Observations in clinical and experimental ocular autoimmunity

de Smet, M.D.

_Citation for published version (APA):_
de Smet, M. D. (2000). Observations in clinical and experimental ocular autoimmunity

**General rights**
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

**Disclaimer/Complaints regulations**
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
Chapter 10

FK506 Treatment of Experimental Autoimmune
Uveoretinitis in Primates

Yujiro Fujino, Chi C Chan, Marc D. de Smet, Naofumi Hikita, Igal Gery,
Manabu Mochizuki, Robert B. Nussenblatt

(by permission © Elsevier Science)
FK 506 Treatment of Experimental Autoimmune Uveoretinitis in Primates

Y. Fujino, C.-C. Chan, M.D. de Smet, N. Hikita, I. Gery, M. Mochizuki, and R.B. Nussenblatt

FK 506 is a neutral macrolide isolated from the fermentation broth of Streptomyces tsukubaensis. It has a pharmacophysiologic action similar to that of cyclosporine A (CyA). It suppresses mixed lymphocyte reactions, the production of T-cell-mediated soluble factors, and the expression of interleukin 2 (IL-2) receptor. It has been extensively investigated using transplantation models. Cyclosporine is currently used in the treatment of various immunologically mediated diseases in the eye, but its usefulness is limited by its adverse side effects.

Experimental Autoimmune Uveitis (EAU) is an experimental model of ocular autoimmune disease that can be induced in different animal species by immunization with a retinal-specific antigen in the presence of an adjuvant. Though the exact mechanism of EAU is still controversial, T cells appear to play a central role. In the Lewis rat, we have shown that FK 506 was effective in preventing the expression of EAU at a dose less than 0.3 mg/kg/d. Within the eye, FK 506 was able to inhibit the expression of IL-2 receptors on T cells and prevent the expression of MHC class II antigens on ocular resident cells. It also significantly delayed the cellular kinetics of EAU, causing a significant increase in the recruitment time of the T-suppressor/cytotoxic cells.

More recently, we reported on the inhibition of clinical EAU in the primate model with doses as low as 0.125 mg/kg/d. The EAU model in the primate more closely resembles certain ocular inflammatory conditions found in man both by the observed clinical course as well as by the histopathology of the ocular lesions. We now report on the histopathologic and immunohistochemical nature of these lesions as well as on the systemic toxicity observed in our treated animals.

MATERIALS AND METHODS

Animals and Immunization Procedures

An extensive description of the study design, immunization schedule, and follow-up is given in our previous report. In summary, 15 rhesus monkeys (Macaca mulatta, 7 males and 8 females) were used in this study. All animals were provided with food and water ad libitum. All animals were obtained from an NIH-approved random source and housed in environmentally controlled rooms with a 12-hour light and dark cycle. The study was approved by the institutional animal care and use committee and complies with the Public Health Service Policy on the Humane Care and Use of Laboratory Animals. The animals were immunized with bovine S-antigen in phosphate buffer saline (PBS) emulsified in complete Freund's adjuvant (CFA, 1:1) containing Mycobacterium tuberculosis H37Ra at 1.25 mg/mL (Difco, Detroit, Mich.). The emulsion was injected as described elsewhere into multiple intradermal sites over the dorsal thoracic region of each animal.

Drug Dosage and Administration

FK 506 was provided by Fujisawa Pharmaceutical Co., Osaka, Japan. The drug was dissolved in PBS and administered intramuscularly on a daily basis starting on day 21 or 23 as previously described. Control animals were similarly injected with the vehicle alone. The treatment was given for 15 to 49 days depending on the experimental group and the animal. Three dose levels were evaluated, namely 0.125, 0.25, and 0.5 mg/kg/d.

Laboratory Determinations

All animals were anesthetized with 1% ketamine/xylazine on a weekly basis and phlebotomized. Laboratory studies included a complete blood count and a chem screen (Mid-Atlantic Regional Laboratory, Rockville Md.).

Histology and Postmortem Examination

The monkeys were sacrificed on day 70 after immunization or as soon as they had lost 15% of their pre-immunization weight. An autopsy was performed on all animals within 2 hours of death. The eyes were enucleated immediately, bisected with one half of the specimen imbedded in O.C.T. and snap-frozen. The other half was fixed in 10% formalin and used to prepare sections for histologic examination.

Immunohistopathology

Using the avidin-biotin-peroxidase complex (ABC) method, 6-μm serial frozen sections of each frozen eye were prepared for immunohistologic study. Adjacent frozen tissue sections were

From Laboratory of Immunology, National Eye Institute (Y.F., C.-C.C., M.D.d.S., N.H., I.G., R.B.N.), Bethesda, Maryland, and Dept. of Ophthalmology (M.M.), Kurume University, Kurume, Japan.

Address reprint requests to Robert B. Nussenblatt, MD, Bldg. 10, Rm. 10N202, NIH, Laboratory of Immunology, National Eye Institute, Bethesda, MD 20892.

© 1991 by Appleton & Lange

0041-1345/91/$3.00 + 0

stained with hematoxylin and eosin (H&E). The primary monoclonal antibodies were OKT4A (CD4), OKT8F (CD8), L. en-14 (CD22), Dako Monoclonal antibody. HLA-DR, and ICAM. All monoclonal antibodies were purchased from Ortho Diagnostic Systems Inc., Raritan, NJ, and Becton Dickinson. Mountain View, Calif. Mouse ascites fluid containing 1 to 2 μg nonspecific protein per mL was used as a control. As a secondary antibody, a biotin-conjugated horse anti-mouse IgG was used (Vector Laboratories, Burlingame, Calif.). The immunoperoxidase was scored as previously described. 19 In brief, positive cells were counted and recorded for the same anatomic area of each section.

Statistics

The significance of differences between groups was determined by Student's t test.

RESULTS

Ocular Histopathologic Findings

Histopathologic findings are summarized in Table 1 for both the control and the FK 506-treated animals. EAU was observed histologically in five of six control animals. Onset of uveitis was between 24 and 33 days after immunization for the control group, whereas in the treated group the disease was limited to 3 of 10 animals: one monkey developed disease 10 days after the termination of FK 506 therapy, while in another the disease developed after a dose reduction to 0.0625 mg/kg/d. The histopathologic changes in the control group resembled those previously reported. 20 These changes included necrosis, gliosis, and lymphocytic infiltration in the retina, particularly surrounding the venules, hypertrophy of the retinal pigment epithelium, and thickening of the choroid with granulomatous lymphocytic infiltrations. Normal ocular morphology was observed in most animals treated with FK 506. In the three animals that developed uveitis, one had extensive changes comparable to those in the control group. His disease started after cessation of therapy. In the other animals, only focal areas of involvement were found with only mild to moderate lymphocytic infiltration limited mainly to the choroid and the subretinal space.

Immunohistochemistry

In the control animals, immunohistochemical staining revealed that the predominant infiltrating cell population were lymphocytes with a ratio of 1:1 between B and T cells. The ratio between the T helper/inducer (CD4) and the T suppressor/cytotoxic (CD8) was 1.5:1. MHC class II antigens were expressed on ocular resident cells: retinal pigment epithelium (RPE), vascular endothelial cells, and retinal glial cells. Intercellular adhesion molecule-1 (ICAM-1) was identified on the vascular endothelium of vessels located in the area of inflammation.

In the treated animals, no inflammatory cells were identified in those animals with no evidence of clinical or histopathological involvement. However, in the three monkeys with disease, there were infiltrating lymphocytes with a relative decrease in the T helper/inducer (CD4) cell population as compared to the T suppressor/cytotoxic (CD8) cell population (mean ratio of 0.8:1). B cells showed a marked decrease with a ratio of 1:4 between B and T cells. ICAM-1 was not visible on either the vascular endothelium or the RPE.

Side Effects of FK 506

Animals treated with 0.5 or 0.25 mg/kg had a 10% to 15% weight loss during the course of therapy as was previously reported. 19 In some animals, the weight loss was accompanied by anorexia, lethargy, and diarrhea, which may well have contributed to the change in weight. Table 2 summarizes both the final measurements and the degree of change from baseline for peripheral blood counts. Adverse effects were largely limited to the animals treated with 0.5 mg/kg/d. A statistically significant decrease in the hematocrit was seen as well as a decrease in the total white cell count. The white cell count was noted to transiently drop, early in the treatment in all groups, followed by a normalization of the count by the end of the third week of therapy.
relative lymphocytopenia was noted in some animals but was less predictable in onset and duration.

Results of the liver-function tests are summarized in Table 3. Statistically significant rises in the serum titers were noted for LDH and SGPT in animals treated with 0.5 mg/kg/d. The rise in LDH was first noted about 14 days into the treatment phase and continued for about 2 weeks after cessation of therapy. No abnormalities of blood glucose were seen in any of the treated animals. Blood urea nitrogen (BUN) was found to be elevated in the animals treated at the 0.5 mg/kg/d dose. This was not associated with a rise in serum creatinine and may have been the result of dehydration as a result of anorexia and diarrhea.

Autopsy showed diffuse multiple granulomas in the liver and kidneys as well as enteritis in the two animals treated at the 0.5 mg/kg/d dose. Abnormal liver pathology was noted in two out of four monkeys treated with 0.25 mg/kg/d, and one of four monkeys treated with 0.125 mg/kg/d. Two of five control animals also had multifocal granulomas in the liver. None of the control animals or the animals treated at the 0.25 or 0.125 mg/kg/d dose had any granulomatous kidney changes. Enteritis was noted in one out of four monkeys treated with 0.25 mg/kg/d, in one out of four monkeys treated with 0.125 mg/kg/d, and in two out of five control monkeys.

**DISCUSSION**

Histopathologic examination of the eyes from primates treated with FK 506 indicates that it can effectively inhibit S-Ag induced EAU in all animals treated at the 0.5 mg/kg/d dose and the majority of the animals treated with 0.25 and 0.125 mg/kg/d. Inhibition of EAU occurred despite starting therapy 3 weeks after the first immunization, at a time when the immunopathogenic response was in an advanced stage of development as judged by the onset of disease in the control animals shortly thereafter. Thus, FK 506 is a powerful inhibitor of the effector limb of the immunopathogenic response. In eyes from animals with only partial response to FK 506, a dynamic shift was observed in the nature of the infiltrating lymphocytes. The increase in the proportion of CD8 and the decrease in the proportion of CD22 cells are assumed to be a result of the inhibitory effects of FK 506. A shift toward infiltrating CD8 cells was also observed with suboptimal doses of CyA in the Lewis rat model. The decrease in the infiltrating B-cell population (CD22) is noteworthy. It is likely to be secondary to a reduction in the recruitment of B cells into the ocular tissue by the reduced number of T helper cells present. A decrease in both the cellular and humoral responses to S-Ag have been observed following administration of FK 506 during the efferent phase. However, the effect of FK 506 on B cells appears to be primarily mediated through its action on T helper cells. A reduction in the expression of class II antigens on ocular resident cells is also noted as is a decrease in the expression of ICAM-1 on the vascular endothelium. Both are important in the propagation of the local inflammatory response. Their decreased expression further limits the recruitment of inflammatory cells.

Side effects in the monkeys treated with FK 506 were largely limited to the animals treated with 0.5 mg/kg/d. Previous reports have suggested that the adverse effects observed varied considerably from one animal species to the next. In rats, little acute toxicity has been observed even at higher doses of 1 to 3 mg/kg/d. In dogs, intussusception and vasculitis have both been observed. Liver involvement was found in some animals with a significant increase in the liver transaminases and alkaline phosphatase. Few toxic changes have been observed to date in primates. Thiru et al. reported vasculitis in transplanted baboons, but this was not found by other investigators. The major toxic changes reported have been limited to the kidneys and to the pancreas. Side effects were observed in our animals only at the highest dose tested. We did not see any hyperglycemia. However, these changes were only observed in animals receiving 1 mg/kg/d or more of FK 506. The increase observed in the BUN in our animals treated with 0.5 mg/kg/d is in keeping with other investigators and was reversible. The change in the LDH has not previously been observed. The LDH may have come from several sources. It is known that Freund's adjuvant can cause muscle atrophy when used repeatedly and might explain the initial high titers seen in all animals. However, in all groups except 0.5 mg/kg/d, there was a decrease in the titer during the course of treatment. In the animals treated with 0.5 mg/kg/d, the rise in SGPT and LDH during therapy suggests that FK 506
may have been mildly hepatotoxic though it was not severe enough to cause any significant impairment of liver function.

Finally, it appears that FK 506 is an effective means of inhibiting EAU. Little acute toxicity is found at doses necessary to inhibit EAU in the majority of animals.

REFERENCES