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Shotgun Lipidomics Elucidates the Lipidome Alterations of the Mcl-1 Inhibitor S63845 in AML Cell Lines with a Focus on Sphingolipids

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ABSTRACT

Objective: Acute myeloid leukemia (AML) is a vigorous type of leukemia requiring effective treatment. Myeloid cell leukemia-1 (Mcl-1) is an anti-apoptotic molecule that is upregulated in AML and is studied as a target for treatment. The specific Mcl-1 inhibitor, S63845, has antiproliferative effects on AML cells. Bioactive sphingolipids have crucial roles in cells and regulate Mcl-1 stability. This study aimed to elucidate the changes in lipid profiles of AML cell lines in response to Mcl-1 inhibitor S63845 treatment, with a special focus on sphingolipids.

Materials and Methods: The cytotoxic effects of S63845 were identified in the AML cell lines MV4-11, HL60, and KG1 using the MTT cell proliferation assay. Lipidome analysis was conducted by quantitative shotgun lipidomics covering 378 individual lipid species in 26 classes within the major lipid categories.

Results: The IC_{50} values of S63845 have been calculated as 7 nM for MV4-11, 53 nM for HL60, and 479 nM for KG1. The lipidome results reveal the S63845 treatment to increase ceramide (Cer) levels in the MV4-11 and KG1 cell lines at the expense of downstream sphingolipids while increasing the hexosylceramide (HexCer) levels in the HL60 cell line at the expense of the Cer and sphingomyelin (SM).

Conclusion: This study showed S63845 to be able to suppress cell proliferation by altering lipid compositions in AML cell lines. More importantly, the study suggested S63845 to differentially affect the lipid profiles of AML cell lines.

Keywords: Mcl-1, small molecule inhibitors, S63845, acute myeloid leukemia, shotgun lipidomics, bioactive sphingolipids

INTRODUCTION

Acute myeloid leukemia (AML) is caused by phenotypic and genetic abnormalities during hematopoietic stem cell differentiation. These abnormalities lead to proliferation and aggregation of immature blood cells (1). Among the acute leukemias, AML represents the most common type and is seen in approximately 80% of adult leukemia patients (1). While the overall survival rate is higher in younger age groups, AML prognosis is poor in older adults, resulting in death in 44% of adults over the age of 75 (2). In the clinic, standard intensive chemotherapy approaches combining cytarabine and anthracyclines are used to combat AML. Other approaches such as targeted therapies have additionally been proposed as being effective in AML therapy with improvements in high-throughput techniques (2). However, the need exists for novel treatment approaches due to the limited current treatment alternatives, poor prognosis, limited tolerance to the treatment in older patients, and the resistance developed against anti-cancer drugs (3). In this context, small molecule inhibitors for Mcl-1 have been proposed as potentially effective agents in AML treatment (4).

Mcl-1 is a member of the Bcl-2 family, which is responsible for the regulation of apoptotic processes (5). In normal cells, Mcl-1 acts as the inhibitor of apoptosis by sequestering BH3-

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only molecules such as Bim, Bid, and Puma or by neutralizing Bax and Bak (5). Mcl-1 inhibits cytochrome c release by disrupting mitochondrial outer membrane permeabilization (6). Overexpression of Mcl-1 results in the disruption of the equilibrium between the anti-apoptotic and pro-apoptotic proteins, subsequently causing an excess proliferation of cancer cells (6). Due to its oncogenic properties and high expression levels in several types of cancers including hematological malignancies and solid cancers, Mcl-1 has been proposed as a powerful target in cancer treatment (6). S63845 was first discovered by Kotschy et al. (4) and has been characterized and reported as an effective small molecule inhibitor of Mcl-1 in many types of cancer. In AraC-resistant acute myeloid leukemia cell lines, S63845 in combination with venetoclax increases anti-leukemic effect, and this combination is strongly synergistic compared to combinations with hypomethylating agents (7). Moreover, S63845 combined with imatinib, nilotinib, dasatinib, or asciminib show synergistically apoptotic effects on chronic myeloid leukemia cells (8). In light of the information on the strong effects of S63845, it has been suggested as a promising therapeutic target for both solid tumors and hematological malignancies (6). However, the exact mechanism of action of S63845 remains unclear.

Lipid compositions are known to be altered in tumor cells, which may affect their cellular response to different agents (9). Fatty acids promote proliferation in tumor cells due to their contribution to the membrane lipid bilayer and additionally have a crucial function in cell signaling as well as in developing resistance against anti-cancer agents (9). Bioactive sphingolipids have important roles in cell proliferation, apoptosis, autophagy, inflammation, and migration (10). The central molecule of this family, ceramide, is related to pro-apoptotic outcomes, whereas the other members, sphingosine-1-P (S1P) and glucosylceramide, are related to anti-apoptotic outcomes and contribute to the development of multidrug resistance in cancer cells (10). Some studies have reported a relationship between sphingolipids and Mcl-1, even suggesting ceramide and S1P to regulate the degradation of Mcl-1 (11, 12). The agents targeting sphingolipid metabolism suppress Mcl-1 expression and subsequently affect Mcl-1 stability (13).

In light of this information, this study aimed to elucidate the effects of the Mcl-1 inhibitor, S63845, on the lipid profiles of three different AML cell lines, MV4-11, HL60, and KG1, with a special focus on sphingolipids. In total 378 individual lipid species have been profiled using mass spectrometry (MS)-based shotgun lipidomics. The results reveal the S63845 treatment influences lipid metabolism depending on the type of AML cell line.

MATERIALS and METHODS

Cell Lines, Chemicals, and Culture Conditions

HL60 and KG1 cell lines were obtained from the German Collection of Microorganisms and Cell Cultures (Germany). The MV4-11 cell line was a kind gift from Dr. A. Adan (Abdullah Gul University, Kayseri, Turkiye). S63845 was obtained from Cayman Chemical (Michigan, USA), and the stock solution (1 mM) was prepared by dissolving it in dimethyl sulfoxide (DMSO, VWR, Pennsylvania, USA). The cell culture medium and additives were obtained from Gibco (CA, USA). The chemical reagents, solutions, and internal lipid standards for the lipidomics analysis were obtained from Sigma-Aldrich (St. Louis, MO, USA), Rathburn Chemicals (Walkerburn, Scotland), Avanti Polar Lipids (Alabaster, AL, USA), and Larodan AB (Solna, Sweden). All cell lines were cultured in RPMI-1640 growth medium containing 10% fetal calf serum (FCS), 2 mM L-glutamine, and 1% penicillin–streptomycin at 37°C in a 5% CO_2 humidified incubator.

MTT Assay

The effects of S63845 on cell proliferation were determined using the MTT cell proliferation assay (Sigma-Aldrich, MO, USA). The protocol was applied for the MTT assay as described previously (14). In each experiment, the final concentration of DMSO exceeded no more than 0.2% in culture. In short, 1×10^4 cells per well from the HL60, KG1, and MV4-11 cell lines were seeded into 96-well plates in 100 µL growth medium. Increased concentrations of S63845 were applied in 100 µL of RPMI-1640 onto the cells, and the cells were incubated at 37°C in 5% CO_2 for 72 h. Next, the cells were incubated for 4 h with 20 µL of MTT (5 mg/mL). After the incubation period, the plates were centrifuged for 10 min at 1800 rpm, and the MTT crystals were dissolved with 100 µL of DMSO. After shaking the plates for 5 min using an orbital shaker, the plates were analyzed under 570 nm wavelengths with a spectrophotometer (Thermo Fisher Scientific Multiskan Spectrum, Finland). IC_{50} values of the S63845 for each cell line were calculated according to the cell proliferation plots.

Treating the Cells with S63845 for the Lipidomics Analysis

The cells were seeded into 6-well plates at 4×10^5 cells per well in 1 mL of growth medium, and treated with IC_{50} values of S63845 for 24 h at 37°C. Because the aim of this treatment is to stimulate the changes in lipid profiles rather than killing the cells, IC_{50} values for the 72-h span were applied for 24 h. After the incubation period, the centrifuge was run at 400 g for 5 min at 4°C, then two washing steps were performed with 500 µL of ice-cold 155 mM ammonium bicarbonate. Next, the 155 mM ammonium bicarbonate were added as pellets to the 4×10^5 cells in 200 µL (2000 cells/µL). The cells were stored at -80°C until the lipid extraction.

Lipid Extraction

The lipid extraction process was executed at 4°C or upon ice (15). Tubes with 200 µL of biological samples containing the 4×10^5 cells were added to a 1,000 µL chloroform/methanol (2:1, *v/v*) and 12.5 µL internal standard solution. The composition of the internal standard solution is presented in Table 1. The lipids were extracted with 20 min of shake at 2000 rpm and then centrifuged for 5 min at 1000 g. The organic phase at the bottom was collected and put into a new tube. After being evaporated for 60 min with a vacuum evaporator, the samples were resuspended in the 200 µL chloroform/methanol (1:2, *v/v*).

Shotgun Lipidomics Analysis

For positive ionization, 10 µL of crude lipid extracts were mixed with 12.9 µL of 13.3 mM ammonium acetate in 2-propanol. For

Table 1. Internal standard solution composition.

Lipid	Amount (pmol)
Cholesteryl ester (CE) 15:0-D7	45.80
Ceramide (Cer) 18:1;2/12:0;0	31.25
Cholesterol (Chol)-D4	356.87
Diacylglycerol (DAG) 12:0/12:0	45.63
Dihexosylceramide (diHexCer) 18:1;2/17:0;0	23.21
Hexosylceramide (HexCer) 18:1;2/12:0;0	28.88
Lysophosphatidic acid (LPA) 17:0	31.15
Lysophosphatidylcholine (LPC) 12:0	25
Lysophosphatidylethanolamine (LPE) 17:1	30.75
Lysophosphatidylglycerol (LPG) 17:1	30.16
Lysophosphatidylinositol (LPI) 13:0	22.91
Lysophosphatidylserine (LPS) 17:1	14.26
Phosphatidic acid (PA) 12:0/12:0	28.35
Phosphatidylcholine (PC) 12:0/12:0	35.94
Phosphatidylethanolamine (PE) 12:0/12:0	39.95
Phosphatidylglycerol (PG) 12:0/12:0	29.31
Phosphatidylinositol (PI) 8:0/8:0	28.38
Phosphatidylserine (PS) 12:0/12:0	25.38
Sphingomyelin (SM) 18:1;2/12:0;0	21.28
Trihexosylceramide (triHexCer) 18:1;2/17:0;0	51.19

negative ionization, 10 µL of crude lipid extract were mixed with 10 µL of 0.2% (*v/v*) methylamine in methanol was used. The mass spectrometric analysis was performed on an Orbitrap Fusion Tribrid mass spectrometer (Thermo Fisher Scientific, USA) coupled to TriVersa NanoMate (Advion Biosciences, USA), a direct nanoelectrospray infusion robot. For the fourier transform (FT)-MS and FT MS/MS analyses, similar features were used as previously reported (16, 17).

Lipid Annotation

Annotation of the lipids was based on sum composition, where glycerolipids and glycerophospholipids are denoted as: <lipid class>< number of carbon atoms in acyl chains >:<number of double bonds in acyl chains> (e.g., PC 34:1). While sphingolipid species are denoted as <lipid class><number of carbon atoms in the sphingoid base and acyl chain>:< number of double bonds in the sphingoid base and acyl chain >;<number of OH groups in the sphingoid base and acyl chain > (e.g., SM 34:1;2) (18-21).

Data Processing

LipidXplorer was used to report the data acquired from the FT MS and FT MS/MS analyses (22, 23). Absolute molar quantities were calculated using LipidQ, an R-based suite of scripts (17).

Statistical Analyses

All experiments were performed over three biological replicates. Statistical significance was determined using the Student's t test and one-way ANOVA, with $p<0.05$ being considered statistically significant.

RESULTS

S63845 Inhibited Cell Proliferation in Three Different AML Cell Lines

The anti-proliferative effects of S63845 in AML cell lines were determined using the MTT cell proliferation assay. The cells were treated with increasing concentrations of S63845 (1-2,000 nM, depending on the cell type) for 72 h, and the MTT cell proliferation assay was executed. S63845 suppressed cell proliferation in each cell line as compared to the untreated control groups. IC_{50} values were calculated as 7, 53, and 479 nM in MV4-11, HL60, and KG1 cell lines, respectively (Figure 1).

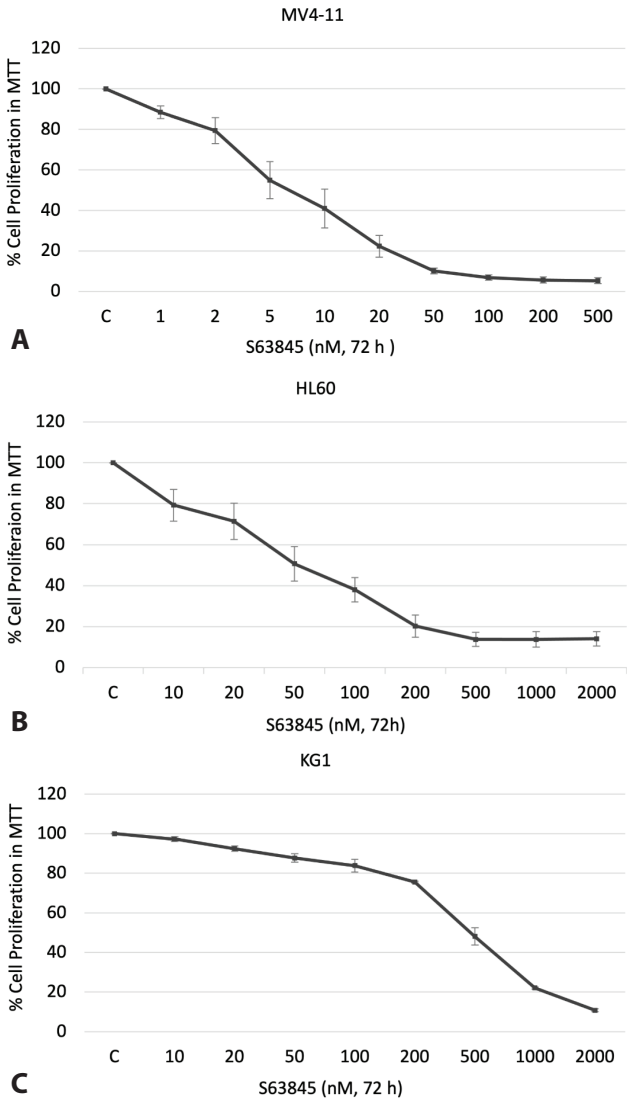

Figure 1. Antiproliferative effects of S63845 on the MV4-11 (A), HL60 (B), and KG1 (C) cell lines. IC_{50} values of S63845 for each cell line were calculated from the cell proliferation plots. Three biological replicates were used for MTT cell proliferation assays, and the results are the means of the independent experiments. The standard deviations are represented with the error bars, with $p<0.05$ being considered statistically significant.

S63845 Differentially Affected Lipid Profiles in Three Different AML Cell Lines

Quantitative shotgun lipidomics analysis was performed on each cell line treated with IC_{50} values of S63845 for 24 h. The lipidomics profiling demonstrated quantification of 26 classes within major lipid categories and enabled profiling of 378 individual lipid species (Figure 2A). The class profile demonstrated a typical mammalian lipidome profile with PC and Chol as the major lipid components of the lipidome with 45.162±3.843 mol% and 17.341±1.311 mol% of all the cells, respectively (Figure 2A). The lipid with lowest detected levels belongs to lysoglycerophospholipid with 0.007±0.013 mol % for LPSO- (Figure 2A). Interestingly, comparing the three cell lines to their treated counterparts demonstrated the HL60 and KG1 class profiles have similar changes in respect to S63845 treatment (Figure 2B). However, the MV4-11 cell lines treated with S63845 demonstrated few similarities with the treated HL60 and KG1 cell lines (Figure 2B).

Due to the presence of a relationship between Mcl-1 and sphingolipid metabolism, we focused on the sphingolipid species to reveal any possible trend with similar changes in all cell lines treated with S63845. We quantified 55 sphingolipid species belonging to five sphingolipid classes: Cer, HexCer, dihexosylceramide (diHexCer), trihexosylceramide (triHexCer), and SM. The cells treated with S63845 did not demonstrate similarities for the species profile within the HexCer and triHexCer classes (Figure 2C). Interestingly, the SM 34:1;2

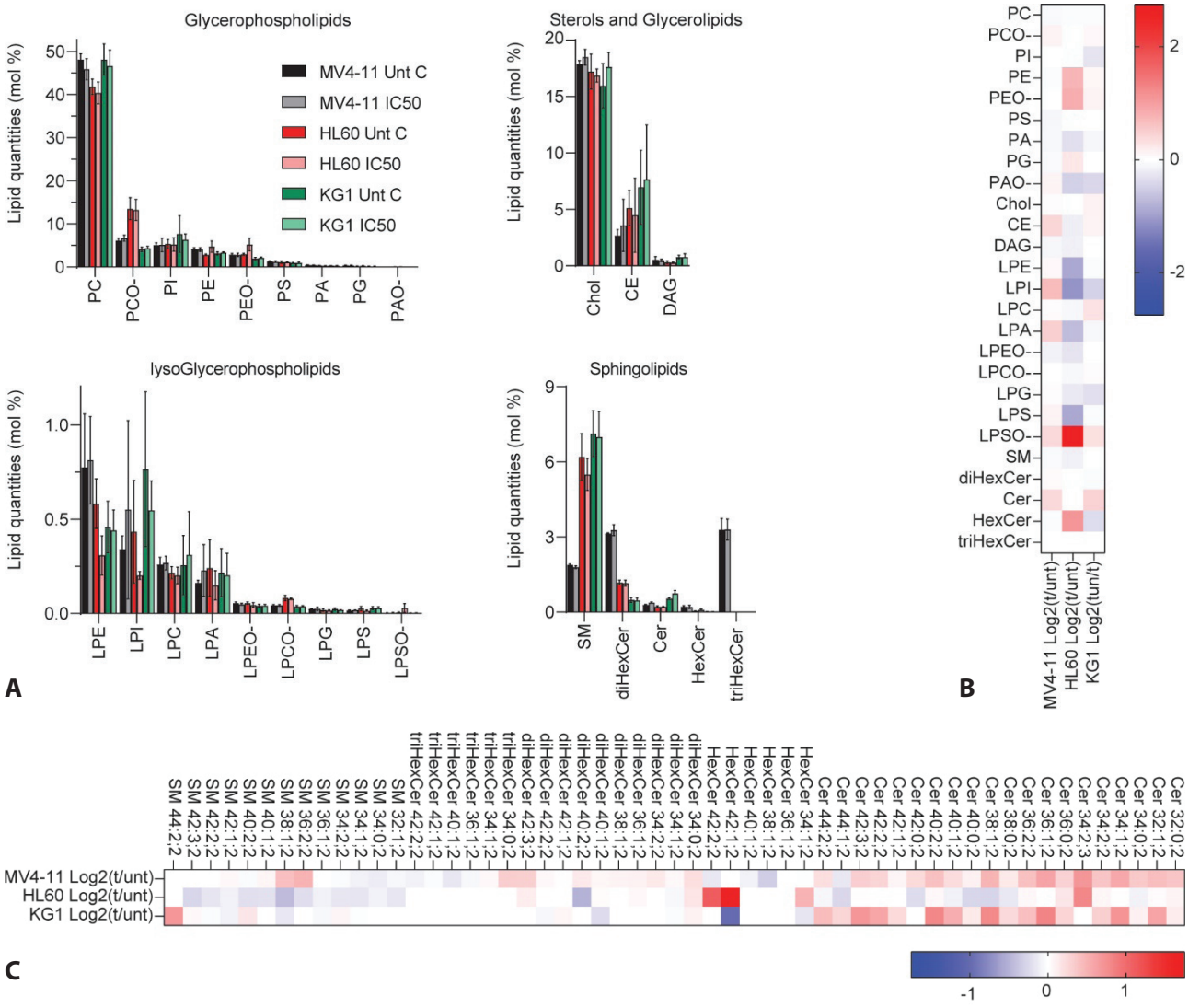

Figure 2. S63845 induced lipidome alterations of the MV4-11, HL60 and KG1 cell lines. A) The bar graph presents the mol % values of the 26 lipid classes. B) The heatmap presents the log2-transformed lipid class abundance ratios of the treated and untreated cell lines. C) The heatmap presents the log2-transformed sphingolipid species abundance ratios of the treated and untreated cell lines.

decreased and diHexCer 34:0;2 increased in all treated cell lines (Figure 2C). However, 10 of the 20 Cer species demonstrated increased levels for all treated counterparts of the MV4-11, HL60, and KG1 cell lines (Figure 2C). While the treated counterparts of the MV4-11 and KG1 cell lines demonstrated similar trends for 18 out of 20 Cer species with increased levels, the treated HL60 cell lines mostly showed an opposite trend compared to the treated MV4-11 and KG1 cell lines (Figure 2C). The HL60 cell line demonstrated increased levels of HexCer, which is one of the downstream sphingolipids of Cer (Figure 2C).

DISCUSSION

Despite of the novel investigations in the treatment of AML patients, approximately 1/3 of the patients can be cured. Therefore, the need still exists for more effective therapeutic approaches in the clinic (24). Targeting Mcl-1 in AML treatment has been proposed to have potential in *in vitro* and *in vivo* studies conducted by many groups (25). S63845 has been reported to have cytotoxic and apoptotic effects in AML cell lines with IC_{50} values less than 1 µM (4).

Lipid composition may change with regard to several types of cancer cells, and these changes affect the cellular responses against chemotherapeutical agents (26). Sphingolipids are important elements of membrane lipids and have crucial roles in cell signaling, regulating several cellular events including cell proliferation, cell death, senescence, invasion, and angiogenesis (10). Many studies have reported the regulatory effects of Cer and S1P on Mcl-1 stability (11). However, no study has reported yet on the mechanism of action of the Mcl-1 inhibitor S63845 regarding the effects on lipid profiles in AML cell lines. Therefore, this study has aimed to elucidate the potential effects of S63845 on lipid profiles in the MV4-11, HL60, and KG1 cell lines using quantitative shotgun lipidomics. Upon treating the cells with increasing concentrations of S63845 for 72 h, the IC_{50} values were calculated as 7, 53, and 479 nM in MV4-11, HL60, and KG1 cell lines, respectively. The study's aim has been to induce lipid alteration rather than suppressing cell proliferation for the lipidomics analysis. Therefore, we treated the cells for only 24 h while using the corresponding concentrations for the 72 h-IC_{50}-values of S63845. The results of profiling 378 individual lipid species in 26 classes showed that most of the Cer species levels increased in response to the S63845 treatment, whereas SM levels decreased (Figure 2A). These results suggest S63845 to be able to induce SM hydrolysis, leading to increases in Cer levels. Hydrolysis of SM is carried out by SMases and results in cell death and suppression of cell proliferation in cancer cells (27). However, S63845 mostly affects the lipidomes over a variety of different actions, depending on the type of cell. For instance, some types of SMs (i.e., SM 36:2;2 and SM 38:1;2) increase in MV4-11 cell lines, whereas they decrease in the other cell lines. SM accumulation has been suggested to possibly be related to the negative impact regarding autophagosomal membrane maturation in early stages (27). This accumulation of SM may suppress autophagy in these cells. Additionally, the S63845 treatment increased HexCer levels in the HL60 cell lines, contrary to the other cells. In many types of cancer cells, increased HexCer levels have been related to the development of multidrug resistance and cell survival. Cer is converted into HexCer through UDP-glucose Cer glucosyltransferase and glucosylCer synthase (10, 28). The effects of these changes may be elucidated by examining the cell death mechanisms and gene expression levels in further studies. Moreover, due to AML being a very heterogenous disease, each type of cell line and patient sample may respond to the anti-cancer agents differently despite having the same disease type, and their lipid profiles may also change in distinct manners (9). The study's results depicting the distinct effects of S63845 depending on cell type also contribute to this rationale.

CONCLUSION

In conclusion, this study revealed the S63845 treatment to be able to inhibit cell proliferation by increasing Cer levels in AML cell lines. This increase in Cer levels may result from the hydrolysis of SM and may then lead to apoptosis. However, each cell line may respond to the S63845 treatment through different mechanisms. The results of the shotgun lipidomics analysis showed that, despite belonging to the same disease type, the same agent may affect lipidomes differently depending on the cell line. This result suggests the potential of using lipidomic profiles in terms of diagnostic and prognostic fingerprints. This study sheds light on the action of the S63845 mechanism. The exact mechanism of the S63845's action resulting in the changes in lipidomes should be elucidated in further studies regarding each cell line.

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Peer-review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study - M.K.Y., M.B.; Data Acquisition - M.K.Y., M.B.; Performing experiments - M.K.Y., M.B.; Data Analysis/Interpretation - M.K.Y., M.B.; Statistical Analyses - M.K.Y., M.B.; Drafting Manuscript - M.K.Y., M.B.; Critical Revision of Manuscript - M.K.Y., M.B.; Final Approval and Accountability - M.K.Y., M.B.

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REFERENCES

1. Padmakumar D, Chandraprabha VR, Gopinath P, Vimala Devi ART, Anitha GRJ, Sreelatha MM, et al. A concise review on the molecular genetics of acute myeloid leukemia. Vol. 111, Leukemia Research. Elsevier Ltd; 2021. [CrossRef]

2. Newell LF, Cook RJ. Advances in acute myeloid leukemia. Vol. 375, BMJ (Clinical research ed.). NLM (Medline); 2021. p. n2026. [CrossRef]
3. Pearson JM, Tan SF, Sharma A, Annageldiyev C, Fox TE, Abad JL, et al. Ceramide analogue SACLAC modulates sphingolipid levels and MCL-1 splicing to induce apoptosis in acute myeloid leukemia. Mol Cancer Res 2020; 18(3): 352-63. [CrossRef]
4. Kotschy A, Szlavik Z, Murray J, Davidson J, Maragno AL, le Toumelin-Braizat G, et al. The MCL1 inhibitor S63845 is tolerable and effective in diverse cancer models. Nature 2016; 538(7626): 477-82. [CrossRef]
5. Bolomsky A, Vogler M, Köse MC, Heckman CA, Ehx G, Ludwig H, et al. MCL-1 inhibitors, fast-lane development of a new class of anti-cancer agents. J Hematol Oncol 2020;13: 173. [CrossRef]
6. Wang H, Guo M, Wei H, Chen Y. Targeting MCL-1 in cancer: current status and perspectives. J Hematol Oncol 2021; 14(1): 67. [CrossRef]
7. Moujalled DM, Pomilio G, Ghiurau C, Ivey A, Salmon J, Rijal S, et al. Combining BH3-mimetics to target both BCL-2 and MCL1 has potent activity in pre-clinical models of acute myeloid leukemia. Leukemia 2019; 33(4): 905-17. [CrossRef]
8. Malyukova A, Ujvari D, Yektaei-Karin E, Zovko A, Madapura HS, Keszei M, et al. Combination of tyrosine kinase inhibitors and the MCL1 inhibitor S63845 exerts synergistic antitumorigenic effects on CML cells. Cell Death Dis 2021; 12(10). [CrossRef]
9. Stefanko A, Thiede C, Ehninger G, Simons K, Grzybek M. Lipidomic approach for stratification of acute myeloid leukemia patients. PLoS One 2017; 12(2). [CrossRef]
10. Ogretmen B, Hannun YA. Biologically active sphingolipids in cancer pathogenesis and treatment. Nat Rev Cancer 2004; 4(8): 604-16. [CrossRef]
11. Powell JA, Lewis AC, Zhu W, Toubia J, Pitman MR, Wallington-Beddoe CT, et al. Targeting sphingosine kinase 1 induces MCL1-dependent cell death in acute myeloid leukemia. Blood 2017; 6: 771-82. [CrossRef]
12. Tan SF, Liu X, Fox TE, Barth BM, Sharma A, Turner SD, et al. Acid ceramidase is upregulated in AML and represents a novel therapeutic target. Oncotarget 2016; 7(50): 83208-22. [CrossRef]
13. Lewis AC, Wallington-Beddoe CT, Powell JA, Pitson SM. Targeting sphingolipid metabolism as an approach for combination therapies in haematological malignancies. Cell Death Discov 2018; 4(1): 72. [CrossRef]
14. Kozanoglu I, Yandim MK, Cincin ZB, Ozdogu H, Cakmakoglu B, Baran Y. New indication for therapeutic potential of an old well-known drug (propranolol) for multiple myeloma. J Cancer Res Clin Oncol 2013; 139(2): 327-35. [CrossRef]
15. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem Physiol 1959; 37(8): 911-7. [CrossRef]
16. Almeida R, Pauling JK, Sokol E, Hannibal-Bach HK, Ejsing CS. Comprehensive Lipidome Analysis by Shotgun Lipidomics on a Hybrid Quadrupole-Orbitrap-Linear Ion Trap Mass Spectrometer. J Am Soc Mass Spectrom 2015; 26(1): 133-48. [CrossRef]
17. Nielsen IØ, Groth-Pedersen L, Dicroce-Giacobini J, Jonassen ASH, Mortensen M, Bilgin M, et al. Cationic amphiphilic drugs induce elevation in lysoglycerophospholipid levels and cell death in leukemia cells. Metabolomics 2020; 16(9): 91. [CrossRef]
18. Ejsing CS, Sampaio JL, Surendranath V, Duchoslav E, Ekroos K, Klemm RW, et al. Global analysis of the yeast lipidome by quantitative shotgun mass spectrometry. Proc Natl Acad Sci USA 2009; 106(7): 2136-41. [CrossRef]
19. Klose C, Surma MA, Gerl MJ, Meyenhofer F, Shevchenko A, Simons K. Flexibility of a eukaryotic lipidome--insights from yeast lipidomics. PLoS One 2012; 7(4): e35063. [CrossRef]
20. Sampaio JL, Gerl MJ, Klose C, Ejsing CS, Beug H, Simons K, et al. Membrane lipidome of an epithelial cell line. Proc Natl Acad Sci USA 2011; 108(5): 1903-7. [CrossRef]
21. Ejsing CS, Moehring T, Bahr U, Duchoslav E, Karas M, Simons K, et al. Collision-induced dissociation pathways of yeast sphingolipids and their molecular profiling in total lipid extracts: a study by quadrupole TOF and linear ion trap-orbitrap mass spectrometry. J Mass Spectrom 2006; 41(3): 372-89. [CrossRef]
22. Herzog R, Schuhmann K, Schwudke D, Sampaio JL, Bornstein SR, Schroeder M, et al. LipidXplorer: A Software for Consensual Cross-Platform Lipidomics. PLoS One 2012; 7(1): e29851. [CrossRef]
23. Herzog R, Schwudke D, Shevchenko A. LipidXplorer: Software for Quantitative Shotgun Lipidomics Compatible with Multiple Mass Spectrometry Platforms. Curr Protoc Bioinformatics 2013; 43(1). [CrossRef]
24. Ewald L, Dittmann J, Vogler M, Fulda S. Side-by-side comparison of BH3-mimetics identifies MCL-1 as a key therapeutic target in AML. Cell Death Dis 2019; 10(12). [CrossRef]
25. Wei AH, Roberts AW, Spencer A, Rosenberg AS, Siegel D, Walter RB, et al. Targeting MCL-1 in hematologic malignancies: Rationale and progress. Blood Rev 2020; 44: 100672. [CrossRef]
26. Escribá P v., Busquets X, Inokuchi J ichi, Balogh G, Török Z, Horváth I, et al. Membrane lipid therapy: Modulation of the cell membrane composition and structure as a molecular base for drug discovery and new disease treatment. Prog Lipid Res 2015; 59: 38-53. [CrossRef]
27. Ogretmen B. Sphingolipid metabolism in cancer signalling and therapy. Nat Rev Cancer 2018; 18(1): 33-50. [CrossRef]
28. Pal P, Atilla-Gokcumen GE, Frasor J. Emerging Roles of Ceramides in Breast Cancer Biology and Therapy. Int J Mol Sci 2022; 23(19): 11178. [CrossRef]