Author's response to reviews

Title: Abnormalities in the meibomian glands in patients with oral administration of anticancer combination drug-capsule TS-1(R)

Authors:

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Author's response to reviews: see over
Re: Revision of Manuscript # 20230529531459856
Date: June 30th, 2015

We are submitting our revised manuscript entitled; “Abnormalities in the meibomian glands in patients with oral administration of anticancer combination drug-capsule TS-1®” by Mizoguchi et al. for publication in BMC Cancer.

We so much appreciate this opportunity of revision, and could satisfy the requirement requested by reviewers. We look forward to hearing from you.
Sincerely yours,

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CC: Shizuya Saika, MD, PhD (Chairman of the Department)
Authors’ response to reviewers’ comments

Reviewer: Naoyuki Morishige

Major points

Comment: (1) The mechanism of meibomian gland disorders by TS-1 is not well-discussed. This is the first report which mentions potential adverse effect of TS-1 on meibomian gland, thus the authors are required to mention about the mechanism in discussion.

Response
Yes, we agree with the comments. Function of meibomian glands is considered to depend on proliferation of its acinar cells. Cell proliferation is mediated a set of variety of enzymes including thymidylate synthase of which expression is upregulated by various growth factors. 5-FU is known to inhibit the activity of this enzyme, leading to attenuation of cell growth. Animal experiments might be useful to uncover the effects of systemic TS-1 on cell proliferation activity in meibomian glands. Discussion has been improved.

(2) TS-1 is orally administrated thus it would affect to meibomian gland evenly. However, the meibomian gland changes are not uniform. How would you explain this?

Response
Yes, we agree with the comments. We think that the size of the duct or orifice affects the obstruction of the orifice of the meibomian glands. Discussion has been improved.

(3) It would be better to include age-matched normal controls to compare the subjects. It may provide the authors valuable information.

Response
Yes, we mostly thank the comment by the reviewer. We quoted about Meibo-score in normal subjects from this article (Arita R, Itoh K, Inoue K, et al: Noncontact infrared meibography to document age-related changes of the meibomian glands in a normal population. Ophthalmology 115: 911-915, 2008). It would be useful to include age-matched normal controls upon IRB approval in the next project. We mostly appreciate the suggestion for a future project.

Minor point


Response
Yes, we agree with the comments. We have cited this article and have added it to the reference list.

Reviewer: Sunyoung Young Jang

Comment: This is a case series to report 3 patients who were diagnosed with Meibomian gland dysfunction (MGD), caused by an administration of TS-1®. The
change of Meibomian gland structure was shown using MeiboPen®. Since seborrheic or obstructive MGD with the morphologic change of Meibomian gland can be commonly encountered at the ophthalmologic clinic, I think that it is mandatory to present a meibography of pre-administration of TS-1®, in order to conclude that TS-1® might cause meibomian gland loss in these patients. If it is not possible, I think 3 cases seems to be insufficient to draw such conclusion and much more patients needs to be gathered to make a reliable conclusion.

Response
Yes, we totally agree with the comment. We would like to suggest the possibility that abnormalities in the meibomian glands in patients with oral administration of anticancer combination drug-capsule TS-1®. So, there is a need for prospective study.

Reviewer: David A Sullivan
Comment: Would the authors add a hypothesis in the Background as to why they thought TS-1 would impact the meibomian gland?

Response
Yes, we agree with the comments. Lacrimal fluid containing 5-FU is secreted on the eye surface and meibomian glands, causing inflammation around orifice of the meibomian gland. This inflammation may subsequently have caused keratinization and fibrosis, leading to obstruction of the orifice of meibomian gland and resulting in meibomian gland obstruction and subsequent tissue damage. Introduction has been improved.

Comment: Human meibomian gland function and secretion is critically dependent upon the active proliferation of glandular epithelial cells (Liu S et al, Invest Ophthalmol Vis Sci 2013; 54:2541-2550). This proliferation is stimulated by several growth factors, a process that may be mediated by their upregulation of thymidylate synthetase gene expression (Series accession numbers GSE18099 and GSE 37089, National Center for Biotechnology Information’s Gene Expression Omnibus, cited in Liu S et al, Invest Ophthalmol Vis Sci 2013; 54:2541-2550). Tegafur (i.e.5-FU), in turn, suppresses the activity of this enzyme, thereby decreasing DNA synthesis and cell proliferation. Would the authors consider discussing this anti-proliferative influence, which may help account for the observed meibomian gland contraction and loss following TS-1 exposure? Such decreased proliferation could also lead to reduced meibum quality and obstructive MGD.

Response
Yes, we agree with the comments. We would suggest that animal experiments might be useful to uncover the effects of systemic TS-1® on cell proliferation activity in meibomian glands. Discussion has been improved.

Comment: Would the authors explain how they determined the “Meibo-score”?
Response
Yes, this information is essential – thanks you so much pointing out. We have quoted the Meibo-score from a publication (Arita R, Itoh K, Inoue K, Amano S : Noncontact infrared meibography to document age-related changes of the meibomian glands in a

**Comment:** The authors state that topical diquafosol treatment elicited improvement in both corneas of the Case 1 patient. Did this therapy also reverse the meibomian gland contracture and loss, and improve the Meibo-score? The authors should add these data to the manuscript.

**Response**
Yes, we agree with the comments. Topical treatment for the damage in the corneal epithelium in Cases 1 was considered to be effective presumably due to direct effects on the corneal epithelial homeostasis, but not on the meibomian glands.

**Comment:** Would the authors define the word “noncorrigunt”?

**Response**
Yes, in the examination of visual acuity, refractive correction did not improve the visual acuity, the term “noncorrigunt” was used here.

**Comment:** The authors state that topical rebamipide and ofloxacin treatment elicited improvement in both corneas of the Case 2 patient. Did this therapy also reverse the meibomian gland contracture and loss, and improve the Meibo-score? The authors should add these data to the manuscript.

**Response**
Yes, we agree with the comments. Topical treatment for the damage in the corneal epithelium in Cases 2 was considered to be effective presumably due to direct effects on the corneal epithelial homeostasis, but not on the meibomian glands.

**Comment:** Were the patients taking any other topical or systemic drugs that may have influenced the ocular surface?

**Response**
Yes, we agree with the comments. All the patients reported here did not receive such drug.

**Comment:** The authors suggest that 5-FU would reach the ocular surface by secretion from the lacrimal gland. Why wouldn’t 5-FU reach the meibomian gland via a direct vascular route?

**Response**
Yes, we mostly thank the comment by the reviewer. We just wanted to suggest that 5-FU would reach the ocular surface by secretion because of the reason of orifice obstruction. As the reviewer suggests, there might be a possibility the access of 5-FU via systemic circulation.

**Comment:** There is no peer-reviewed evidence that the human meibomian gland becomes inflamed in obstructive MGD (Knop E et al, Invest Ophthalmol Vis Sci
Response
Yes, we mostly thank the comment by the reviewer. A Japanese group reported that repeated administration of TS-1® induced focal tissue necrosis and inflammation in conjunctiva in dogs (Hayashi T, Yamaguchi S, Kito S, Tanaka G, Kurokawa K, Hirota T: An Oral repeated dose toxicity study of a new antineoplastic agent S-1 in dogs: I. A 13-week repeated dose toxicity study, II. An ophthalmologic toxicity recovery study. J Toxicol Sci 1996, 21: 527-544 (In Japanese)). We therefore 5-FU in tear fluid might induce local inflammation and resultant focal tissue scarring in/around orifice of the glands.

Comment: A very recent online abstract (Sullivan DA et al), which will be presented at the upcoming May 2015 meeting of the Association for Research in Vision and Ophthalmology (www.arvo.org) in Denver, Colorado, USA, reports that UTP (the diquafosol parent) and rebamipide have no effect on the proliferation or differentiation of human meibomian gland epithelial cells. Would the authors speculate, then, how their patients’ corneas (and meibomian glands?) improved after these treatments in Cases 1 and 2?

Response
Yes, we agree with the comments. Topical treatment did exert a therapeutic effect on the corneal epithelium. In the cases here reported such topical treatment did not show obvious therapeutic effects on meibomian glands.