Combining Nelfinavir With Chloroquine Inhibits In Vivo Growth of Human Lung Cancer Xenograft Tumors

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Abstract. Background/Aim: Nelfinavir is a human immunodeficiency virus protease inhibitor that is currently being repositioned as an anticancer drug. Chloroquine, an anti-malarial lysosomotropic drug, inhibits autophagy. It has been reported that the combination of nelfinavir and chloroquine significantly enhances endoplasmic reticulum (ER) stress and induces selective cell death in multiple cell line models (in vitro). Materials and Methods: We assessed the effects of the combination of these drugs on human NSCLC cell lines in vitro using cell proliferation assay and performed preclinical treatment studies using cell line-derived xenograft mouse models in vivo. Results: In vitro, this combination enhanced inhibition of NSCLC cell proliferation with increased proteotoxicity, including ER stress and apoptosis. In vivo, the growth of human NSCLC xenograft tumors was inhibited, which correlated with increased apoptosis and induction of ER stress as well as NSCLC growth in vitro. Conclusion: Our findings suggest that the induction of proteotoxicity provides a promising new target for developing anticancer drugs.

Our results demonstrated that combining nelfinavir with chloroquine enhances proteotoxicity and apoptosis in NSCLC cells. This finding suggests that combining nelfinavir with chloroquine might provide a promising new target for developing anticancer drugs.

Materials and Methods

Cell culture. H157 and A549 NSCLC cell lines were obtained from the National Cancer Institute (NCI)/Navy Medical Oncology (Bethesda, MD, USA) and the American Type Culture Collection (Manassas, VA, USA), respectively, and maintained as described previously (2).

Reagents. Nelfinavir was obtained from the National Institutes of Health (NIH) AIDS reagent repository (Bethesda, MD, USA) and Pfizer Inc. (New York, NY, USA). Chloroquine was purchased from MP Biometrics, Inc. (Solon, OH, USA). Primary antibodies for ubiquitin, cleaved/total poly ADP-ribose polymerase (PARP), and phospho (p)-eukaryotic initiation factor 2α (eIF2α) (Ser51) were...
Combining nelfinavir with chloroquine enhances NSCLC growth inhibition and proteotoxicity. To assess their effects on cellular proliferation, nelfinavir and chloroquine were tested. The combination of nelfinavir and chloroquine reduced proliferation more than either drug alone in both H157 and A549 cells (Figure 1A). To investigate the mechanism of growth inhibition, PARP cleavage was assessed by immunoblotting analysis (Figure 1B). The combination induced greater PARP cleavage than either drug alone, suggesting apoptotic cell death.

Moreover, to assess whether the combination enhanced proteotoxicity, we evaluated ubiquitinated proteins. Our findings showed that the combination enhanced the level of ubiquitin more than either drug alone, and the results represent proteotoxicity in the cytosol (Figure 1B). Additionally, the expression of ATF3, an ER stress marker, also increased more with the combination than with either drug alone, and this was evident by 24 h. LC-3 II expression, a marker for autophagosomes, was not enhanced in the combination treatment compared with chloroquine alone. This finding suggests that nelfinavir and chloroquine do not affect autophagy in these NSCLC cells. Taken together, these data show that combining nelfinavir with chloroquine enhances proteotoxicity in a time-dependent manner in two different cell lines.

Combining nelfinavir with chloroquine inhibits NSCLC tumor growth in vivo. To determine whether the combination of nelfinavir and chloroquine enhanced the antitumor effects of either drug alone, we treated athymic NCr-nu/nu mice bearing H157 or A549 xenograft tumors with 50 mg/kg nelfinavir, 50 mg/kg chloroquine, or their combination. The combination of nelfinavir and chloroquine was well-tolerated and reduced tumor growth of H157 by nearly 75% (Figure 2A, upper panel) and of A549 by nearly 85% (Figure 2A, lower panel, and 2B) compared with the mice treated with vehicle. To evaluate the correlation between the antitumor effects and the mechanisms identified in vitro, markers of apoptosis and ER stress were evaluated in the tumors. The combination significantly increased the expression of cleaved PARP compared with the control in H157 xenograft tumors (Figure 2C). Because ATF3 did not increase in the group treated with the combination, we evaluated p-eIF2α, another ER stress marker. The combination significantly increased the expression of p-eIF2α compared with that in the control. Taken together, these findings suggest that the combination inhibits tumor growth of NSCLC and enhances apoptosis induced by ER stress in vivo.

Discussion

Our data show that combining nelfinavir with chloroquine enhances proteotoxicity and inhibits in vivo growth of human NSCLC xenograft tumors as well as in vitro growth of NSCLC cells, which supports our hypothesis. Previously, we...
reported that combining nelfinavir with bortezomib enhances the disruption of protein homeostatic balance as a proteotoxic effect in NSCLC and MM cells in vitro and in vivo (3), suggesting the importance of proteotoxicity as a new target for developing anticancer drugs. In this study, we reconfirmed that nelfinavir can enhance proteotoxic effects when combined with drugs that induce proteotoxicity, such as chloroquine. These findings support our previous phase I clinical trial of nelfinavir as an anticancer agent for advanced solid tumors, including NSCLC (9). Taken together, the combination of nelfinavir and chloroquine may be an effective clinical strategy to maximize patient response to nelfinavir.

To our knowledge, this is the first study to describe in vivo growth inhibition of human NSCLC xenograft tumors by combining nelfinavir with chloroquine. Johnson et al. reported the efficacy of this combination in the H460 cell
Figure 2. Combining nelfinavir (NFV) with chloroquine (CQ) inhibits tumor growth of non-small cell lung cancer (NSCLC) in vivo. A: Tumor growth of H157 (upper panel) and A549 (lower panel) NSCLC cells as xenografts in athymic NCr-nu/nu. Data are the mean±SD. B: Representative photographs of two different mice bearing A549 xenograft tumors from each group after treatment for 13 days. Circles indicate tumors. C: Apoptosis and endoplasmic reticulum stress markers in vivo. The indicated markers were evaluated in the tumors using immunoblotting analysis as described in the Materials and Methods. V: Vehicle, N+C: nelfinavir + chloroquine. Data are the mean±SD of five mice. Significantly different at *p<0.05 and **p<0.01 compared with vehicle treatment.
line in an in vitro study (6). In this study, we utilized two other NSCLC cell lines: H157 cells with KRAS (Q12R) and phosphatase and tensin homolog (PTEN) (7), and A549 cells with a KRAS (Q12S) mutation (10). We also expanded our in vitro findings to an in vivo study. Our data show that combining nelfinavir with chloroquine enhances growth inhibition of H157 and A549 cells in vitro and in vivo, supporting the findings of Johnson et al. (6).

Autophagy plays a compensatory role in drug-induced proteotoxicity by activating autophagic degradation of misfolded proteins that results in harmful cellular effects (11). Chloroquine inhibits lysosome fusion with autophagosomes, resulting in faults in late autophagy and increased LC-3 II. Therefore, we expected that in combining nelfinavir with chloroquine, autophagy would be inhibited and result in enhanced proteotoxicity, compared with treatment with either drug alone. However, LC-3 II expression following combination treatment was similar to that following chloroquine administration alone (Figure 1B). This finding suggests that the proteotoxic mechanisms that result from combining nelfinavir with chloroquine might proceed in an autophagy-independent manner, as described by Johnson et al. (6).

In conclusion, we demonstrated that combining nelfinavir with chloroquine enhances inhibition of NSCLC growth and proteotoxicity in vitro and in vivo. Our findings suggest that the induction of proteotoxicity by activating autophagic degradation of misfolded proteins that results in harmful cellular effects (11).

Conflicts of Interest
The Authors report no financial or other interests that could be construed as conflicts of interest.

Authors’ Contributions
Conceived and designed the experiments: J.L., S.K., J.J.G. and P.A.D. Performed the experiments: J.L. and S.K. Analyzed the data: J.L., S.K. and J.J.G. Wrote the article: S.K. and P.A.D.

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