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Investigating the Synergistic Effects of Two Curcuminoids and Cisplatin on Cancer Cell Migration

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INVESTIGATING THE SYNERGISTIC EFFECTS OF TWO CURCUMINOIDS AND CISPLATIN ON CANCER CELL MIGRATION

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By

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I dedicate this thesis to my parents, Rick and Rita, and my brother, Tyler, for supporting me during my collegiate career. Also, I dedicate this to all those who are fighting cancer and to the families of those with the disease.

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ABSTRACT

Cisplatin is a common chemotherapy drug used to treat various cancers; however, it is relatively ineffective against many cancers, including several types of lung cancer. One approach that could improve cisplatin's effect is to combine it with another drug that produces a synergistic response greater than either drug alone. Curcumin, a naturally occurring plant compound, has been investigated for synergisms in conjunction with cisplatin chemotherapy, but curcumin use is hampered by its low bioavailability. This project investigated whether two synthetic curcumin analogs, EF-24 and CLEFMA (curcuminoids), which have greater solubility than curcumin, could, when combined with cisplatin, decrease the migration rate of the non-small cell lung cancer cell line, A549. Dishes of A549 were treated with media only control, cisplatin, EF-24, CLEFMA or combinations of cisplatin with either curcumin analog. Then, a scratched clearing was introduced through the cells and photographs were taken at timed intervals to determine if either curcuminoid increased the effect of cisplatin by slowing cell migration into the cleared area. We found that when combined with cisplatin, both curcuminoids increased the effectiveness of cisplatin against cancer cell migration and reduced wound recovery.

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INTRODUCTION

Nearly 1.7 million people were diagnosed with cancer in the United States in 2017, and around 600,000 of them are projected to die from this disease (Siegel et al., 2017). The sheer number of these cancer statistics suggests that further improvements to current cancer treatments are required.

One hallmark of cancer is its ability to metastasize or migrate to surrounding tissue or to other parts of the body (Hanahan & Weinberg, 2011), and this aspect of cancer is now a target for many therapeutic interventions. Cisplatin is a commonly used platinum-based chemotherapy that is effective against bladder, ovarian, and testicular cancer (Dasari & Tchounwou, 2014). This platinum compound can also slow cancer cell migration (Yin et al., 2018). However, cisplatin typically is not effective against many cancers, including non-small cell lung cancers, often due to the development of resistance to this drug (Shen et al., 2012). Therefore, there is considerable interest in improving cisplatin's effectiveness, including enhancing its effect against migration.

Curcumin is a naturally occurring liposoluble pigment that is extracted from the rhizome of *Curcuma longa*, and is commonly used in Asian cuisine (Ali et al., 2017; Liu et al., 2017; Shen et al., 2017). It also has been used as a pharmacological agent in Chinese medicine due to its anti-inflammatory, anticoagulant, and antioxidant properties. In addition, it possesses anticancer effects by suppressing the mTOR/PI3K/AKT pathway which induces apoptosis and autophagy (Wang et al., 2017). Curcumin is currently being tested on non-small cell lung cancers including the cancer cell line, A549 (Liu et al., 2017; Shen et al., 2017; Shi et al., 2017a; Wang et al., 2017). However, curcumin exhibits low-bioavailability, i.e. low solubility, which reduces its effectiveness (Skoupa et

al, 2017). Due to this, there is considerable interest in developing synthetic curcumin analogs (curcuminoids) that exhibit higher bioavailability but retain curcumin's anticancer efficacy.

The two curcuminoids studied in this project were (3E,5E)-3,5-bis[(2-fluorophenyl)methylene]-4-piperidinone (EF-24), and 4-[3,5Bis[(2-chlorophenyl) methylene]-4-oxo-1-piperidinyl]-4-oxo-2-butenoic acid (CLEFMA). These two curcuminoids have higher bioavailability than curcumin, and both have anticancer effects (Sahoo et al, 2012; Skoupa et al., 2017). EF-24 has been shown to induce apoptosis in K562 myelogenous leukemia cancer cells, and CLEFMA has been shown to induce apoptosis in H441 lung adenocarcinoma cancer cells (Sahoo et al, 2012; Skoupa et al., 2017). Although these curcuminoids do have anticancer effects, their effect against cancer cell migration and in combination with cisplatin has yet to be determined.

The purpose of my research project was to investigate if EF-24 and CLEFMA have a synergistic effect with cisplatin against A549 cancer cell migration. A549 is a non-small cell lung carcinoma cell line that is commonly used in cancer research, and it has been used to study curcumin and cisplatin (Baharuddin et al., 2015; Tung et al., 2016). A cell migration assay was used to measure if the curcuminoids increased cisplatin's effect against A549 cellular migration into an area of a dish cleared of cells. There were six different treatment groups: control, cisplatin, EF-24, CLEFMA, cisplatin and EF-24, and cisplatin and CLEFMA. The percent wound recovery rate was measured to see what the effect of cisplatin, and the curcuminoids alone or in combination with cisplatin had on cancer cell migration.

It was found that the cisplatin only, EF-24 only, and CLEFMA only treatment groups did not significantly reduce the cell migration rate. However, the combination groups (cisplatin and EF-24; cisplatin and CLEFMA) did significantly reduce the migration rate. My results suggest that the two curcuminoids increased cisplatin's effectiveness against cancer cell migration.

METHODS

Cell Migration Assay

This study used a cell migration assay to investigate how the curcuminoids alone or with cisplatin treatment affected cancer cell migration. The A549 cell line was grown in 10 cm diameter dishes in F12K media in an incubator (37 °C and 5% CO_2). The cell line was grown to approximately 90% confluence, and then the media was aspirated out. Then, a scratch was made with a 200 µL pipet tip through the cell monolayer. Once the wound was made, the cells were immediately treated with one of the following for 24 hours: 15 µM CLEFMA only, 2 µM EF-24 only, 10 µM cisplatin only, 10 µM cisplatin with 15 µM CLEFMA, 10 µM cisplatin with 2 µM EF-24, and a media control. These concentrations are the IC_{50} values determined in our lab during another project for the A549 cell line. For the cisplatin with curcuminoid treatments, the curcuminoid was added three hours after the cisplatin. This was to ensure that the cisplatin could enter the cells and their nuclei (Park et al., 2012) before the curcuminoid vehicle, DMSO, which can inactivate cisplatin (Hall et al., 2014), was introduced. For treatments where cisplatin and a curcuminoid were combined, the wound was not made until after curcuminoid addition. Each treatment category was performed in triplicate.

A Nikon camera (DS-5M) attached to a Nikon TMS microscope was used to take pictures of the wound in the dishes at zero hours, the time the wound was made, and at twenty-four hours after wound introduction (Figure 1). Then, the area of the wound was measured using Adobe Photoshop's lasso function. The percent wound recovery was then calculated by subtracting the cleared area at 24 hours from the cleared area at 0

hours and then dividing the resultant value by the original cleared area and converting to a percentage.

Statistical Analysis

A one-way ANOVA with a Dunnett's multiple comparison test was used to analyze all migration assay results. The *p* value was set at 0.05.

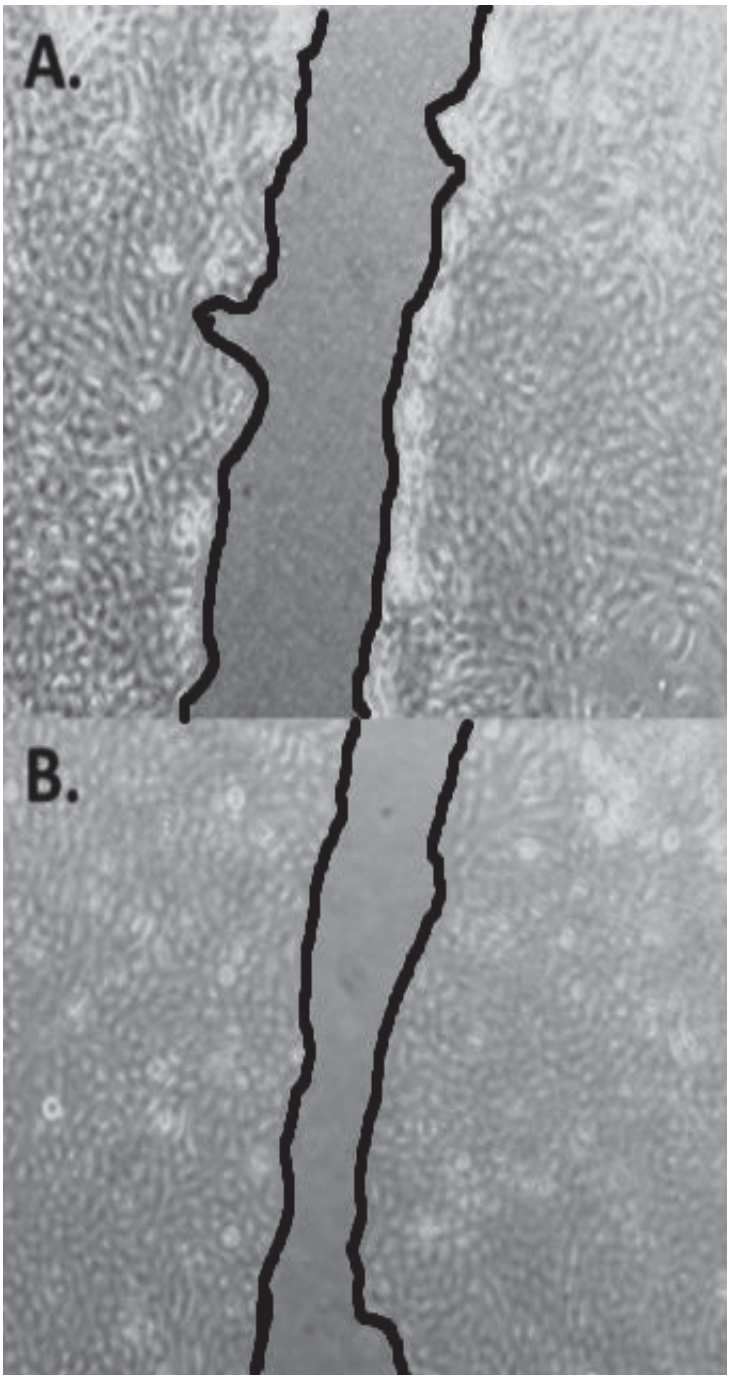

Figure 1: A549 cellular migration over a 24 hour treatment interval. A.-B. Photographs taken from the same coordinate at a location of initial total A549 confluence in dishes treated with F-12K media (vehicle control). A. t = 0 hours post-wound. B. t = 24 hours post-wound.

RESULTS

This study used a cellular migration assay to assess whether cisplatin, EF-24, CLEFMA or a combination of cisplatin with either curcuminoid altered A549 cellular migration. Figure 2 shows wound examples corresponding to each treatment group. The percent wound recovery for the cisplatin treatment group was 19.1%, which was not statistically different ($p > 0.05$) than the control treatment group's percent wound recovery of 25% (Figure 3). The EF-24 and the CLEFMA only treatments had a percent wound recovery of 21.9% and 22.8%, respectively. Both of these values, like cisplatin, were also not statistically different ($p > 0.05$) from the control (Figure 4). However, when cisplatin treatment was followed by either curcuminoid, the percent wound recovery was significantly reduced ($p < 0.05$) compared to control (Figure 5). The cisplatin and EF-24 treatment group had a percent wound recovery of 13.3%, and the cisplatin and CLEFMA treatment group had a percent wound recovery of 12.8%. When compared to cisplatin only, the combination treatments did not have a significant difference in percent wound recovery (Figure 5). When the cisplatin only treatment group is compared to the cisplatin and EF-24 group, there is a 5.8% difference, and when compared to the cisplatin and CLEFMA treatment group, there is a 6.3% difference. The difference between EF-24 only and the combination treatment of cisplatin and EF-24 is 8.6%; and the difference between CLEFMA only and the cisplatin and CLEFMA group is 10%. Figure 6 shows a summary of all the treatment groups and their percent wound recovery.

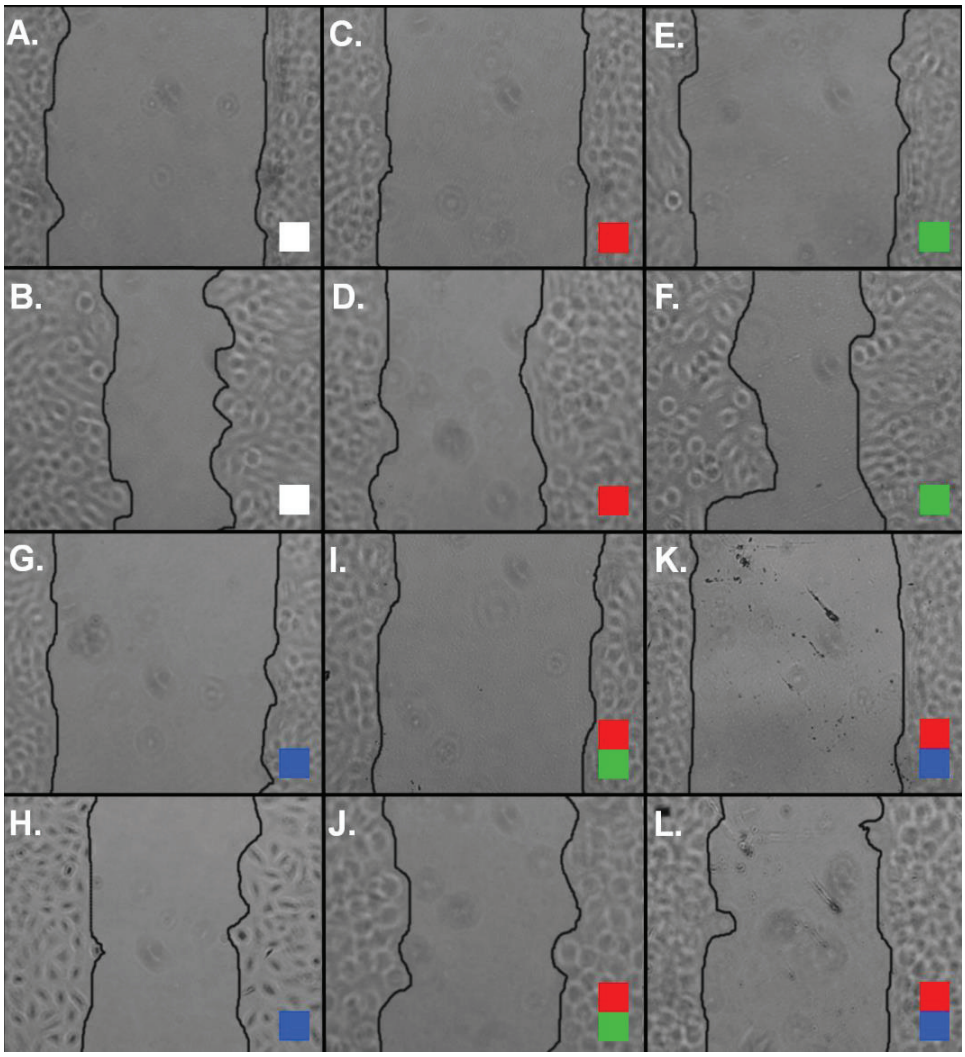

Figure 2: Combining cisplatin with a curcuminoid caused A549 cell migration to decrease. Color code (white box = control; red box = cisplatin only; green box = EF-24 only; blue box = CLEFMA only; red and green box = cisplatin with EF-24; red and blue box = cisplatin with CLEFMA. A. Control (0 Hr). B. Control (24 Hr). C. Cisplatin (0 Hr). D. Cisplatin (24 Hr). E. EF-24 (0 Hr). F. EF-24 (24 Hr). G. CLEFMA (0 Hr). H. CLEFMA (24 Hr). I. Cisplatin + EF-24 (0 Hr). J. Cisplain + CLEFMA (0 Hr). L. Cisplatin + CLEFMA (24 Hr).

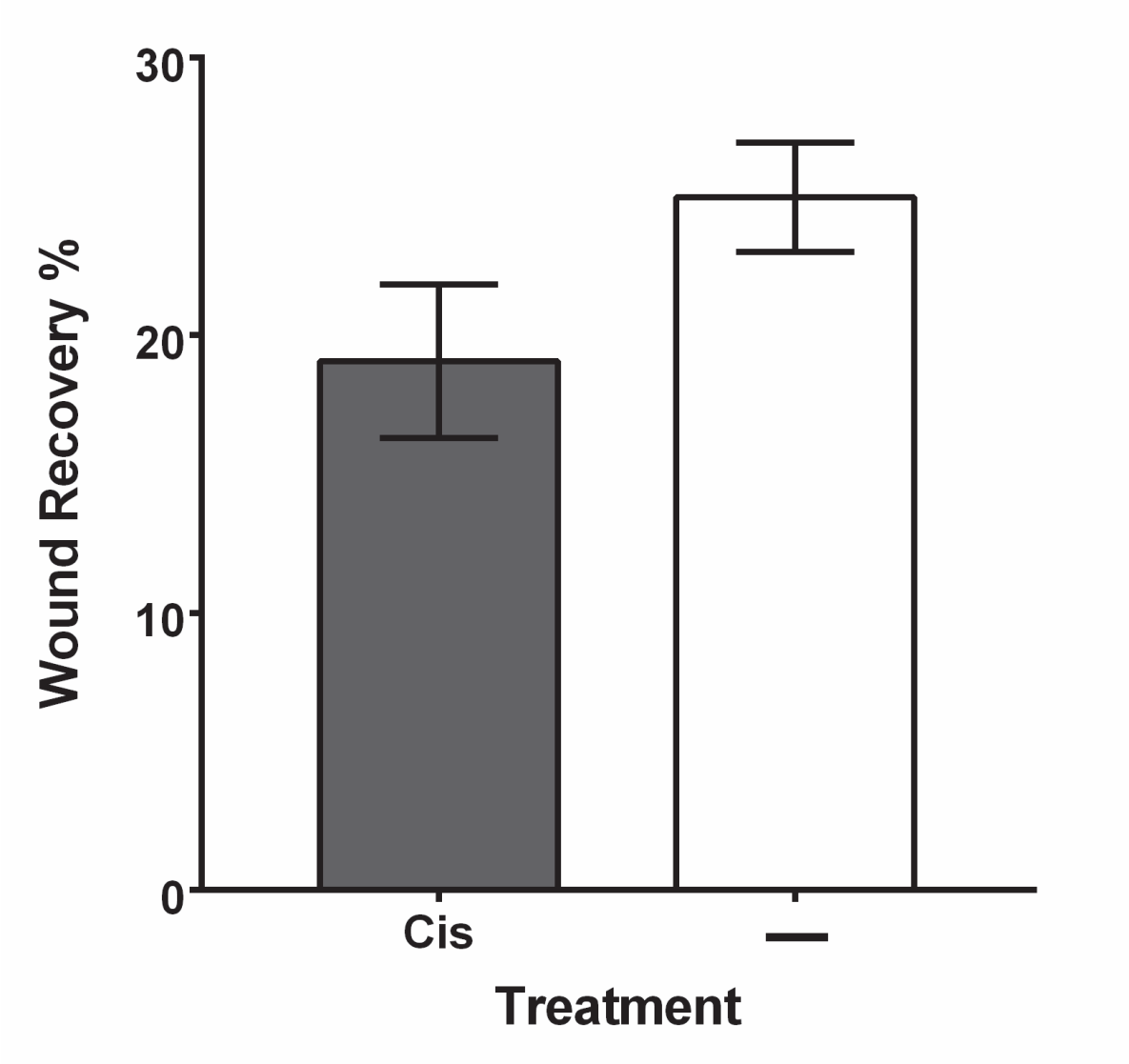

Figure 3: Cisplatin treatment does not reduce A549 migration compared to control. N = 3; $p > 0.05$.

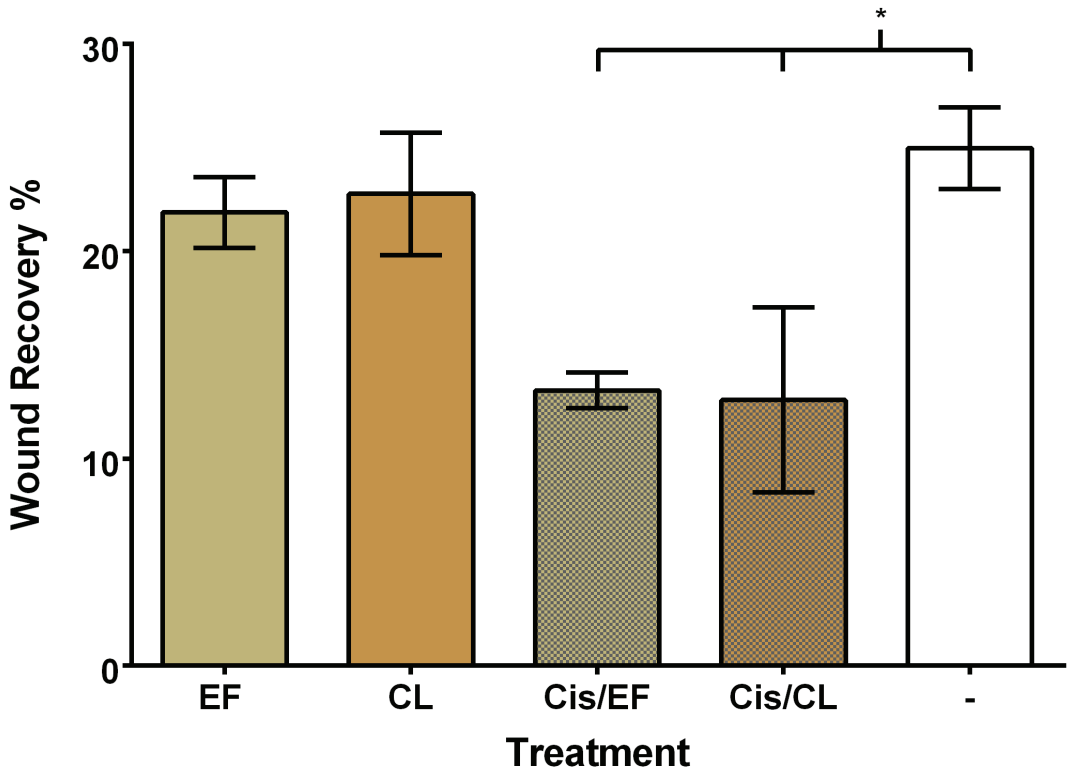

Figure 4: Combination of cisplatin and a curcuminoid decreased A549 wound recovery. Curcuminoid treatment alone was not statistically different than vehicle control. Combination of cisplatin with either EF-24 or CLEFMA significantly reduced migration. N = 3; * $p < 0.05$.

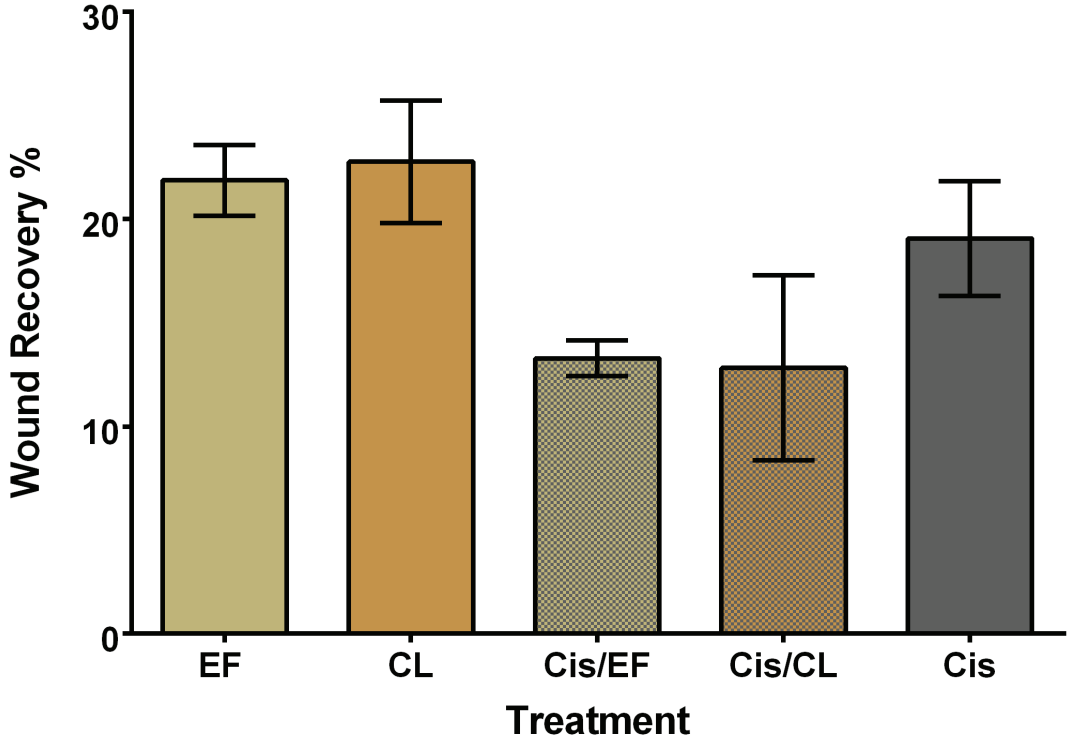

Figure 5: Curcuminoid and curcuminoid-cisplatin treatments do not alter A549 wound recovery compared to cisplatin alone. N= 3; $p > 0.05$.

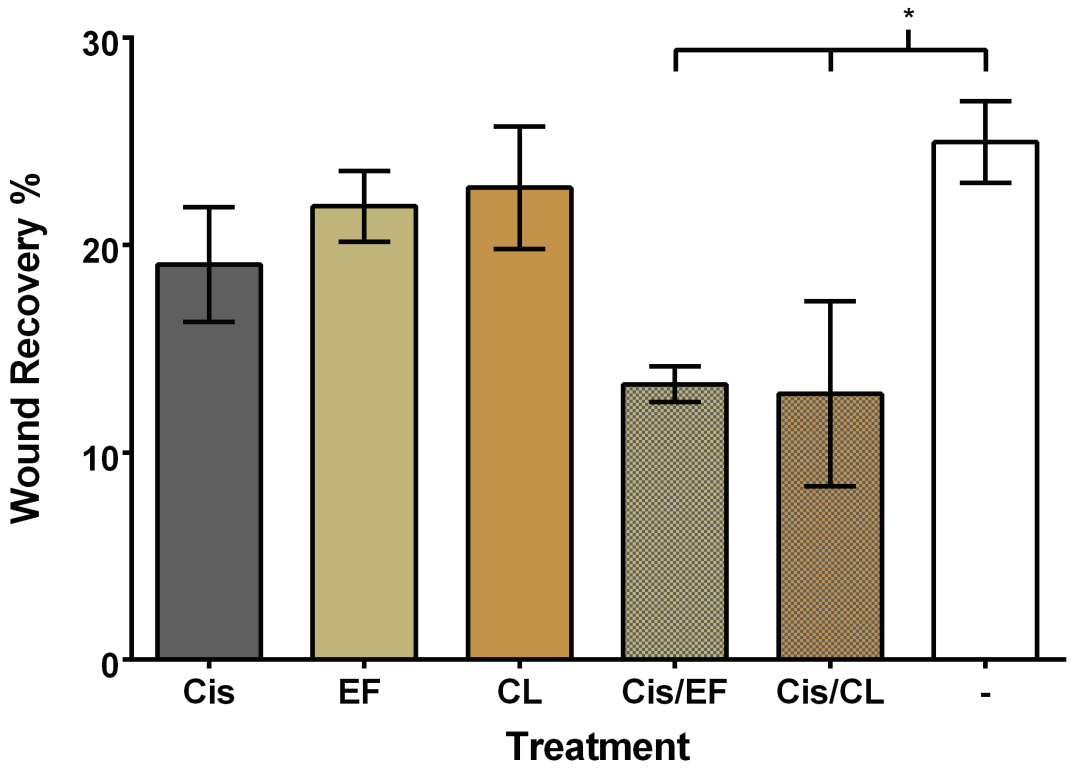

Figure 6: Only the combination of cisplatin with a curcuminoid reduces A549 wound recovery relative to the negative control. N = 3; * $p < 0.05$.

DISCUSSION

Combining chemotherapy drugs that target different cell pathways could increase their individual effects against cancer cell migration. Cisplatin treatment can decrease migration in the A549 lung cancer cell line but can become ineffective due to the development of resistance (Tung et al., 2016; Kim et al., 2017). Similarly, curcumin has an effect against A549 lung cancer cell migration (Baharuddin et al., 2016; Jiao et al., 2016). However, curcumin exhibits solubility issues, and therefore is inefficient to use in cancer treatment (Skoupa et al., 2017). Because of this, we reasoned that the curcuminoids EF-24 and CLEFMA, which are more soluble structural analogs of curcumin (Skoupa et al., 2017; Sahoo et al., 2012), might have an effect against cancer cell migration. Further, combinations of curcumin with cisplatin in the A549 cell line caused migration to decrease (Baharuddin et al., 2016) which suggested to us that treatment with both a curcuminoid and cisplatin might reduce cancer cell motility. We used a cell migration assay to assess the effect of cisplatin, EF-24, CLEFMA, and combinations of cisplatin with either curcuminoid against A549 lung cancer cell migration.

We initially treated A549 cells with cisplatin or one of the curcuminoids to assess if these compounds could individually reduce cell migration. Cisplatin did not significantly reduce migration compared to vehicle control treatment (Figure 3; $p > 0.05$). The percent wound recovery for the cisplatin treatment was 19.1%; whereas, vehicle recovery was 25%. Similarly, both curcuminoids did not significantly reduce migration (Figure 4; $p > 0.05$). The EF-24 only treatment group had a percent wound recovery of 21.9%, and the CLEFMA only treatment group had a percent wound recovery of 22.8%.

These results indicate that cisplatin and curcuminoid treatment are ineffective against cancer cell migration, but their combination does exhibit an increased effect against migration (Figure 6; $p < 0.05$).

Both cisplatin and curcuminoid treatment together could decrease cancer cell migration by targeting separate pathways that control motility. Cisplatin signals through multiple pathways to effect cancer cell migration. For example in A549 cells, cisplatin signals through a pathway incorporating transforming growth factor β1 (TGF-β1; Kim et al., 2017). Sox2 and Wnt/β catenin signaling have also been implicated in A549 cell migration during cisplatin treatment (He et al., 2017a). The microRNA, miR-146a, modulates migration in cisplatin treated A549 cells through regulation on cyclin J, which controls cell mitosis in oncogensis (Shi et al., 2017b). Like cisplatin, curcumin effects cancer cell migration by signaling through discrete pathways. In A549 cells, treatment with curcumin can reduce migration by modulating the microRNA, miR-330-5p, and pathways incorporating mitogen-activated protein kinase, TGF-β and Wnt (Zhan et al., 2017). Reduced migration due to modulation of c-Met, Akt, mTOR, and S6 in curcumin treated A549 cells has also been reported (Jiao et al., 2016). As both cisplatin and curcumin target some common cellular migration pathways, e.g., TGF-β and Wnt, in A549, combining these two compounds could produce synergistic effects against motility. Indeed, combinations of curcumin and cisplatin have reduced the invasive property of A549 cells by downregulating Bcl-2 while upregulating Bax (He et al., 2017b). Also, curcumin can improve cisplatin's effect against A549 migration by downregulating cyclin D1 and upregulating p21 mRNA expression (Baharuddin et al., 2016). Further, the curcuminoid, H-4073, can enhance cisplatin's effect against head and

neck cancer migration by inhibiting the STAT, FAK, Akt, and VEGF pathways (Kumar et al., 2014). The results obtained from combining cisplatin with curcumin or the curcuminoid, H-4073, suggest that other curcuminoids such as EF-24 and CLEFMA might also be able to enhance the effect of cisplatin against cancer cell migration.

Our investigation of the effects on migration from combining cisplatin with either EF-24 or CLEFMA showed that together they significantly reduced A549 cell migration. Cisplatin with EF-24 and CLEFMA significantly reduced cell migration to 13.3% and 12.8% of control treatment respectively (Figure 4; $p < 0.05$). Although, cisplatin-curcuminoid treatment did not reduce migration significantly below that of cisplatin alone (cisplatin-EF-24 = 5.8% and cisplatin-CLEFMA = 6.3%) (Figure 5; $p > 0.05$), a greater, but still not significant reduction was seen between the individual curcuminoids and their combination with cisplatin (EF-24 vs. cisplatin-EF-24 = 8.6% and CLEFMA vs. cisplatin-CLEFMA = 10%) (Figure 4-5; $p > 0.05$). These results indicate that combining cisplatin with either curcuminoid can enhance the effect of cisplatin alone against A549 cancer cell migration, although this effect does not appear to be either synergistic or additive in nature.

Our results from combining EF-24 and CLEFMA suggest that they could signal through different cellular motility pathways than cisplatin and enhance the platinum compound's effect against cancer cell migration. Although a comparative signal transduction analysis of cisplatin and CLEFMA has not yet been performed, studies have shown that EF-24 and cisplatin can signal through different pathways or target distinct components of the same pathway. In cisplatin sensitive and resistant human ovarian cancer cells, cisplatin does not induce caspase-3 expression; however, EF-24 induces the

expression of this protein (Tan et al., 2010). In malignant mesothelioma cells, cisplatin caused anti-apoptosis gene Bcl2 mRNA expression to decrease, but EF-24 did not have this effect (Onen et al., 2015). Cisplatin and EF-24 might be able to effect migration by acting on different components of the same pathway. EF-24 can form an adduct with the antioxidant glutathione in leukemia cancer cells, which could be responsible for increased ROS expression in these cells (Skoupa et al., 2017). However, cisplatin can reduce the activity of another component of this antioxidant pathway, glutathione S-transferase, an enzyme that binds glutathione to reactive oxygen species (Mukherjea and Rybak, 2011). Thus, these studies suggest that cisplatin and one of our curcuminoids, EF-24, can target distinct pathways or the same pathway by acting on different signaling components.

The cisplatin-curcuminoid treatments exhibited a significant decrease in migration compared to control, but not when compared to either cisplatin or the curcuminoids alone (Figure 6; $p < 0.05$). Even though cisplatin or the curcuminoids individually did not reduce A549 migration, these treatments did cause a detectable reduction in migration (Figure 6). If either cisplatin or a curcuminoid causes a non-significant effect against migration in the same or separate pathways, then combining these compounds might increase the effect in the same pathway or through summating signals traveling in separate pathways, and then, the combination could induce a significant reduction in cancer cell migration. Evidently, future work needs to be done to elaborate the precise pathways that cisplatin, EF-24, and CLEFMA target in the A549 cell line. Having this information will allow us to better understand how these compounds influence different cancer cell migration signaling pathways and how these pathways intersect and regulate each other.

FUTURE DIRECTIONS

More research needs to be done on how these two curcuminoids increase cisplatin's effectiveness against cancer cell migration. Research conducted recently in our laboratory has shown that combining either curcuminoid with cisplatin does not cause a synergistic effect against A549 cancer cell viability. As increased reactive oxygen species (ROS) release is implicated in cancer cell death, we have also investigated whether cisplatin and the curcuminoids increased ROS release in A549 cells, and found that the curcuminoids alone do not cause this effect and that combining cisplatin with a curcuminoid reduced ROS levels (unpublished data). In the future, caspase and Western blot assays will be used to identify which apoptotic pathways are modulated by cisplatin, the curcuminoids and their combination in the A549 cell line. We hope that the knowledge obtained from this research could prove to be valuable in the development of future pharmaceutical interventions for treating lung and other cancers.

REFERENCES

Aggarwal, G., & Aggarwal, S. K. (2017). Evolution and progress in the application of radiation in cancer diagnosis and therapy. *Current Science (00113891)*, *113*, 413-421. doi:10.18520/cs/v113/i03/413-421

Ali, N. M., Yeap, S. K., Abu, N., Lim, K. L., Ky, H., Pauzi, A. Z. M., Yong, Ho, W.Y., Tan, S.W., Alan-Ong, H.K., Zareen, S., Alitheen, N.B., & Akhtar, M. N. (2017). Synthetic curcumin derivative DK1 possessed G2/M arrest and induced apoptosis through accumulation of intracellular ROS in MCF-7 breast cancer cells. *Cancer Cell International*, *17*, 30. doi:10.1186/s12935-017-0400-3

Baharuddin, P., Satar, N., Fakiruddin, K. S., Zakaria, N., Lim, M. N., Yusoff, N. M., Zakaria, Z., & Yahaya, B. H. (2016). Curcumin improves the efficacy of cisplatin targeting cancer stem-like cells through p21 and cyclin D-1 mediated tumour cell inhibition in non-small cell lung cancer cell lines. Oncology Reports, *35*, 13-25. doi:10.3892/or.2015.4371

Bisht, S., Schlesinger, M., Rupp, A., Schubert, R., Nolting, J., Wenzel, J., Holdenrieder, S., Brossart, P., Bendas, G., & Feldmann, G. (2016). A liposomal formulation of the synthetic curcumin analog EF24 (Lipo-EF24) inhibits pancreatic cancer progression: towards future combination therapies. *Journal of Nanobiotechnology*, *14*, 1-15. doi:10.1186/s12951-016-0209-6

Dasari, S., & Tchounwou, P. B. (2014). Cisplatin in cancer therapy: molecular mechanisms of action. *European Journal of Pharmacology*, *5*, 364–378. doi:10.1016/j.ejphar.2014.07.025

Hall, M. D., Telma, K. A., Chang, K., Lee, T. D., Madigan, J. P., Lloyd, J. R., Goldlust, I.S., Hoeschele, J.D., & Gottesman, M. M. (2014). Say no to DMSO: dimethylsulfoxide inactivates cisplatin, carboplatin, and other platinum complexes. *Cancer Research, 74*, 3913-3922. doi:10.1158/0008-5472.CAN-14-0247

He, J., Shi, J., Zhang, K., Xue, J., Li, J., Yang, J., Chen, J., Wei, J., Ren, H., & Liu, X. (2017a). Sox2 inhibits Wnt-β-catenin signaling and metastatic potency of cisplatin-resistant lung adenocarcinoma cells. *Molecular Medicine Reports*, *15*, 1693-1701. doi:10.3892/mmr.2017.6170

He, B., Wei, W., Liu, J., Xu, Y., & Zhao, G. (2017b). Synergistic anticancer effect of curcumin and chemotherapy regimen FP in human gastric cancer MGC-803 cells. *Oncology Letters, 14*, 3387-3394. doi:10.3892/ol.2017.6627

Jiao, D., Wang, J., Lu, W., Tang, X., Chen, J., Mou, H., & Chen, Q. (2016). Curcumin inhibited HGF-induced EMT and angiogenesis through regulating c-Met dependent PI3K/Akt/mTOR signaling pathways in lung cancer. *Molecular Therapy Oncolytics*, *3*, 16018. doi:10.1038/mto.2016.18

Kim, S., Park, J., Zhang, D., Cho, S., Yi, H., Cho, S., Chang, B., Kim J., Shim, J., El-Aty, A., & Shin, H. (2017). Sustainability of CD24 expression, cell proliferation and migration, cisplatin-resistance, and caspase-3 expression during mesenchymal-epithelial transition induced by the removal of TGF- β1 in A549 lung cancer cells. *Oncology Letters, 14*, 2410-2416. doi: 10.3892/ol.2017.6398

Kumar, B., Yadav, A., Hideg, K., Kuppusamy, P., Teknos, T., & Kumar, P. (2014). A novel curcumin analog (H-4073) enhances the therapeutic efficacy of cisplatin treatment in head and neck cancer. *PloS ONE, 9*, e93208. doi:10.1371/journal.pone.0093208

Liu, F., Gao, S., Yang, Y., Zhao, X., Fan, Y., Ma, W., Yang, D., Yang, A., & Yu, Y. (2017). Curcumin induced autophagy anticancer effects on human lung adenocarcinoma cell line A549. *Oncology Letters,14*, 2775-2782. doi:10.3892/ol.2017.6565

Mou, S., Zhou, Z., He, Y., Liu, F., & Gong, L. (2017). Curcumin inhibits cell proliferation and promotes apoptosis of laryngeal cancer cells through Bcl-2 and PI3K/Akt, and by upregulating miR-15a. *Oncology Letters*, *14*, 4937-4942. doi:10.3892/ol.2017.6739

Mukherjea, D., & Rybak, L. P. (2011). Pharmacogenomics of cisplatin-induced ototoxicity. *Pharmacogenomics*, *12*, 1039–1050. doi:10.2217/pgs.11.48

Onen, H.I., Yilmaz, A., Alp, E., Celik, A., Demiroz, S.M., Konac, E., Kurul, I.C., & Menevse, E.S. (2015). EF24 and RAD001 potentiates the anticancer effect of platinum-based agents in human malignant pleural mesothelioma (MSTO-211H) cells and protects nonmalignant mesothelial (MET-5A) cells. *Human and Experimental Toxicology*, *34*, 117-126. doi:10.1177/0960327114542965

Pang, L., Lu, J., Huang, J., Xu, C., Li, H., Yuan, G., Cheng, X., & Chen, J. (2017). Upregulation of miR-146a increases cisplatin sensitivity of the non-small cell lung cancer A549 cell line by targeting JNK-2. *Oncology Letters, 14,* 7745-7752. doi:10.3892/0l.2017.7242

Park, G. Y., Wilson, J. J., Song, Y., & Lippard, S. J. (2012). Phenanthriplatin, a monofunctional DNA-binding platinum anticancer drug candidate with unusual potency and cellular activity profile. *Proceedings of the National Academy of Science of the United States of America,109*, 11987-11992. doi:10.1073/pnas.1207670109

Reid, J., Buhrow, S., Gilbert, J., Jia, L., Shoji, M., Synder, J., & Ames, M. (2014). Mouse pharmacokinetics and metabolism of the curcumin analog, 4-Piperidone,3,5-bis[(2-fluorophenyl)methylene]-acetate(3E,5E) (EF-24; NSC716993). *Cancer Chemotherapy and Pharmacology, 73*, 1137-1146. doi:10.1007/s00280-014-2447-3

Sahoo, K., Dozmorov, M. G., Anant, S., & Awasthi, V. (2012). The curcuminoid CLEFMA selectively induces cell death in H441 lung adenocarcinoma cells via oxidative stress. *Investigational New Drugs*, *30*, 558–567. doi:10.1007/s10637-010-9610-4

Shen, D.-W., Pouliot, L. M., Hall, M. D., & Gottesman, M. M. (2012). Cisplatin resistance: a cellular self-defense mechanism resulting from multiple epigenetic and genetic changes. *Pharmacological Reviews*, *64*, 706–721. doi:10.1124/pr.111.005637

Shen, J., Chen, Y., Jia, Y., Zhao, W., Chen, G., Liu, D., . . . Liu, X. (2017). Reverse effect of curcumin on CDDP-induced drug-resistance via Keap1/p62-Nrf2 signaling in A549/CDDP cell. *Asian Pacific Journal of Tropical Medicine, 10*, 1190-1196. doi:10.1016/j.apjtm.2017.10.028

Shi, J., Zhang, X., Shi, T., & Li, H. (2017a). Antitumor effects of curcumin in human bladder cancer in vitro. *Oncology Letters*, *14*, 1157-1161. doi:10.3892/ol.2017.6205

Shi, L., Xu, Z., Wu, G., Chen, X., Huang, Y., Wang, Y., Jiang, W., & Ke, B. (2017b). Up-regulation of miR-146a increases the sensitivity of non-small cell lung cancer to DDP by downregulating cyclin J. *BMC Cancer, 17,* 138. doi:10.1186/s12885-017-3132-9.

Siegel, R. L., Miller, K. D., & Jemal, A. (2017). Cancer statistics, 2017. *CA: A Cancer Journal for Clincians, 67*, 7-30. doi:10.3322/caac.21387

Singh, V., Arora, D., Satija, N., Khare P., Roy S., & Sharma, P. (2017). Intricatinol synergistically enhances the anticancerous activity of cisplatin in human A549 cells via p38 MAPK/p53 signalling. *Apoptosis, 22*, 1273-1286. doi:10.1007/s10495-017-1404-0.

Skoupa, N., Dolezel, P., Ruzickova, E., & Mlejnek, P. (2017). Apoptosis induced by the curcumin analogue EF-24 is neither mediated by oxidative stress-related mechanisms nor affected by expression of main drug transporters ABCB1 and ABCG2 in human leukemia cells. *International Journal of Molecular Sciences*, *18*, 1-18. doi:10.3390/ijms18112289

Tan, X., Sidell, N., Mancini, A., Huang, R., Wang, S., Horowitz, I., Liotta, D., Taylor, R., & Wieser, F. (2010). Multiple anticancer activities of EF24, a novel curcumin analog, on human ovarian carcinoma cells. *Reproductive Sciences, 17,* 931-940. doi:10.1177/1933719110374239

Tung, C., Jian, Y., Chen, J., Wang, T., Chen, W., Zheng, H., Chang, P., Liao, K., & Lin, Y. (2016). Curcumin downregulates p38 MAPK-dependent X-ray repair cross-complement group 1 (XRCC1) expression to enhance cisplatin-induced cytotoxicity in human lung cancer cells. *Naunyn-Schmiedeberg's Archives of Pharmacology, 389*, 657-666. doi:10.1007/s00210-016-1235-5

Wang, A,. Wang, J., Zhang, S., Zhang, H., Xu, Z., & Li, X. (2017). Curcumin inhibits the development of non-small cell lung cancer by inhibiting autophagy and apoptosis. *Experimental & Therapeutic Medicine*, *14*, 5075-5080. doi:10.3892/etm.2017.5172

Yang, C., Liu, Y., Ma, Y., Xue, Y., Liu, D., Ren, Y., Liu, X., Li, Y., & Li, Z. (2012). Curcumin blocks small cell lung cancer cells migration, invasion, angiogenesis, cell cycle and neoplasia through Janus kinase-STAT3 signalling pathway. *PLoS One, 7*, e37960. doi:10.1371/journal.pone.0037960

Yin, P., Song, G., & Jiang, Z. (2018). Cisplatin suppresses proliferation, migration and invasion of nasopharyngeal carcinoma cells in vitro by repressing the Wnt/β-catenin/Endothelin-1 axis via activating B cell translocation gene 1. Cancer Chemotherapy and Pharmacology, *81*, 1-10. doi:10.1007/s00280-018-3536-5

Zhan, J., Jiao, D., Wang, Y., Song, J., Wu, J., Wu, L., Chen, Q., & Ma, S. (2017). Integrated microRNA and gene expression profiling reveals the crucial miRNAs in curcumin anti-lung cancer cell invasion. *Thoracic Cancer, 8*, 461-470. doi:10.1111/1759-7714.12467.