6-Thioguanine inhibits herpes simplex virus 1 infection of eyes

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Dr. Zhiwei Wu
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Re: Spectrum00646-21 (6-Thioguanine inhibits herpes simplex virus 1 infection of eyes)

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Clinton Jones
Editor, Microbiology Spectrum

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Reviewer comments:
Reviewer #2 (Comments for the Author):

The purpose of this project was to evaluate 6-TG for its anti-herpes simplex virus activity and mechanism of action. The authors present data on the potency and cytotoxicity of 6-TG in several cell types. 6-TG was potent and synergized with ACV, indicating that it likely acted through a different pathway. The effects of HSV-1 and 6-TG on the Rac1 protein were investigated in cells and infected corneal tissue. 6-TG was shown to be tolerated in rabbit eyes at high concentrations, so mouse studies were undertaken. 6-TG gel prevented HSK as well as GCV gel in mouse eyes. This project includes many aspects of antiviral evaluation. It is clearly written, although the Abstract needs more details of the results and less background information.

The Abstract contains information that belongs in the Introduction (what drugs are used in which countries, drug resistance) and lacks information from the Results (IC50 of 6-TG, effective dose in mice). It is not helpful to state that 6-TG is "better" than ACV and GCV because it is more potent. If 6-TG is potent but not absorbed well, then it is a "worse" drug. Stating that 6-TG is more potent than ACV and GCV is the better term. However, 6-TG is only slightly more potent than ACV (EC50 approximately 0.5 micromolar), so this sentence should probably be moved to the Discussion.

Line 72-73: TFT is not given orally for HSV keratitis. Trifluridine is usually administered as a topical gel (Viroptic) for HSV keratitis. It is administered orally as a combination drug trifluridine/tipiracil for cancer treatment.

Line 109-110: Correct the grammar in this sentence. Consider this change: 6-TG mediates its immunosuppressive effects by interfering with Rac1 protein function (20).

Figure 2: In Fig. 2a, the EC50 of 6-TG in Vero cells seems much lower, around 0.01 micromolar than in HCEC cells (0.1 micromolar). This point should be raised in the discussion. In Fig. 2b, the y-axis label and the figure legend do not describe the units of the plaque assay. Does the axis show the Log10 of the pfu/mL? What are the error bars? How many replicates? In Fig. 2d, the scale bar on the fluorescence micrographs is too small to see and there is no DNA stain to indicate how many cells are in each field. Fig. 2d micrographs and bar graph are not necessary and should be removed.

Fig. 5e: the concentrations of 6-TG on the western blot, (0 5 10) need a label on the right side (6-TG µM) for clarity. It would also help if it was indicated on the graph that these cells were not infected with HSV-1.

Fig. 7: The cytokine analysis is difficult to interpret because the labels on the graphs are too small. Since the data are negative, meaning 6-TG did not induce inflammation, these graphs can be moved to the Supplemental data section and enlarged enough to see the axes. Alternatively, the entire figure can be moved to the Supplement.

Fig. 8: Make the colors for each group of mice the same. Why are the colors in 8e different than 8f and 8g? Use one color for PBS, another color for 6-TG, and another color for GCV. Make all graphs consistent.

Staff Comments:

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Thank you for submitting your paper to Microbiology Spectrum.
September 10, 2021

Prof. Zhiwei Wu  
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Re: Spectrum00646-21R1 (6-Thioguanine inhibits herpes simplex virus 1 infection of eyes)

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Supplemental Material: Accept
