Format for ANSWERING REVIEWERS

November 26, 2014

Dear Editor,

Thank you and the reviewers for your excellent comments and considering our manuscript, “Pseudopemphigoid as Caused By Topical Drugs and Pemphigus Disease.” Please find enclosed the edited manuscript in Word format (File name: Pseudopemphigoid 12075-edited Final Revision.doc). We appreciate the opportunity to publish in the World Journal of Ophthalmology.

We have made the changes suggested by the reviewers and responded in a point by point fashion.

Title: Pseudopemphigoid as Caused By Topical Drugs and Pemphigus Disease

Authors:

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Name of Journal: *World Journal of Ophthalmology*

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Comments:

REVIEWER 02502338:

1) In this review article, the authors have summarized all clinical and immunological information for pseudopemphigoid. Although cicatricial conjunctivitis are caused by various factors, the authors focus on drug-induced conjunctival cicatrizatin (DICC) and two pemphigus diseases, including ocular pemphigus vulgaris (OPV) and paraneoplastic pemphigus (PNP). This is a well-written review, containing large information and ample of references. Organization of the manuscript is good, and English are adequate. However, I have several comments, which may be helpful to improve the manuscript. (1) The description for the relationship between DICC and mucous membrane pemphigoid (MMP) is interesting and intriguing. Although MMP is not considered to be drug-induced disease, there may be a possibility that drug might be main cause of MMP. The authors should discuss about and emphasize this issue.

Thank you for this comment. Emphasis on drug-induced MMP has been added to the paper as below:

Page 7, Paragraph 3 and Page 8, Paragraph 2

DICC can develop as a non-progressive, self-limiting "toxic" reaction to an offending topical drug or as a progressive, immunological process that continues despite cessation of the offending drug.[10-12] Although increased activity of fibroblasts has been implicated as a possible effect of topical drugs on the local immune system, the exact mechanism by which offending drugs directly induce cicatricial conjunctivitis remains unknown.[13]

When immunoglobulin localized to the conjunctival epithelial basement membrane zone are found, then autoimmune phenomenon are suggested.[13, 14] Practolol, an oral beta-blocker, and its derivative metipranolol, a topical beta-blocker that treats glaucoma, have been implicated to induce immunologically-mediated DICC.[15-18] This is related to the chemical structure and pharmacologic metabolism in the body – both compounds require deacetylation for metabolic activation, which produces a toxic aniline derivative in practolol and a slightly less toxic phenol derivative in metipranolol.[15] When oxidized, these derivatives become highly reactive and are normally neutralized in the body by the addition of glucuronic acid or sulfate. However, this mechanism is insufficient in patients that have a lower capacity for enzymatic detoxification.[15] When this occurs, these reactive oxidative products can be bound by proteins to create antigens.[15] Therefore, the toxicity potential of practolol and metipranolol to produce immunologically-mediated cicatricial conjunctivitis occurs in patients who are susceptible to these reactions required for metabolic activation of the drug due to its pharmacologic structure. Drug chemical structure has not been implicated in the mechanism of cicatricial conjunctivitis induced by other offending topical drugs and

in many cases of DICC, a toxic or immune-mediated reaction cannot be further defined.

And Page 9, Paragraph 2

Incidences of MMP developing in uninvolved eyes of patients that did not receive the inciting drug may indicate an immunological etiology.[10] On the other hand, instances of unilateral changes histologically and immunologically identical to MMP that occur in only the eye that received an offending drug is considered to be drug-induced.[14] The absence of bilateral ocular involvement, other mucosal or cutaneous manifestations, and disease that is non-progressive after cessation of the offending drug suggests a drug-induced reaction. Therefore, DICC may involve either a toxic mechanism of damage or an autoimmune etiology where inciting topical medications sensitize predisposed individuals to developing a more rapid onset of ocular MMP.

2) In the discussion for the relationship between DICC and MMP, the authors described about epitope spreading. In their story, epitope spreading is an event that the damage to cornea by drug exposed certain antigens, which activate T and B cells. However, epitope spreading usully mean the expansion of autoantibodies to previous epitopes to autoantibodies to other epitopes, which are either intramolecular or extramolecular. The authors should consider the theory of epitope spreading, and rewrite the section, if necessary.

Thank you for this important clarification. This theory of epitope spreading has been added to the paper, as below:

Page 8, Paragraph 3

Epitope spreading is one possible theory that may elucidate the mechanism behind autoimmune phenomenon as induced by topical drugs. Epitope spreading[19, 20] *refers to the phenomenon of autoimmune reactivity not only against one protein, but also against other epitopes on the same protein or other proteins in the same tissue. Intramolecular epitope spreading that occurs between different epitopes on the same protein is often used to explain the molecular pathogenesis and severity of disease in bullous pemphigoid.*[21] *Additionally, epitope spreading may occur due to tissue damage that causes certain antigens to become newly exposed to autoreactive T or B cells, thus producing an autoimmune disease in predisposed individuals.*[19, 22] *This mechanism of epitope spreading can be promoted by injury that exposes previously sequestered antigens, causing activation of antigen presenting cells that attract autoreactive lymphocytes in these individuals.*[20] *Intermolecular epitope spreading that occurs between two different proteins has been cited to explain the conversion of one autoimmune disease into another. Pemphigus autoimmune disease converting into pemphigoid disease, or conversions between other autoimmune blistering diseases either simultaneously or separated by a few years, is hypothesized to occur when tissue damage exposes protein parts that are normally undetected by the*

immune system.[23, 24] *In a similar manner, ocular mucosal injury due to Stevens-Johnson syndrome, Lyell Syndrome, or direct chemical injury from drugs may be implicated to expose normally hidden antigens to processing and presentation by activated T-cells, resulting in the formation of MMP.*[10, 19, 22]

3) In dermatological field, OPV is not very common, and is not well characterized. Therefore, the authors should describe in more detail for the pathophysiology of OPV and for the difference between OPV and pemphigus vulgaris (PV) without ocular involvement.

Thank you for this comment. This important point is now addressed in an additional paragraph as below.

Page 13, paragraph 2:

Ocular involvement in PV is rare and its low incidence in the literature may be related to the course of disease or due to underreporting. Desmoglein 3 is heavily expressed in the basal layer of conjunctival epithelium along with strong expression of desmocollin 3, and desmoplakin 1 and 2, throughout the conjunctiva.[47, 48] *The mechanism on why ocular involvement in PV is rare despite the presence of anti-desmoglein 3 autoantibodies in disease is unclear. Suggestions include that the ocular surface is less exposed to trauma than other tissues normally affected by PV*[49]*; that there is inactivation of desmoglein 3 in ocular epithelium that is readily*

compensated by other desmosomal proteins thereby leaving only a minority of patients susceptible to disease if compensation cannot be attained[47]*; or that conjunctival involvement in PV is simply underreported.*

4) Although we know that a few PV patients show lid margin erosion and conjunctival hyperemia, we have not seen PV patients with conjunctival ulceration pseudomembrane formation. Therefore, I suspect that the diagnoses of PV in the previous reports of OPV were not correct, and such patients might have either MMP or PNP. The authors should discuss about this possibility.

Thank you for this comment. The possibility of dual diagnoses is now addressed in the paper, specifically in the discussion of the study performed by Chirinos et al, as below.

Page 14, Paragraph 3:

Although all patients in this series had immunopathological diagnoses of PV, additional serology studies and/or secondary confirmatory biopsies were not performed to determine the coexistence of MMP. Dual diagnoses of MMP and PV have been previously reported in the literature[64, 65] *and therefore remain a possibility in this series.*

5) In Table 5, the authors described the molecular weight of desmoglein 1 as 165 kDa. However, it is now usually descried as 160 kDa in most study, and the authors should

change it.

Thank you for this important comment. We have modified the molecular weight of desmoglein 1 to 160 kDa in Table 4 (Page 43) and in all other instances it is mentioned in the paper.

REVIEWIER 00505284:

1) The authors present an informative and timely review of a quite devastating ocular disease family. The review provides the reader with a fairly well thought out up-to-date comparison of the epidemiology, clinical findings, diagnosis, and treatments for mucous membrane pemphigoid, pseudopemphigoid, drug-induced cicatricial conjunctivitis, pemphigoid vulgaris and paraneoplastic pemphigoid. We wholeheartedly agree with their conclusion. Minor suggestions: 1. The format of the outline and text as well as reference should be checked. (There may have been some shifting of margins during transmission of the manuscript.)

Thank you for this suggestion. The outline, text, and reference formatting has been checked and, to the best of our knowledge, is consistent with World Journal of Ophthalmology guidelines.

2) The order of the tables as they appear in the text is out of sequence.

Thank you for this important comment. Table 1 (Page 39) and Table 2 (Page 40-41) are now re-ordered according to appearance within the text.

3) In several places an acronym is provided, but not used in the subsequent text.

Thank you for this comment. To the best of our knowledge, we have now modified all acronyms to make its use consistent in the subsequent text including DICC (drug induced conjunctival cicatrization), PV (pemphigus vulgaris), OPV (ocular pemphigus vulgaris), DIF (direct immunofluorescence), IIF (indirect immunofluorescence), and IEM (immunoelectron microscopy).

4) Fig 1; The high magnification photos are sufficient.

Thank you.

5) Pathology slides showing differential conjunctival staining pattern would add significantly to the paper.

Thank you for this comment. We have added photos of conjunctival biopsies with positive direct immunofluorescence staining (DIF) from patients with mucous membrane pemphigoid, pemphigus vulgaris, and paraneoplastic pemphigus (Please see Figure 2A-C, Page 46). Additionally, a photo of a negative DIF staining pattern from a patient with pseudopemphigoid (most likely

drug-induced) is added (Please see Figure 2D, Page 46).

EDITOR REVIEW:

1) Please define the title and running title again. Note: 1.The title concisely summarizes the main topic of the study and is not too long (no more than 12 words). The use of words such as ‘exploration’, ‘research’, ‘analysis’, ‘observation’, and ‘investigation’ is avoided. The title does not start with ‘The’ or Arabic numbers, and does not include uncommon abbreviations.

2. A running title is provided (no more than 6 words).

Thank you for this comment. We have changed the title to be “Pseudopemphigoid as Caused By Topical Drugs and Pemphigus Disease” and the running title to be “Pseudopemphigoid: A Review.”

2) Please not allow to cite the reference in the Abstract part.

Thank you for this comment. This citation has now been removed (previously on Page 2).

3) Please revise your Heading 1 like this.

4) Please revise your Heading 2 like this.

Thank you for this suggestion. The headings within the text are now modified according to the suggestions with changes highlighted.

5) Please list all the abbreviations in the tables. Thank you!

Thank you for this important suggestion. All abbreviations have now been listed as below, Page 44:

MMP: Mucous membrane pemphigoid. PNP: Paraneoplastic pemphigus. OPV: Ocular pemphigus vulgaris. DIF: Direct immunofluorescence. IIF: Indirect immunofluorescence. IEM: Immunoelectron microscopy. LAD-1: Linear IgA bullous dermatosis autoantigen 1.

We thank the editors and reviewers for the helpful and thorough comments.

Thank you again for the opportunity to publish in World Journal of Ophthalmology.

Sincerely yours,

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