

Effect of Pioglitazone & Metformin Treatments on Oral Leukoplakia Mustafa Ali, Beverly Wuertz, and Frank G. Ondrey Molecular Oncology Program, Department of Otolaryngology, University of Minnesota, Minneapolis, MN

INTRODUCTION

Oral leukoplakia exhibits as a white or gray-appearing patch; commonly found on the buccal mucosa and side regions of the tongue and is frequently associated with tobacco and alcohol use.⁴ Unfortunately, such an oral plaque has the ability and molecular makeup to mutate into a malignant form of cancer known as oral squamous cell carcinoma. Oral squamous cell carcinoma is a very serious and problematic form of cancer, due to its tendency towards locoregional invasion, often spreading to nearby tissues, bones, lymph nodes, etc.⁸ Its progression is aided by the asymptomatic nature of oral leukoplakia. As of now, the means of treatment, such as surgical removal, cryoprobe, or antiviral medications, are less than effective; since the rate of recurrence is between 7.7% to 38.1%.^{11,12} However, constant care and oversight of one's post-treatment often lowers chances of cancerous progression.

Nonetheless, we propose an alternative treatment/solution. We believe pioglitazone and metformin have notable properties that can be advantageous as a treatment for an individual with oral leukoplakia depending on the concentration and duration between each dosage. Their unique molecular makeup of such drugs permit interactions with various receptors that generate distinctive chemical signals; thus, having an influence of the cell division process (dependent on the concentration).¹⁷ As of now, both drugs are tested and FDA-approved diabetic drugs that currently manage hyperglycemia of an individual which have minimal, concerning side effects.^{15,16}

MATERIALS & METHODS

In order to maintain a healthy population of cells within the flask prior to treatment, continuous cell culturing was applied in order to minimize cell death that would counteract the purpose and goals of the study. First and foremost, trypsin medium was utilized to digest the proteins within the cells. The cells were then centrifuged at 1000 RPM to separate the sample into a supernatant and cell pellet. Furthermore, the cell pellet were resuspended equally throughout the solution, using the respective medium. Last but not least, a relatively small amount of the resuspended cell pellet were plated and incubate to permit further cell growth. Thus, a healthy population of MSK-Leuk 1 cells were achieved and can be employed with respect to treatments and trials

Cell Lines

MSK-Leuk 1: MSK leukoplakia cells were used and donated by Dr. Peter Sacks from Memorial Sloan Kettering Cancer Center, New York.

Treatments:

Cells were treated with either metformin or pioglitazone. A total of two trials were conducted in which each trial culture plates were used to plate a computed concentration of MSK-Leuk I cells and were treated with the following dosages: *Pioglitazone*: 5, 10, 20, and 40 μ M + a control well: DMSO *Metformin:* 10 mM, 1 mM, 100 μ M, and 10 μ M + a control well of media alone

Clonogenic Assays

In order to determine cell proliferation after treatments with various concentrations of both pioglitazone and metformin, a clonogenic assay was done. Such a mechanism allows for a clearer observation of cell growth and measurement via the employment of various staining properties.

RESULTS

Figure 1: An illustration of the structure prior to and after reduction of a soluble MTT reagent that yields an insoluble formazan dye.

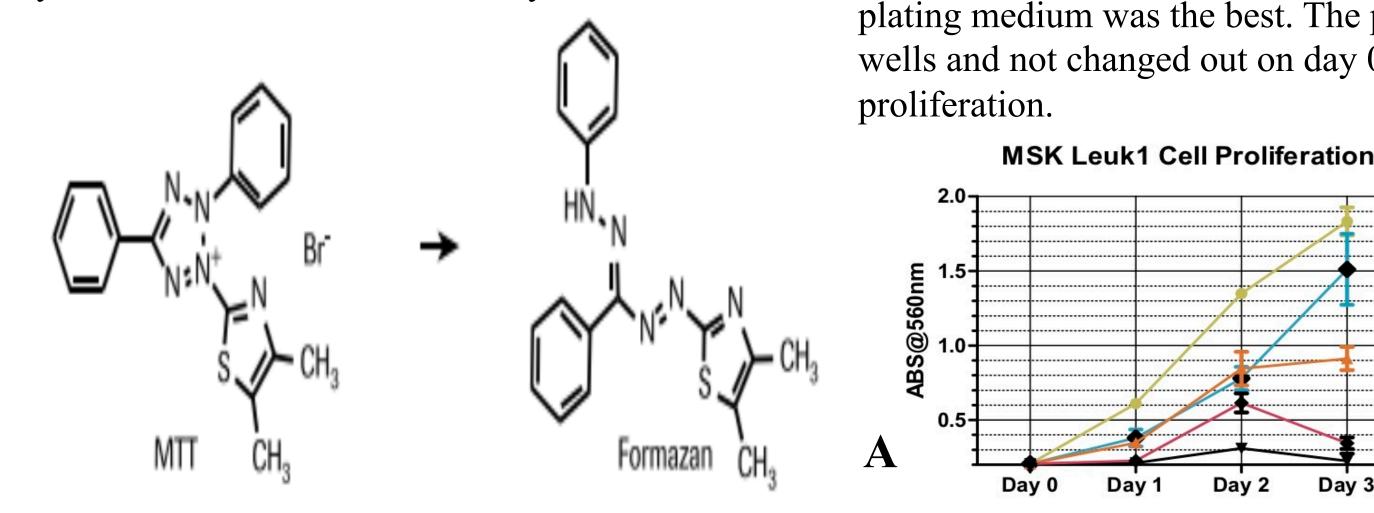


Figure 3: (A,C) MSK-Leuk 1 cell proliferation from day 0-3 after exposure to 4 concentrations of pioglitazone treatment and measured at an absorbance of 560 nm. (A) At day 3, pioglitazone significantly decreased cell proliferation at both 20 μ M and 40 μ M, each with a p value of < 0.001. (C) At day 3, pioglitazone had a statistically insignificant effect on 5 µM (p=0.9549). (B,D) MSK-Leuk 1 cell proliferation from day 0-3 after exposure to 4 concentrations of metformin treatment, also measured at an absorbance of 560 nm. (B) At day 3, metformin significantly decreased cell proliferation at 1 mM (p=0.0335) and 10 mM (p < 0.001). (D) At day 3, metformin had minimal effect on cell proliferation at 10 µM (p=0.0886, >0.5 thus statistically insignificant). Dimethyl sulfoxide (DMSO) added to KGM-2 media and Keratinocyte Growth Medium-2 (KGM-2) alone were used for pioglitazone and metformin treatments, respectively. For the sole purpose, of acting as a solvent control to use as a standard of comparison.

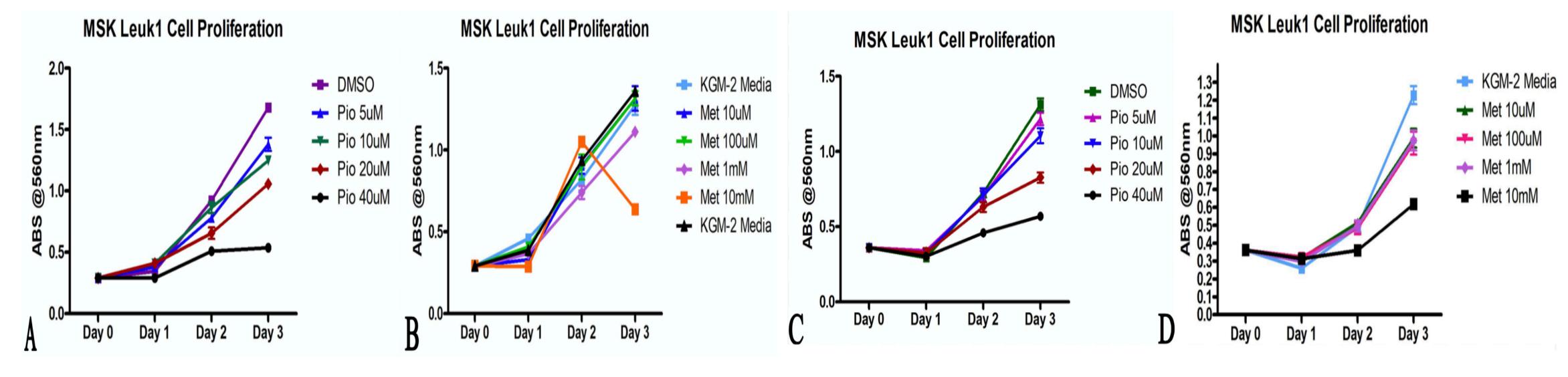


Figure 4: A 6 (3x2) circular well plate being utilized to perform a clonogenic assay in which a series of staining properties and fixatives were implemented allowing for a clear observation of MSK-Leuk 1 clonogenic potential, following treatment of both pioglitazone and metformin. Cell wells A, B, A', and B' were used as a control to act as a standard of comparison to wells with treatment.

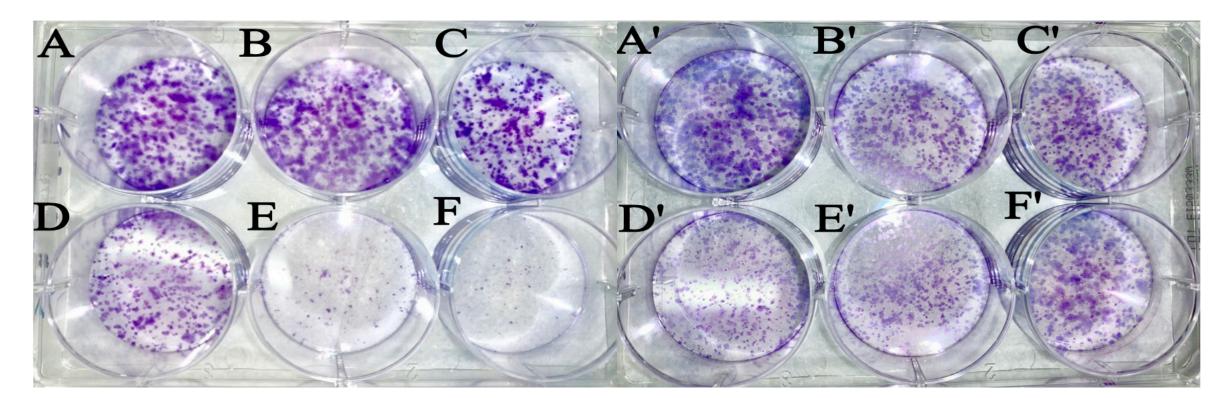
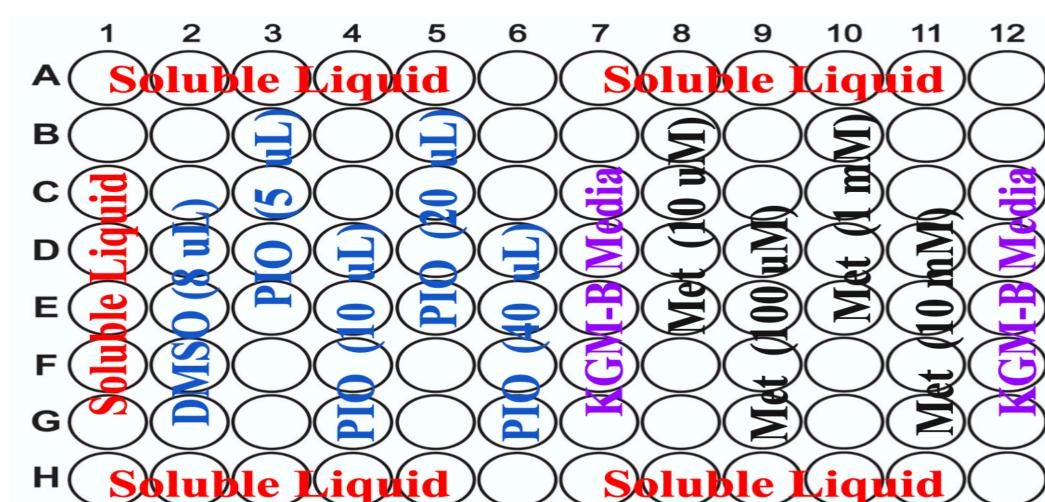


Figure 2: (A & B) An example of the few MTT trials ran to elucidate the best medium to ensure reproducible, reliable growth rates for MSK-Leuk 1 cell proliferation, measured at an absorbance level of 560 nm. In this round of trials, the worst performing medium being Fibroblast, while the plating medium was the best. The plating medium is the KGM-2 medium but was left within the cell wells and not changed out on day 0; in order to observe what effects such a factor would have on

MSK Leuk1 Cell Proliferation - Plating Media Plating Media ← KBM-2 ⊆ 0.8-🛨 Dermalife - Dermalife - Fibroblast Media + KSFM KSFM

Figure 5: An illustration that depicts an example of the organization within the 96-well tissue culture plates utilized within the course of the experiment.



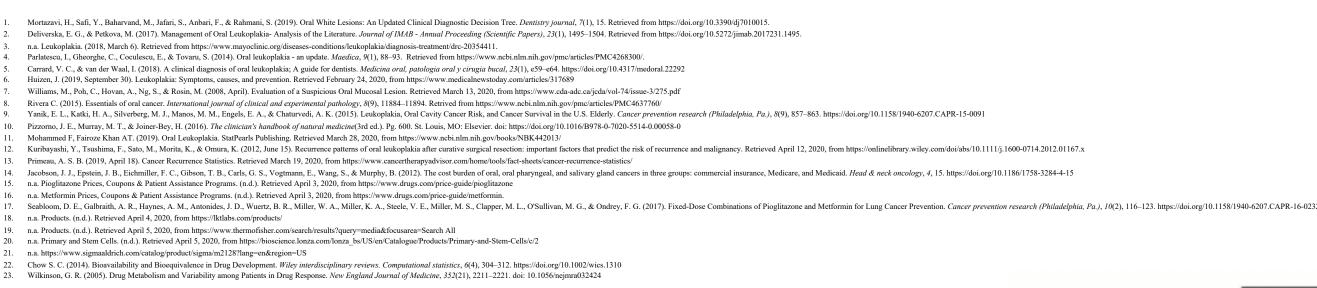
Analysis of Results During the course of this experiment, we have gathered concrete evidence which suggests that continuous exposure of pioglitazone and metformin to MSK-Leuk 1 cells results in a decrease in cell proliferation. Of the two treatments, higher concentrations of pioglitazone yields a much more substantial decrease of proliferation, when compared to the higher concentrations of metformin. In fact, referring to figure 3A, at day 3, 40 µM pioglitazone resulted in MSK-Leuk 1 cells to decrease to a value of ~0.508 (abs @560 nm). Whereas, referring to figure 3D, at day 3, 10 mM concentration of metformin resulted in MSK-Leuk 1 cell to only decrease to a value of ~0.597 (abs @560 nm). Amounting to about a 85% increase; suggesting that the molecular makeup of pioglitazone has unique properties which allows it disrupt the cell pathways of cancerous cells in a more efficient and an effective manner.

As mentioned before, studies have shown that "the rate of recurrence is between 7.7% to 38.1%."^{11,12} One would look at such odds as being negligible, however, let's compare it with the recurrence rate of 5 of the top 10 most common cancers in the U.S (skin, lung, prostate, breast, and colorectal cancer), for simplicity.^G With the aid of a study, the average rate of recurrence of such cancers is around 28.4%.¹³ Thus, allowing one to view such a number from a different perspective. In addition to the fact that the proposed treatment is not only ineffective but causes an individual to endure a painful experience (as far as 3 weeks post-op), as well as a financial burden (especially those without medical insurance). In fact, according to a study, the "total annual health care spending for OC (oral cancer) patients during the year after the index diagnosis was \$79,151... "¹⁴

Therefore, our treatment option is quite the better alternative, even though it's at the early clinical trial stages. Thus, there has been no data collection of the recurrence rate, but so far has shown remarkable results within patients and experiments within laboratory trials. Also, being a fraction of cost compared to the current treatment option, with the added benefit of taking the place of painful surgery.

With regards to further investigation, clinical studies were recently established in our lab and are soon to be subjected to further modification when we understand and study the synergistic effects of both drugs, in order to maximize such results. All in all, this proposed revolutionary treatment option is not only cost effective, efficient, but with further research we may be able to treat other cancerous cell types!

This project would also not be feasible without the aid of my two mentors, Dr. Frank Ondrey (principal investigator) and Mrs. Beverly Wuertz (laboratory manager). They have guided me throughout each step and instilled a sense of knowledge of various laboratory studies that have been very essential in undergoing such an experiment.





CONCLUSIONS

ACKNOWLEDGEMENTS



(Philadelphia, Pa.), 8(9), 857-863. https://doi.org/10.1158/1940-6207.CAPR-15-0091

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