Combinatory Treatment with the antiviral remdesivir and the endolysosomal host-SARS-CoV-2interface by clinically licensed functional inhibitors of acid sphingomyelinase (FIASMA) including the antidepressant fluoxetine

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Abstract

The Coronavirus Disease 2019 (COVID-19) pandemic caused by the Severe Acute Respiratory Syndrome Related Coronavirus 2 (SARS-CoV-2) is a global health emergency. As only very limited therapeutic options are clinically available, there is an urgent need for the rapid development of safe, effective, and globally available pharmaceuticals that inhibit SARS-CoV-2 entry and ameliorate COVID-19 severity. In this study, we explored the use of small compounds acting on the homeostasis of the endolysosomal host-pathogen interface, to fight SARS-CoV-2 infection. We find that fluoxetine, a widely used antidepressant and a functional inhibitor of acid sphingomyelinase (FIASMA), efficiently inhibited the entry and propagation of SARS-CoV-2 in the cell culture model without cytotoxic effects and also exerted potent antiviral activity against two currently circulating influenza A virus subtypes, an effect which was also observed upon treatment with the FIASMAs amiodarone and imipramine. Mechanistically, fluoxetine induced both impaired endolysosomal acidification and the accumulation of cholesterol within the endosomes. An antiviral effect that can be improved by the combinatory use of fluoxetine and the viral RNA-polymerase inhibitor remdesivir which displayed synergistic antiviral effects in commonly used reference models for drug interaction. As the FIASMA group consists of a large number of small compounds that are well-tolerated and widely used for a broad range of clinical applications, exploring these licensed pharmaceuticals may offer a variety of promising antivirals for host-directed therapy to counteract enveloped viruses, including SARS-CoV-2.

Results

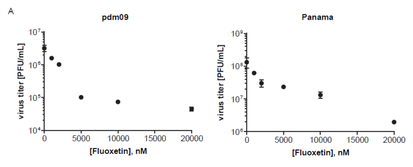


Figure 1. Antiviral potential of fluoxetine treatment against IAV subtypes pdm09 and Panama in Calu-3 cells. (A) Virus titers determined in Calu-3 cells infected with the respective IAV subtype at 0.01 MOI for 24 h. Cells were pretreated with solvent or fluoxetine for 16 h. Data points present mean virus titers ± SEM of three independent experiments. (B) Released viral titers normalized to the control condition and log-transformed fluoxetine concentrations were used to generate the dose-response curves. EC50 and EC90 values were determined using the 4PL nonlinear regression model.

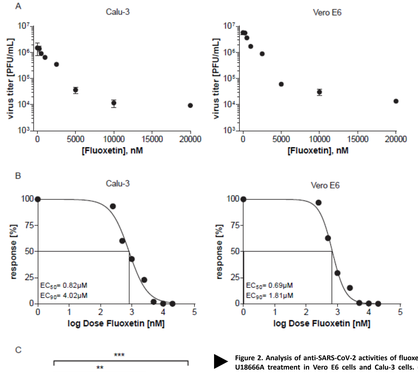


Figure 2. Analysis of anti-SARS-CoV-2 activities of fluoxetine and U18666A treatment in Vero E6 cells and Calu-3 cells. (A) Virus titers determined in Calu-3 and Vero E6 cells infected with SARS-CoV-2 at 0.1 MOI for 48 h. Treatment of infected cells with solvent or fluoxetine was started 1 h p.i. Data points present mean virus titers ± SEM of three independent experiments. (B) To generate the dose-response curves, virus release was normalized to the control condition, fluoxetine concentrations were log-transformed, and nonlinear regression and a 4PL model was used to fit the curves and to determine the EC50 and EC90 values. (C) Polarized Calu-3 cells grown on semipermeable supports were infected with SARS-CoV-2 isolate at 0.1 MOI for 48 h. Cells were treated 1 h p.i. with 20 µM fluoxetine, and 2 or 10 µg/mL U18666A. Bar graphs represent the mean viral titers ± SEM of three independent experiments. One-way ANOVA followed by by Dunnett's multiple comparison test. **p ≤ 0.01, ***p ≤ 0.001.

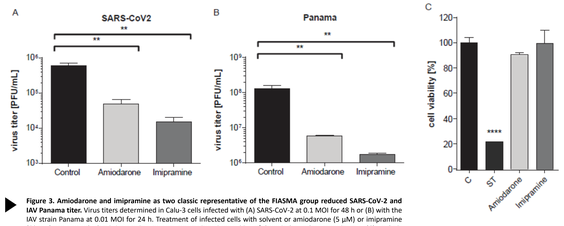
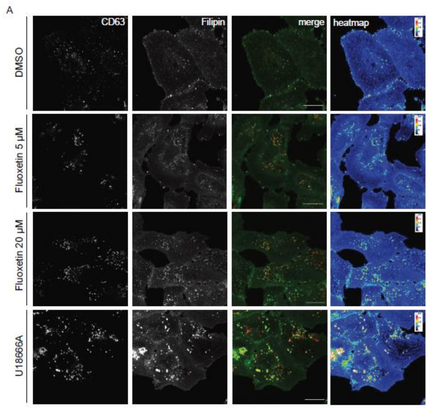


Figure 3. Amiodarone and imipramine as two classic representative of the FIASMA group reduced SARS-CoV-2 and IAV Panama titer. Virus titers determined in Calu-3 cells infected with (A) SARS-CoV-2 at 0.1 MOI for 48 h or (B) with the IAV strain Panama at 0.01 MOI for 24 h. Treatment of infected cells with solvent or amiodarone (5 µM) or imipramine (50 µM) was started 1 h p.i. Data points present mean virus titers ± SEM of three independent experiments. (C) Analysis of cell viability. MTT assay of Calu-3 cells treated with the solvent DMSO (C), amiodarone (5 µM) or imipramine (50 µM) for 48 h. The protein kinase inhibitor staurosporine (ST), a strong inducer of cytotoxicity, served as a positive control. Bar graphs represent the mean viral titers ± SEM of three independent experiments. One-way ANOVA followed by Dunnett's multiple comparison test; **p ≤ 0.01, ***p ≤ 0.001.



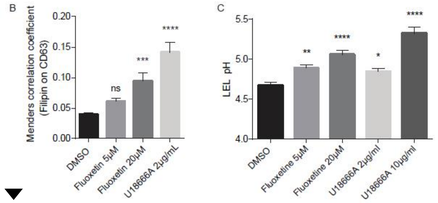


Figure 4. Increased endolysosomal cholesterol storage and dysregulated acidification upon fluoxetine treatment. Vero E6 cells were treated for 16 h with either the solvent DMSO, fluoxetine, or U18666A at the indicated concentrations. (A) Representative 2D maximum intensity projections of entire z-stacks obtained by confocal imaging. LELs were identified via immunodetection of the LEL marker protein CD63 and unesterified cholesterol was visualized using filipin. Representative pseudocolored digital images are shown. To generate the heat maps, filipin-positive pixels were color-encoded according to their intensity values. Scale bar, 20 µm. (B) For each cell, the colocalization of filipin with CD63 quantitated across the entire z-stack was calculated as Manders' coefficient. Bar graphs represent means ± SEM of 3 individual cells per condition from three independent experiments. (C) Endolysosomal pH values in Calu-3 cells were measured by ratio imaging. Bar graphs present mean pH values ± SEM of 56 cells for each condition from three independent experiments. One-way ANOVA followed by Dunnett's multiple comparison test; ns, not significant, **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001.

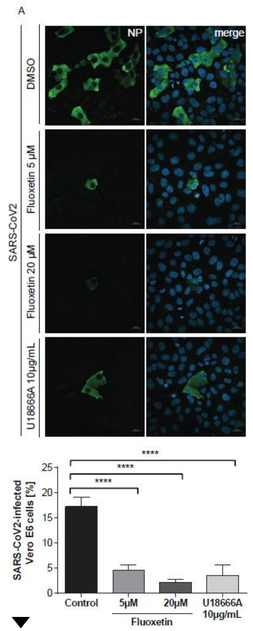


Figure 5. Impact of fluoxetine and U18666A on SARS-CoV-2 infection success within the first cycle of replication. (A) Vero E6 cells pretreated with the drugs at the indicated concentrations were infected with SARS-CoV-2 at 1 MOI for 1 h. Nuclei were visualized with DAPI. To determine infection rates, NP-positive cells were detected by immunofluorescence imaging. Mean percentages ± SEM of NP-positive cells were calculated from 3 independent experiments. One-way ANOVA followed by by Dunnett's multiple comparison test. ****p ≤ 0.0001.

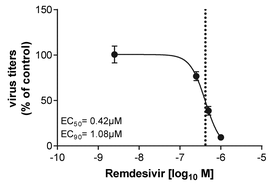


Figure 6: Analysis Dose-Response curve of remdesivir treatments in Calu-3 cells. Calu-3 cells were infected with 0.1 MOI of SARS-CoV-2 for 1 h and treated with the indicated drug combinations for 48 h. Mean percent inhibition ± SEM of SARS-CoV-2 replication, with mean virus titer in control cells (treated with the solvent DMSO) set to 100%; n = 5. LogEC50 and LogEC90 values were determined by fitting a non-linear regression model. (Calu-3: EC50 = 0.42 µM, EC90 = 1.08 µM).

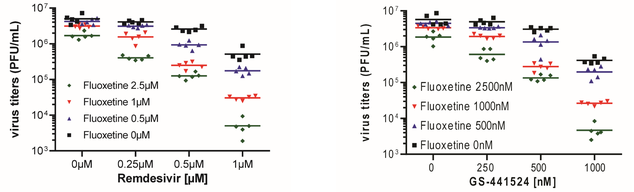


FIGURE 7. Antiviral activities of treatments. Infectious virus production in polarized Calu-3 cells treated with two-drug combinations as indicated 2 hpi. Each symbol represents plaque-forming units (PFU) per mL detected in a single experimental sample, lines indicate means; n = 5/treatment.

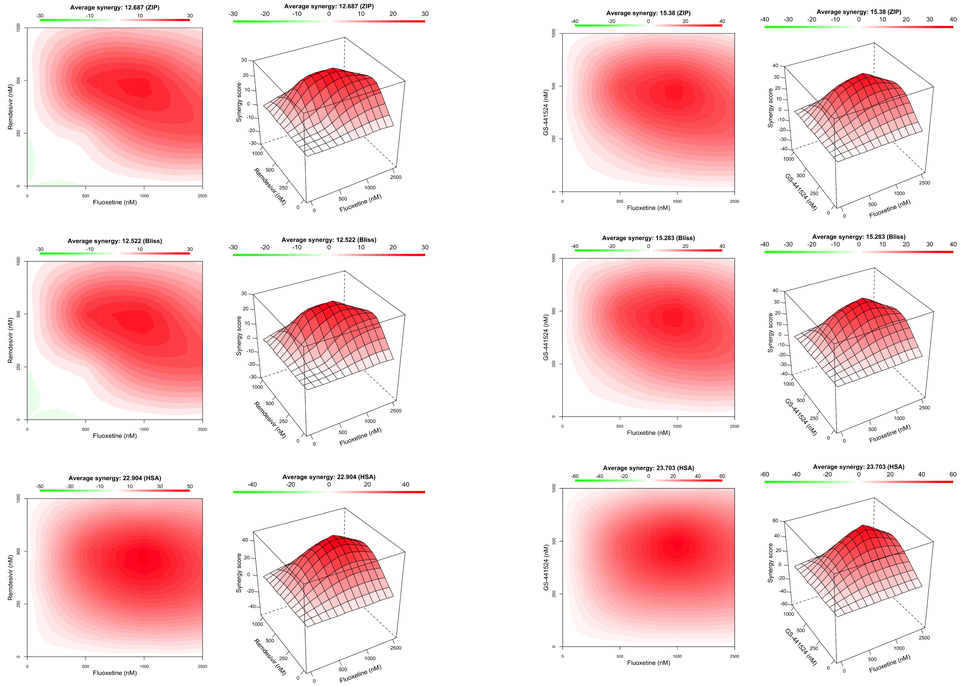


Figure 8. Evaluation of the pharmacological interactions of fluoxetine and remdesivir (FluoRem). ZIP, Bliss independence, and Highest single agent (HSA) reference models were used to assess the interaction landscapes and to identify areas of synergy. Interaction surfaces are color-coded according to the synergy scores of the responses.

Figure 9. Evaluation of the pharmacological interactions of fluoxetine and GS-441524 (FluoGS). ZIP, Bliss independence, and Highest single agent (HSA) reference models were used to assess the interaction landscapes and to identify areas of synergy. Interaction surfaces are color-coded according to the synergy scores of the responses.

Conclusion

Here we report that ItraRem and FluoRem drug combinations, in both cases targeting the host cell and the virus independently, showed stronger antiviral activities against SARS-CoV-2 than the remdesivir monotherapy. Moreover, the overall therapeutic effect of the combinations was larger than the expected sum of the independent drug effects and underlying synergistic effects were determined, allowing for lower concentrations of the individual drugs. Of note, their reported plasma concentrations are well within these ranges. Our analysis on the antiviral activity of combinatory drug combinations via commonly used interaction models argue for an enhanced efficacy that is based on synergistic drug interaction and suggests promising novel options for SARS-CoV-2 treatment.

Schloer S., Brunotte L., Mecate Zambrano A., Zheng S., Tang J., Ludwig S., Rescher U. Drug synergy of combinatory treatment with remdesivir and the repurposed drugs fluoxetine and itraconazole effectively impairs SARS-CoV-2 infection. **BJP** 2021

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