Doxycycline injected intravitreally appeared safe at concentrations of 125 µg/0.1 ml or less in albino rabbits. Intravitreal doxycycline may be beneficial, and is an inexpensive alternative drug which could be used in the treatment of bacterial endophthalmitis particularly against resistant *Staphylococcus aureus* organisms (*Arch Soc Esp Oftalmol* 2007; 82: 223-228).

Key words: Doxycycline, endophthalmitis, intravitreal injection, retinal toxicity.

inyectados con 125 µg/0,1 ml o niveles menores de doxiciclina.

Conclusión: Las inyecciones intravítreas de doxiciclina parecen seguras en un rango igual o menor a 125 µg/0,1 ml en el conejo albino. La doxiciclina intravítrea podría ser una alternativa barata y beneficiosa para el tratamiento de la endoftalmitis, especialmente frente a los *staphylococcus aureus* resistentes.

Palabras clave: Endoftalmitis, doxiciclina, inyección intravítrea, toxicidad retiniana.

INTRODUCTION

Endophthalmitis is a serious infectious and inflammatory process, which arises due to a traumatic or iatrogenic inoculation of infectious agents. The total prevalence of endophthalmitis after cataract surgery in the United States is approximately 0.1% (1). In spite of the progress in surgery and treatment, this is devastating post-op complication causes a severe loss of visual equity in 30% of cases (2) and blindness in 18% (3). However, with early diagnostic and treatment, we can achieve satisfactory visual results.

In recent years, intravitreal injections of antibiotics have proved to be the most efficient way to maintain adequate levels of intra-ocular treatment (4,5). The most frequently used antibiotics for intravitreal injection against endophthalmitis are vancomycine, la amikacine and ceftazidime. Vancomycine is considered to be the treatment of choice for covering endophthalmitis due to gram positive organisms (GP) (6). Aminogluco-sides and ceftazidime a broad range antibody at six with antibacterial activity over the gram negative organisms (GN). In addition, aminogluco-sides exhibits a synergic effect when associated to vancomycine against cocci GP (5,7). In order to cover a large spectrum of microorganisms, vancomycine is usually combined with caftazidime or amikacine, but there is evidence of an increased resistance to these agents (8). In fact, the resistance of *Streptococcus viridans* to tetracyclines has been demon-

strated, including sensitivity to vancomycine and chloramphenicol (9). Doxycycline, a broad range tetracycline, has been used successfully in the treatment of community acquired pneumonia (CAP) and other respiratory, genital/urinary, gynecological and ocular infections (10-13). In the light of the above findings, we consider that doxycycline could be an alternative treatment to vancomycine due to the low minimum inhibiting concentration (MIC) against GP cocci. Even though this antibiotic is well known in oral or parenteral treatment, there are no studies about its intravitreal use or toxicity. Accordingly, this study has been carried out to assess the ocular toxicity of doxycycline intravitreal injections.

SUBJECTS, MATERIAL AND METHOD

Animals

Fourteen New Zealand albino rabbits were used, having a weight of 2-3 kg. The animals were treated according to the agreement of the *Association of Research in Vision and Ophthalmology* (ARVO). A biomicroscopy (BMC) and indirect ophthalmoscopy (IO) were performed before beginning the study, after the intravitreal injection, and before sacrificing the animals. All those which exhibited medium opacity or retinal lesions were excluded from the study.

Intravitreal injections

The rabbits were anesthetized prior to all the procedures with 1 ml intramuscularly of a mixture of ketamine (50 µg/kg) and xylazine (5 µg/kg). Their pupils were dilated with an ophthalmic solution of phenylephrine 2.5% (Colircusí Fenilefrina®. Alcon Cusí, SA. El Masnou. Barcelona, Spain) and tropicamide 0.5% (Colircusí Tropicamida® Alcon Cusí, SA. El Masnou. Barcelona. Spain). Proparacaine 0.5% was applied (ANESALCONMR; Alcon Laboratorios Argentina, S. A.; Buenos Aires; Argentina) as topical anesthesia.

The procedures were performed under sterile conditions, using a surgical microscope. An incision in the anterior chamber was made with a 25 G needle to evacuate 0.1 cc of aqueous humor, thus reducing intra-ocular pressure (IOP) and avoiding on the other hand, reflux of the antibiotic after the injection. A dose of doxycycline was injected with a 30 G needle at 2 mm from the sclero-corneal limbus. The rabbits were divided into six groups, injecting 2 right eyes of each group with one of the following concentrations of doxycycline diluted from an initial concentration of 100 µg with dextrose 5%: 2,000 µg/0.1 ml; 1,000 µg/0.1 ml; 500 µg/0.1 ml; 250 µg/0.1 ml; 125 µg/0.1 ml and 67.5 µg/0.1 ml. Two eyes used as controls received 0.1 mL of dextrose 5%.

Electrical and physiological test

Electroretinograms (ERG) were performed in utilizing the UTAS-2000 system (LKC Technology; Gaithersburg; USA) and the standard ERG protocol before the intravitreal injection and two weeks thereafter. The rabbits were adapted to darkness for 30 minutes after pupil dilatation. Unipolar contact lens were fitted (ERG electrodes) in each cornea utilizing goniosol (IOLab Corporation; Claremont, CA.; USA). The negative electrode was placed in the frontal subcutaneous space and the ground electrode was placed in the year with gel. The scotopic response to adaptation to light was recorded (step 1), the scotopic response to flash (step 2) and the photopic response to adaptation to light (step 3). For each step, the average of five curves was determined, calculating the differences between waves a and b for each step. The base ERG was compared (prior to the intravitreal injection) with the final

one two weeks after the injection. Reductions in responses over 30% were taken to be significant. In the groups treated with lower concentrations of doxycycline we found reductions and below 30%, which were accordingly non-significant.

Histological Assessment

After the final ERG, session, all the rabbits were sacrificed with an intravenous injection of sodium pentobarbital. The eyes were enucleated and fixed in karnovsky 48 hours for a subsequent processing and dyeing with hematoxiline-eosine for the histological study.

RESULTS

Clinical assessment

IO was performed in all the eyes after the intravitreal injection, and just before euthanasia. We did not find medium opacity, vitreous hemorrhage, retina detachment or atrophy of the optic nerve. In any of the eyes which were injected with dosages below 1,000 µg/0.1 ml. With dosages of 1,000 µg/0.1 ml and 2,000 µg/0.1 ml remains of crystallized drug were found in the vitreous.

Electrical and Physiological Test

Amplitude reductions between the base and final ERG over 30% were considered to be significant. The ERGs of the groups treated with 2,000 µg/0.1 ml; 1,000 µg/0.1 ml; 500 µg/0.1 ml and 250 µg/0.1 ml of doxycycline exhibited a significant reduction in the amplitude of waves A and B (fig. 1).

Histological Assessment

Histology was performed for all the eyes treated with doxycycline, finding evidence of retinal toxicity in the eyes injected with 250 µg/0.1 ml and higher concentrations of doxycycline (fig. 2). No alterations were found in any of the eyes injected with concentrations of 125 µg/0.1 ml or less.

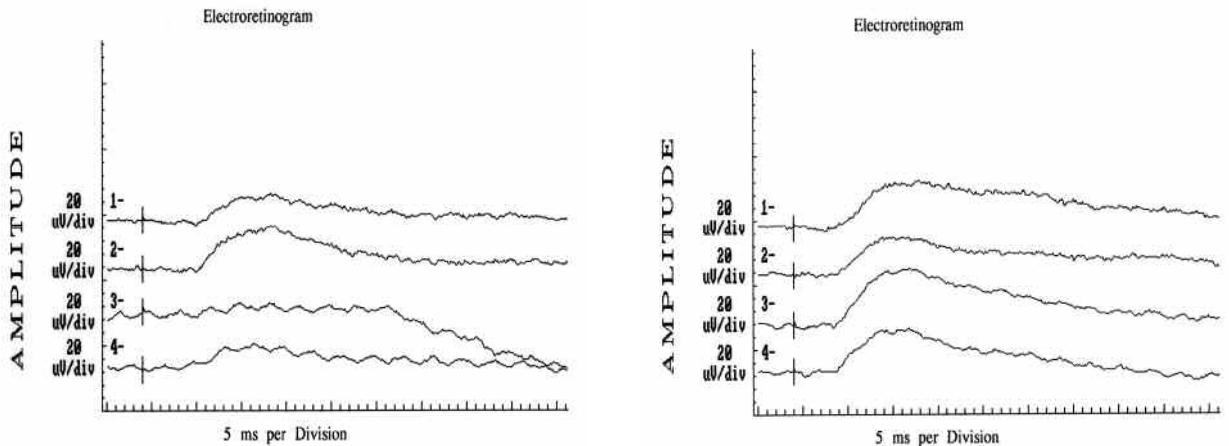


Fig. 1: A. Initial ERG (1 and 2) and after two weeks of the intravitreal injection (3 and 4) of two eyes which received 250 µg/0.1 ml of doxycycline. The final ERG shows amplitude reductions as evidence of retinal toxicity. B. Initial (1 and 2) and final ERG (3 and 4) of two eyes that received 125 µg/0.1 ml of doxycycline, without a finding significant reductions in the amplitude.

DISCUSSION

The antibiotic with the lower MIC over a bacterial group is the most potent one. Low MIC and high concentrations of the antibiotic are required in tissues for effective therapy which does not give rise to resistance. It is considered then an effective treatment for endophthalmitis requires at least 2-10 times the MIC-90 for the microorganism causing the condition (13). Retinal toxicity was observed in the eyes that were injected with 250 µg/0.1 ml and higher concentrations of doxycycline (fig. 2). However, no evidence of toxicity was found in any of the eyes that were injected with concentrations of 125 µg/0.1 ml or lower. Fluorquinolones are broadly used as intravitreal treatment of endophthalmitis. They exhibit a broad spectrum of action, covering the majority of gram positive and gram negative organisms.

Fourth generation fluorquinolones (gatifloxacin and moxifloxacin) have improved the spectrum of action over gram positives in comparison to second generation Fluorquinolones (14). On the other hand, we have shown alterations in ERG at concentrations of 320 µg/0.1 mL and in the clinical assessment with concentrations above 160 µg/0.1 mL (15).

At present, intravitreal injection of antibiotics is considered to be the standard management for endophthalmitis (16,17). The literature also has described increases in the prevalence of methicillin-resistant *Staphylococcus aureus* (EA) (18,19).

The increase in the resistance of GP microorganisms to methicillin and first and second generation fluorquinolone, as well as the risk of toxicity of Aminoglycosides when utilized in highly dosages, has led researchers to assess new antibiotics. Accordingly, many doctors have made vancomycin the only alternative to cover GPs. Doxycycline has been used with great success for treating severe infections (10-12) but, before this study, its intra-ocular toxicity had not been assessed.

Doxycycline has a great potency, a broad spectrum of action, long mean life and high bioavailability in comparison with macrolides, cephalosporins and trimethoprim-sulphamethoxazole.

The combination of tigecycline and gentamicin increases activity against vancomycin-resistant enterococci and EA (20). Aldridge KE et al (21) proved that doxycycline and norfloxacin are as active against methicillin-resistant Eas as vancomycin. Doxycycline has demonstrated a good activity. Against the majority of the methicillin-resistant staphylococci strains (coagulase-negative EA and Staphylococci, including *S. Epidermidis*) tested with 100 strands inhibited with 2 µg/0.1 of the antibiotic. The MIC of *S. pneumonia*, *Haemophilus influenzae*, and *Moraxella catarrhalis* was below 2 µg/0.1 ml (22,23). The results of MIC tests in the quality control ranges proposed (QC) in the use of doxycycline for *S. pneumonia*, SA and *E. faecalis* were 0.016-0.12 µg/ml; 0.12-0.5 µg/ml; and 2-8 µg/ml respectively (24).

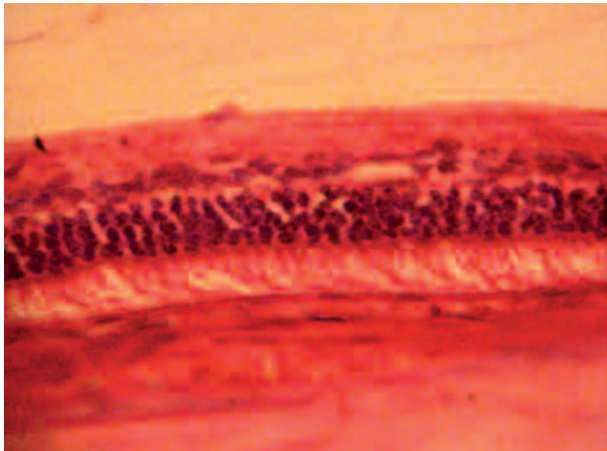
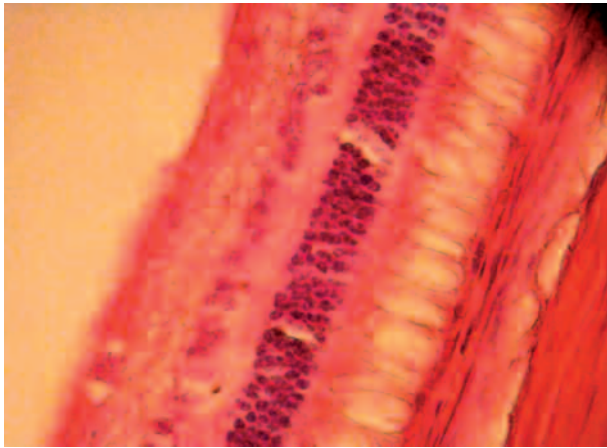
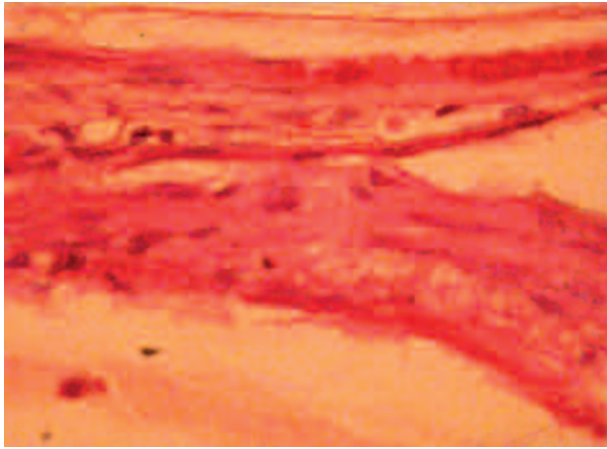


Fig. 2: Histological retina sections dyed with hematoxyline-eosine with 400 increases of rabbits from the groups injected with A. 250 $\mu\text{g}/0.1$ ml B. 125 $\mu\text{g}/0.1$ ml and C. 67,5 $\mu\text{g}/0.1$ ml of doxycycline. All the neurosensory retina layers were found intact without signs of toxicity, except for the group treated with a dosage of 250 $\mu\text{g}/0.1$ ml.

On the other hand, the pressure-reducing effect of tetracycline after intravitreal injection in rabbits has been demonstrated, and this could reduce its scope of application. This is that appears after a few days, but it persists a long time, with an intraocular pressure reduction of up to 12 mmHg in some cases (25).

Assuming that the volume of vitreous humor in the rabbit is 1.5 ml (26) and that the drug is homogeneous to distributed after intravitreal injection, the concentrations of 67.5 and 125 $\mu\text{g}/0.1$ ml of doxycycline were estimated at 42 and 78 $\mu\text{g}/0.1$ ml respectively. Even the minimum doses exceed the necessary effective dosage to inhibit the resistant GP strains (coagulase-negative EA and *Stafilococci*, including *S. Epidermis*) and GNs.

In conclusion, our study demonstrates that intravitreal injections of doxycycline in a concentration of 125 $\mu\text{g}/0.1$ ml or lower are safe for albino rabbits. The intravitreal doxycycline can be a cheap and beneficial alternative to vancomycine for treating bacterial endophthalmitis, with a broad spectrum of action and a low rate of resistance.

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