

An Assessment of Corn Oil as a Vehicle for Cyclosporin A (CsA) at Varied Injection Sites in Preventing Rejection of Rat Laryngeal Allografts

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Justin Wu, Hasan Jalisi, C. Esther Yang, Robert E. Harley, Gilbert Brodsky, Marshall Strome (Joint center for Otolaryngology, Brigham and Women's Hospital and Beth Israel Hospital, Surgical Research Laboratories, Harvard Medical School, Boston, Ma. 02115.)

(This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the Harvard Medical School).

Abstract

This study was designed to determine the efficacy of corn oil as an alternative vehicle to olive oil for emulsifying cyclosporin (C5A) in preventing rejection of transplanted rat larynges. The issue of varied site absorption was also addressed. Thirty animals were transplanted to get 5 viable transplants at two weeks for three varied sites of administration; intramuscular (IM), subcutaneous (SQ) and intraperitoneal (IP). Five mg/kg of CsA in corn oil was the dose administered based on earlier data generated in our laboratory. Postulating selective absorption, the indirect measure of laryngeal histopathology, i.e. rejection, was chosen over blood levels for evaluation. In the IM group 2 graft mild rejection whereas 3 showed marked cellular and vascular rejection. The SQ group had 1 mild, 1 moderate and 3 with severe rejections. The IP group had one moderate rejection and 4 severe rejections. Qualitatively the IM and SQ groups were similar. The IP group histologically evidenced far greater cellular rejection. CsA 5mg/kg emulsified in corn oil did not differ substantively in histologic scope or pattern of rejection from CsA in olive oil in experimental rat laryngeal transplantation. Further, the data did not support a change in the administration of CsA from an intramuscular site (JPMA 44: 14, 1994).

Introduction

The discovery in 1970 of two new strains of fungi imperfecta that produced anti-fungal metabolites led to the isolation of cyclosporin A (CsA) three years later¹. This potent immunosuppressant remains the mainstay in organ transplantation, both in experimental animals and humans. CsA is the beginning of a third generation of clinical immunosuppressive agents characterized by selective immune regulation. Specifically, CsA acts preferentially on humoral and cell-mediated immunity by inhibiting T helper cells through the suppression of the macrophage derived monokine, interleukin-1 (IL-1) and of the T helper-derived lymphokine, interleukin-2 (IL-2); it spares the neutrophils and more importantly the T suppressor cells². CsA is not lymphotoxic because the immunosuppressive effect is reversible³. The latter is demonstrative of the progress made with immunosuppressive agents. The first generation (e.g., cyclophosphamide, azathioprine, methotrexate) exerted indiscriminate cytotoxicity against all dividing cells, including the immune competent cells. The second generation was the lymphocytotoxic agents (e.g., anti-lymphocyte serum, L- asparaginase) which restricted most of its action to the immune competent cells. A recent new animal model was used with CsA to prevent rejection of heterotopic laryngeal allografts from LBNF-1 to Lewis rats - Strome et al. ¹. Intramuscular (IM) administration of

CsA is the established method of GsA delivery in rats. Wassef, however, has shown that- CsA can be administered to rats at different sites - IM, oral (PO), intraperitoneal (IP) and subcutaneous (SC), all with adequate plasma levels⁴. He also noted that the IM route offered the greatest bioavailability while the SC route exhibited the least variation of CsA serum levels over the 24 hour period following administration of CsA. Despite this finding, no standardized protocol for GsA administration has been established and a variety of routes, e.g., IM⁵, IP⁶ and SC⁷ have been used in rat organ transplantation. Thus a paucity of data exists as to the preferred route of CsA delivery. This study was designed to investigate the best delivery of GsA in our current rat model for laryngeal transplantation. We have used GsA powder emulsified in olive oil in earlier experiments. Unpublished information suggests that CsA dissolved in corn oil might offer more uniform absorption. This postulate was explored.

Materials and Methods

Three experimental groups, each consisting of 10 rats, received laryngeal allogeneic transplants. In all groups, male LBNF-1 hybrids weighing 250-350g served as donors and male inbred LEW rats weighing 300-350g were the transplant recipients. All animals were obtained from Harlan Sprague-Dawley, Hasslett, MI. The rat model utilized for these experiments is described in greater detail elsewhere⁸. In brief, the donor larynx, with both of its vascular pedicles (right and left superior thyroid arteries and the external/common carotids) were harvested and then transplanted into the left side of the recipient's neck. Daily doses of 5 mg/kg of CsA in corn oil were injected intramuscularly (group I), intraperitoneally (group II) and subcutaneously (group III), all for a period of two weeks. In addition, the rats received 120,000 units of IM penicillin postoperatively and were maintained on a tetracycline/water supply before their sacrifice. The laryngeal grafts were evaluated for vascular patency as well as for macroscopic and histologic signs of rejection. They were compared to a group in our earlier series that had received 5 mg/kg of intramuscular GsA emulsified in olive oil. Vascular obstruction was defined clinically as a visible thrombus or failure of the vessel to refill after occlusion and release. Clinical rejection was defined on a graded continuum ranging from an intact allograft with no apparent signs of inflammatory reaction to complete destruction of the laryngeal musculature and cartilage. The grafts were then excised and fixed with 10% formalin, sectioned horizontally and stained with hematoxylin and eosin. The morphology and degree of rejection of the cartilaginous structure and mucosa were assessed microscopically.

Results

In each of the three groups, five transplants resulted in patent (vascularized) grafts. In group I (TM), two patent grafts had intact cartilaginous and muscular structures, intact mucosa and were classified as exhibiting mild rejection (Figure 1).

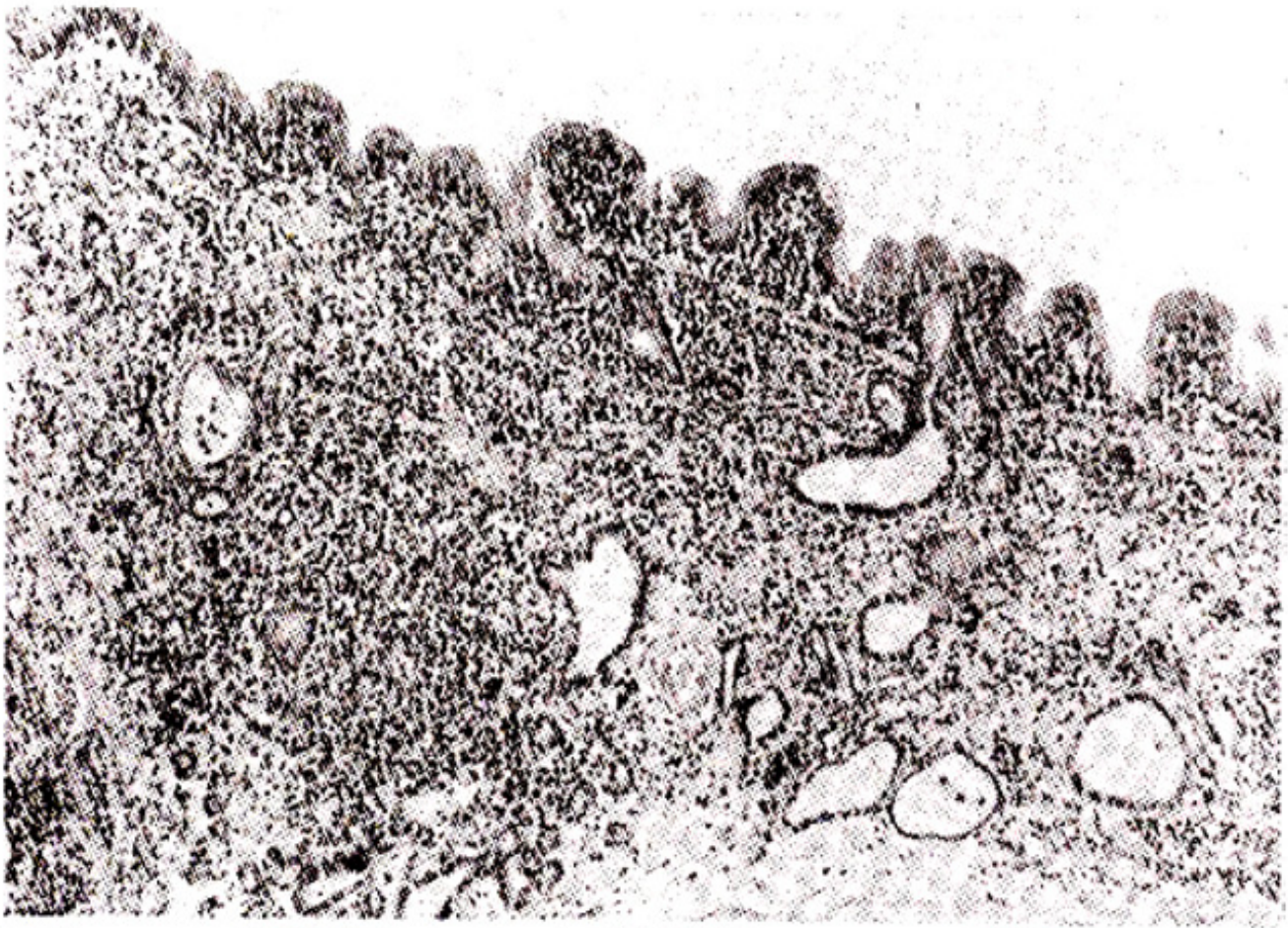


Figure 1. IM-CsA 5 mg/kg x 2 weeks. Mild rejection - intact brush border, mild-moderate cellular infiltrate preservation of glandular elements.

The other three, however, had evidence of marked cellular and vascular rejection. Some residual mucosa was identified, but exhibited chronic inflammation with abundant lymphocytes, histiocytes and a partial loss of minor salivary glands. This heterogeneity in the histopathological results is similar to that in the olive oil 5 mg/kg GsA TM group in our first GsA study⁹. That data showed mild-moderate rejection (3/6 transplants) and moderate-severe rejection (3/6 transplants). In group II, one animal had moderate rejection with a moderate cellular infiltrate and an absence of glandular elements. The rest had severe rejection, worse than those in group I (Figure 2).

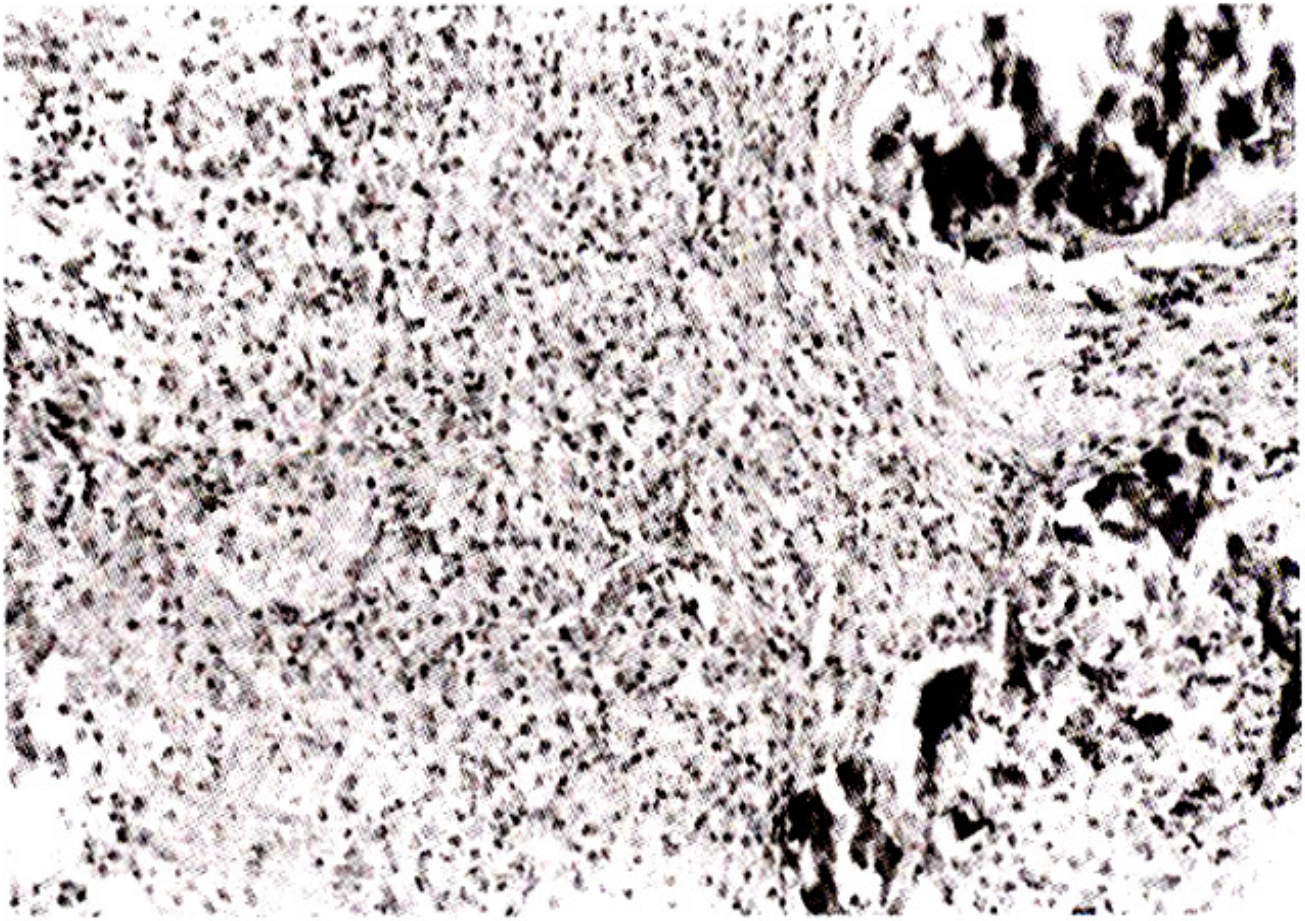


Figure 2. IP-CsA 5 mg/kg x 2 weeks. Severe rejection - destruction of most architecture - granulation tissue, loss of salivary acini.

These grafts evidenced infarction, absence of salivary acini and granulation tissue in the lumen. One developed a rejection cocoon (a shell of granulation tissue) and there was no identifiable laryngeal framework. The histopathology in group III was similar to group 1. Two of the five patent laryngeal grafts were labelled as mild and moderate rejection (Figure 3).



Figure 3. SC-CsA 5 mg/kg x 2 weeks. Mild rejection - intact brush border, minimal cellular infiltrate, intact acini.

The mild specimen had an intact brush border, a minimal cellular infiltrate and intact glands. The moderate specimen had an intact cartilaginous structure, a lamina propria replaced by chronic inflammation, normal surface epithelium with lymphocytic infiltration and some atrophy of the minor salivary glands. Three, however, revealed a marked inflammatory response and severe rejection in the cartilaginous and mucosal elements.

Discussion

Our recently completed study showed that 5 mg/kg of CsA in olive oil given intramuscularly resulted in varied rejection patterns of laryngeal allografts from LBNF-1 donors to Lewis recipients. 50% of the transplants evidenced mild to moderate graft rejection while the other 50% evidenced moderate to severe rejection¹. In this study, the histopathological results in groups land III were similar to those in our earlier study. Both of these experimental groups had a 40% mild to moderate rejection pattern and a 60% marked to severe cellular and vascular rejection pattern. Therein, the histologic correlates from the laryngeal grafts receiving 1) TM injections of 5 mg/kg of CsA dissolved in corn oil, 2) SC injections of 5mg/kg of CsA dissolved in corn oil, or 3) TM injections of 5mg/kg of CsA dissolved in olive oil are quantitatively similar. Although the latter rejection grouping (mild-moderate-severe) are similar, qualitative cellular differences within each subset favour TM CsA in olive oil. Yet with small numbers, the most that can be inferred from this data is that there is no clear advantage to either vehicle (corn oil or olive oil) in rat laryngeal transplantation surgery. Similarly, there was no clear benefit evident

between the IM or SG route using corn oil as a vehicle. Group TI (IP) comparatively had far worse results in that 4 of 5 transplanted larynges evidenced severe rejection. Histologically, a severe inflammatory response, absence of acini and infarction were evident. 5mg/kg of GsA in corn oil given IP is less efficacious than either the TM or SC route in preventing rat laryngeal graft rejection. We had postulated that small variations in absorption related either to the site of administration or the vehicle, manifest only at lower doses of GsA administration, were potentially responsible for the varied histopathological spectrum observed in our prior study of 5 mg/kg CsA-rat laryngeal transplants. Changing both the vehicle and the site did not substantiate that hypothesis. Thus, even in inbred recipients, some variability in immunogenicity is suggested.

Conclusion

We conclude; therefore, that there is no need to change our administration of CsA in future rat laryngeal transplant studies from an intramuscular site to either SC or TP sites and that CsA powder can be either emulsified in olive oil or corn oil without a significant difference in graft rejection. Given that our earlier trials used olive oil as a vehicle, for continuity, it will remain our vehicle of choice.

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