ARAŞTIRMA/ORIGINAL ARTICLE

Gülhane Tıp Derg 2016;58: 153-156

doi: 10.5455/gulhane. 167354

Farklı dozlarda sığır serumu albümini ile oluşturulan deneysel üveit: Bir ön çalışma

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ÖZET

Çalışmamızda, farklı dozlarda sığır serum albümin (SSA) çözeltileri ile indüklenen deneysel üveit modellerinin histopatolojik analizi gerçekleştirilerek en uygun dozun belirlenmesi amaçlanmıştır. Çalışmada 4 tavşanın sağ gözleri kullanıldı. Üç farklı SSA çözeltisi hazırlandı: 100 µg/0.1 ml, 1 mg/0.1 ml ve 10 mg/0.1 ml SSA çözeltileri sırasıyla tavşan 1, 2 ve 3'e intravitreal yoldan uygulandı. Tavşan 4 kontrol olarak kullanıldı. Üveit oluşumu klinik ve anjiyografik olarak takip edildi. Bir hafta sonra tavşanlar sakrifiye edildi ve gözler histopatolojik analiz için enükle edildi. Tavşan 1'de hafif ön üveit bulguları görüldü. Tavşan 2'de vitreus, retina ve koroidde orta düzeyde enflamasyon varken siliyer cisimde yoğun enflamatuar hücre infiltrasyonu vardı. Tavşan 3'de siliyer cisimde ciddi düzeyde enflamatur reaksiyon ve konjesyon vardı. Ayrıca vitreus, retina ve koroidde de yoğun enflamatuar hücre infiltrasyonu görüldü. Sonuç olarak, 1 mg/0.1 ml SSA çözeltisinin tek doz intavitreal uygulanması anjiyografik ve histopatolojik olarak anlamlı düzeyde arka üveitik reaksiyon oluşturabilir.

Anahtar Kelimeler: *Sığır serum albümini; deneysel üveit; enflamasyon; intravitreal enjeksiyon.*

SUMMARY

Experimental uveitis ınduced by bovine serum albumin in various doses: A pilot study

We aimed to perform histopathological analysis of models of experimental uveitis (EU) induced with bovine serum albumin (BSA) solutions at various doses in order to determine the most appropriate dose of BSA. Right eyes of four rabbits were used. Three different BSA solutions were prepared: 100 µg/0.1 ml, 1 mg/0.1 ml and 10 mg/0.1 ml BSA solutions were applied intravitreally to the first rabbit, second rabbit and third rabbit respectively. Fourth rabbit was used as control. Uveitis formation was followed clinically and angiographically. Rabbits were sacrificed and eyes were enucleated at the end of the first week for histopathological analysis. In the first rabbit, mild anterior uveitic signs were seen. In the second rabbit, there was a moderate inflammation in the vitreous, retina and choroidea, as well as severe inflammatory cell infiltration in the ciliary tissue. In the third rabbit, there was a severe inflammatory reaction and congestion in the ciliary tissue. Severe inflammatory cell infiltration in the vitreous, retina and choroidea was also seen. The results suggested that intravitreal application of a single dose of 1 mg/0.1 ml BSA solution may induce a significant posterior uveitic reaction both angiographically and histopathologically.

Key words: *Bovine serum albumin, experimental uveitis, inflammation, intravitreal injection.*

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Makalenin Geliş Tarihi: Aug 23, 2014 • Kabul Tarihi: Mar 01, 2015 • Çevrim İçi Basım Tarihi:10 Haziran 2016

Introduction

Complicated mechanisms have been used to try to explain the physiopathology of uveitis seen in humans. The findings related to these mechanisms have been mainly determined from experimental uveitis (EU) models. Researchers have applied different factors to induce EU models resembling uveitis in humans. Due to its similarity to autoimmune eye diseases and human uveitis, EU is an important model (1-3). Antigenic proteins such as retinal S-antigen, interphotoreceptor retinoid-binding protein, rhodopsin, and bovine serum albumin (BSA) are used to induce EU (4). Immunization with these antigens causes specific T-cell activation. These inflammatory cells are activated within 10-20 days after immunization and attack the retinal proteins (5). EU is a T-cell dependent illness (6). Infiltration of the photoreceptor layer, granuloma formation, perivasculitis, retinal detachment, and destruction of retinal cells can be monitored histopathologically (4). In the acute phase, T-lymphocytes and polymorphonuclear leucocytes (PMNL) are the dominant cells, but macrophages can also be seen (7). Iris hyperemia, ciliary injection, fibrin reaction, and synechia are frequently observed clinically. The infiltration of inflammatory cells to the anterior chamber (AC) causes lens opacity in rats. Anterior segment involvement in mice is minimal, but in fundus examination, vasculitic signs such as perivascular infiltrates, disc edema, and retinal hemorrhages are detected (8). To induce EU in previous studies, various doses of BSA solutions have been applied. EU formation with a single intraocular BSA injection is one of the most known methods (9, 10). Ultrastructural alterations after a single intravitreal injection of BSA to non-sensitized rabbits have been analyzed previously (7, 11-13).

We aimed to histopathologically analyze EU models induced with BSA solutions at various doses in order to determine the most appropriate BSA dose.

Material and Methods

In the study, four male albino New Zealand rabbits with approximate weights of 2000-3000 g were used. The study was performed according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The study was performed with the permission of Animal Experiments Ethics Committee and contributions of the Departments of Pathology and Ophthalmology at Gulhane Military Medical Academy (GATA). During the study, the animals were kept in special cages with proper nutrition conditions in the Center of Research and Development at GATA. Only one eye (right eye) per animal was used.

All procedures were performed under general anesthesia.

During anesthesia and analgesia, the combination of intramuscular 50 mg/kg ketamine hydrochloride (Ketalar, Eczacıbaşı, Turkey) and 5 mg/kg xylazine hydrochloride (Rompun, Bayer, Turkey) was used. Intraviteral injections were applied with 30-gauge needles 4 mm behind the limbus without injuring the lens and retina.

BSA (Sigma, St Louis, MO, USA) was used to induce EU. Three different BSA-% 0.9 NaCl solutions in the concentrations of 100 µg/0.1 ml, 1 mg/0.1 ml and 10 mg/0.1 ml were prepared and intravitreally injected into the eyes of the first, second and third rabbit respectively. No injection was performed to the fourth rabbit's eye (control). Uveitis formation and progression was observed six hours, one day (Day 1), three days (Day 3) and seven days (Day 7) after BSA injection clinically. Ciliary injection, iris hyperemia, miosis, AC cells, flare, fibrin membrane and cataract formation were examined. The severity of uveitis in the rabbits were evaluated clinically as defined beforehand (14). Posterior uveitis formation and its progression was evaluated on Day 3 and on Day 7 by fundus fluorescein angiograpy (FFA) (HRA; Heidelberg Engineering, Heidelberg, Germany) via 0.5 ml 5% fluorescein injections to the ear veins of the rabbits .

The animals were sacrificed and the right eyes were enucleated at the end of the first week (Day 7) for histopathological analysis. Eyeballs were fixed in 10% formalin and processed for routine histologic sectioning. Paraffin blocks were prepared, and 5 µm sections were cut with a microtome and subsequently stained with hematoxylin and eosin (HE). They were evaluated under light microscope and digital photographs were taken. The pathological alterations in iris, ciliary body and choroidea were graded according to the previously defined criteria (15).

Results

Three different doses of BSA solutions were applied to three rabbits in this study to determine the appropriate BSA dosage that induces a sufficient uveitic reaction. The fourth rabbit did not receive an injection of BSA; only the FFA test was performed for comparison. Uveitis formation was followed clinically and angiographically. The rabbits were sacrificed and right eyes were enucleated for histopathological analysis on Day 7.

In the first rabbit, mild anterior uvetic signs were seen during the observation week. On Day 3, minimal hyperemia in iris and +1+2 tyndall and posterior synechia existed (Figure 1A). There were no vascular leakage or any other posterior uveitic signs seen in angiography performed on Days 3 and 7 (Figure 1B). In the second rabbit, iris hyperemia, +3+4 tyndall, flare and miosis were seen on Day 1; posterior synechia and moderate fibrin reaction occurred on Day 3 (Figure 1C). FFA applied on Day 3 showed that there were vascular leakage and optic disc hyperfluorescence (Figure 1D). In the third rabbit, posterior synechia and dense fibrin reaction were observed on Day 1. Cornea edema, dense ciliary injection, fibrin membrane, and lens opacification were seen on Day 3 (Figure 1E). Cataract had occurred on Day 7. During the FFA test performed on Day 3, a good image could not be obtained due to the fibrin, lens opacification, and vitritis (Figure 1F).

Enucleations were performed one week after BSA injections. Histopathological grading is shown in Table 1. The histopathology of the rabbits were compared with the control. The histopathology of the first rabbit showed there were no signs

Table 1. Histopathological grading.

	First rabbit	Second rabbit	Third rabbit	Fourth rabbit
Iris	-	+1	+3	-
Ciliary tissue	+2	+3	+3	-
Choroidea	-	+2	+3	-
Retina	+1	+2	+2	-

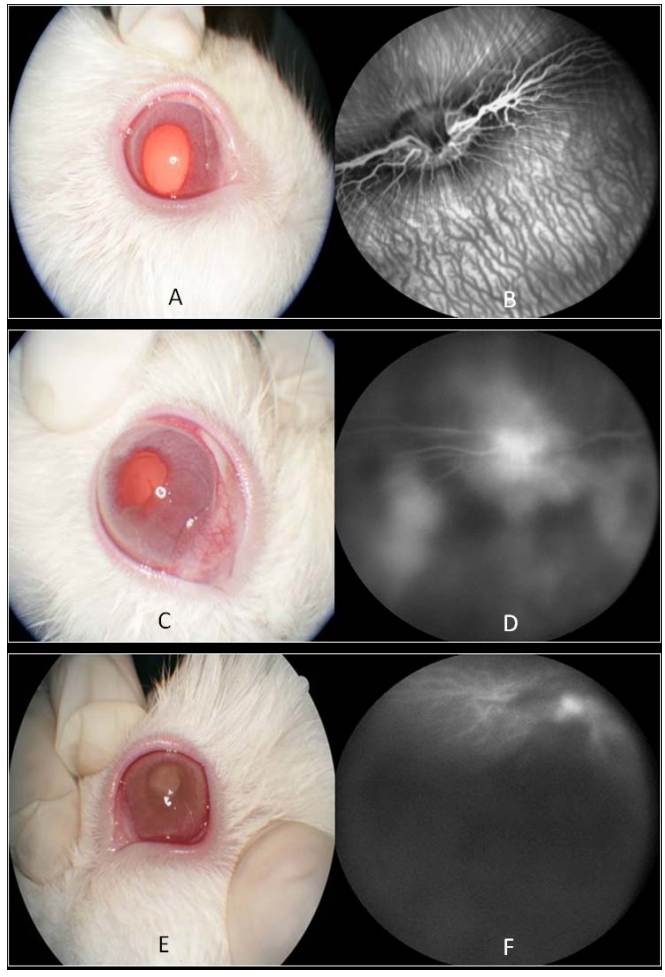

Figure 1. Anterior segment and FFA images of the rabbits on Day 3. (A) There was a minimal hyperemia in iris and (B) no vascular leakage in angiography in the first rabbit. (C) Anterior segment imaging revealed iris hyperemia, posterior synechia, and (D) angiography showed vascular leakage and optic disc hyperflourescence in the second rabbit. (E) In the third rabbit there was a severe lens opacification in biomicroscopy and (F) hardly visible hyperflourescence in angiography.

of inflammation in the cornea and iris. Congestion and mild mononuclear cell (MNC) infiltration were observed in ciliary tissue (Figure 2A). No inflammation was seen in the choroidea and retina (Figure 2B). There were moderate MNC and PMNL infiltration in the vitreous at the histopathology of the second rabbit. Additionally, there were severe inflammatory cell infiltration in the ciliary tissue (Figure 2C) and moderate infiltration in the retina and choroidea (Figure 2D). There were severe inflammatory reaction and congestion in ciliary tissue at the histopathology of third rabbit (Figure 2E). Severe leukocyte and

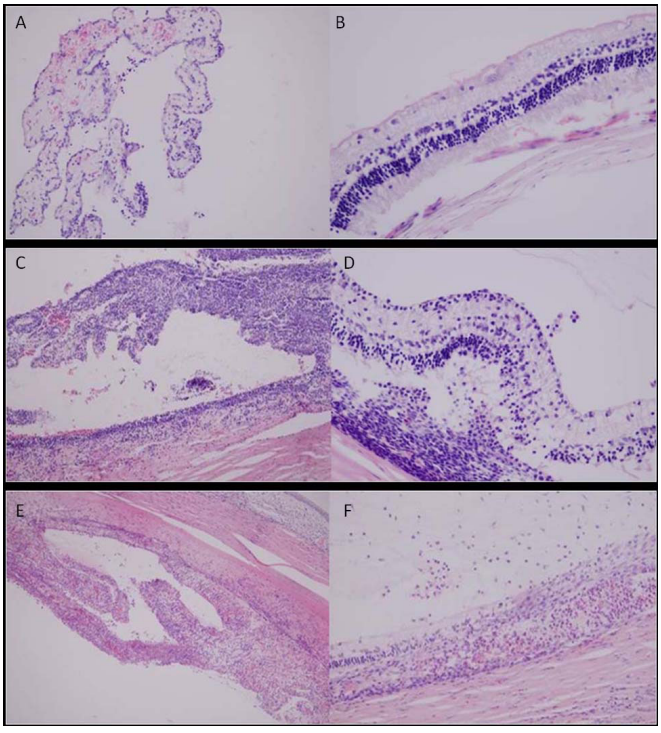

Figure 2. Histopathology of the rabbits on Day 7. The histopathology of the first rabbit showed (A) congestion and mild mononuclear cell (MNC) infiltration in ciliary tissue (HE; magnification x200) and (B) no inflammation in the choroidea and retina (HE; magnification x400). In the second rabbit (C) there was severe inflammatory reaction in ciliary tissue (HE; magnification x100) and (D) moderate infiltration in the retina and choroidea (HE; magnification x400). The histopathology of the third rabbit revealed (E) severe inflammatory reaction and congestion in ciliary tissue (HE; magnification x100) and (F) severe leukocyte and MNC infiltration in the vitreous, retina, choroidea, and sclera (HE; magnification x200).

MNC infiltration in the vitreous, retina, choroidea, and sclera, as well as severe congestion in the choroidea, were also seen (Figure 2F). There was an inflammatory cell infiltration in optic nerve head (ONH), especially in the region adjacent to the vitreous, interstitial tissue, and around the vascular structures.

Discussion

The history of EU induced via intravitreal foreign serum antigens began in the early 1900s (16, 17). Since then, various study types have been used frequently. Recent research has focused on the histopathology of EU induced by serum antigens. Zimmerman and Silverstein (18) first revealed MNC infiltration in the uvea in 1959. This result was later supported by other studies (19, 20).

To induce EU, BSA solution in various doses have been applied before. EU formation via a single intraocular BSA injection is one of the most well known methods (9, 10). Ultrastructural alterations after a single intravitreal injection of BSA to non-sensitized rabbits were analyzed previously (7, 11-13). In a 1974 study, a single dose of intravitreal BSA solution (10mg/0.1ml) was applied to non-sensitized rabbits; 4-12 hours later, there were uveitic signs such as iritis, hypotonia, flare, and tyndalisation (21). The other eyes of the animals were used as control (saline was applied), but there were not any detected uveitic reaction (21). Therefore, it was stated that the early uveitic reactions observed in eyes to which BSA solutions applied were not due to the traumatic effect of injections (21). Uveitis formation was followed only clinically; neither angiographic nor ophthalmoscopic analysis were done in that sudy. In our study, we evaluated the uveitic reactions after injections of different doses of BSA. We determined that the uveitic reaction induced by a 10 mg BSA injection was very severe clinically and posterior uveitis could not be monitored angiographically. Therefore, we think that monitoring of the posterior segment may be limited with this BSA dosage due to excessive uveitic reaction.

In another study on non-sensitized guinea pigs, it was shown that 100 µg/0.1 ml BSA solution induced a sufficient uveitic reaction clinically and histopathologically (22). In this study, uveitis formation was evaluated by clinical and histopathological grading, but angiographic evaluation was not performed. We applied 100 µg BSA intravitreally to the right eye of the first rabbit in our study. We observed mild anterior uveitis clinically, but there was not enough posterior uveitic reaction in angiographical and histopathological evaluation. Therefore, 100 µg BSA may not be capable of inducing posterior uveitis in rabbit uveitis studies.

Uusitalo (23) studied EU in sensitized rabbits previously; he applied 25 mg/0.5 ml BSA solution subcutaneously beforehand; two weeks later 2 mg/0.05 ml BSA solution was applied intravitreally and primary uveitic signs were observed 3 hours after the intravitreal injection. Additionally in this study, three hours after the BSA injection, perivascular PMNL and MNC infiltrations were observed in the ciliary body and iris, and strands of fibrin-like material were seen on the surface of the ciliary processes using a scanning electron microscope. It was revealed that the dominant cell type was MNC on the first day. Two days later, although MNC infiltration decreased slowly, it was observed that a mild MNC infiltration was still occurring in the ciliary body and iris two weeks after BSA injection (23). Intravitreal injection itself can cause traumatic reaction (24). However, it was shown that primarily PMNL infiltration had developed only in BSA-applied eyes of the sensitized rabbits (23). Nonetheless, in the contralateral eye to which intravitreal BSA was not applied, there was minimal MNC infiltration in the ciliary tissue due to the fact that they had been sensitized before. [23] We did not choose to induce EU in sensitized animals due to the long duration of uveitis formation and ultrastructural alterations in the contralateral eye.

Uveitis induced via BSA in rabbits is related to increased activity of both humoral and cellular immunity (3). In EU models, antigen-presenting cells in the eye serve antigens to other inflammatory cells. Furthermore, lymphocyte migration is accelerated by the cytokines released to the environment. Lymphocytes adhere to retina pigment epithelium and retinal vascular endothelium via ICAM-1 and migrate to the area of inflammation (25). It was previously stated that ten days after immunization, lymphocytic and monocytic infiltration would begin in EU and would cause the destruction of photoreceptor and neuroretinal layers (5). Nonetheless, before the signs of uveitic tissue damage, the findings of blood-retina barrier breakdown such as local retinal edema and subretinal exudation occur in the early phases of EU (8). In the acute phase of EU, the PMNLs are the dominant cell type, followed by MNCs. PMNLs and MNCs have the main role in the pathogenesis of EU (7, 23). Before inflammatory cell migration and independent from leukocytes, a fibrin-like material can be seen on the surface of ciliary processes (23). MNC infiltration and the fibrin network in ciliary epithelium form a morphological barrier and

reduce the release of aqueous humor that causes hypotonia in the late phases of uveitis (21, 23). In another EU model induced with intravitreal BSA solution in sensitized rabbits, it was shown that acute and chronic inflammation had occurred in the ciliary body and iris in all rabbits, and inflammatory cell infiltration also had occurred in the choroidea and retina (26).

We observed first uveitic signs clinically six hours after the injection of 1 mg BSA solution in the second rabbit. On Day 3, anterior and posterior uveitis could be observed clinically and angiographically. In histopathology performed on Day 7, there was a moderate inflammation in the ciliary body, choroidea, and retina; MNCs were dominant, but PMNLs could still be seen in the vitreous.

Due to the ethical considerations we used a limited number of animals in this study. Our aim was to determine the appropriate BSA dosage for our next EU study, so we performed a pilot study with this limitation. Despite the limited number of animals, the data obtained from our study are consistent with previous studies (5, 7, 23, 26).

In conclusion, we think the intravitreal application of a single dose of 1 mg BSA solution may be appropriate to induce sufficient uveitic reaction in rabbits clinically, angiographically and histopathologically, but further studies are needed as well.

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