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Vulnerabilities of the SARS-CoV-2 virus to proteotoxicity – opportunity for repurposed chemotherapy of COVID-19 infection

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Abstract

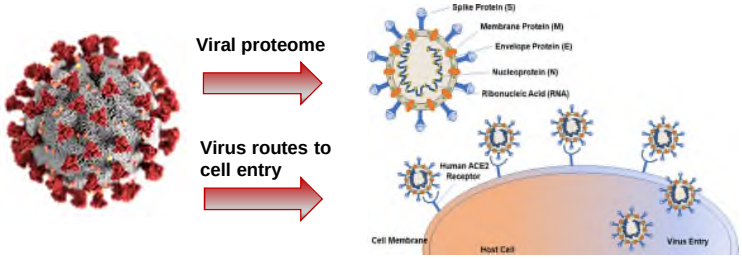
The global pandemic of COVID-19 disease caused by infection with the SARS-CoV-2 coronavirus**,** has produced an urgent requirement and search for improved treatments whilst effective vaccines are developed. A strategy for improved drug therapy is to increase levels of endogenous reactive metabolites for selective toxicity to SARS-CoV-2 by preferential damage to the viral proteome. Key reactive metabolites producing major quantitative damage to the proteome in physiological systems are: reactive oxygen species (ROS) and the reactive glycating agent methylglyoxal (MG). We found a 4.6-fold enrichment of arginine residues, targets of MG modification, in functional domains of the SARS-CoV-2 viral proteome; in contrast, there was 0.8-fold enrichment or depletion of cysteine, targets of ROS, in functional domains. This suggests SARS-CoV-2 is sensitive to inactivation by MG but resistant to ROS-mediated oxidative damage. We examined arginine residues activated for MG modification in functional domain in the SARS-CoV-2. We found 25 such arginine residues, including 2 in the spike protein and 10 in the nucleoprotein. These sites were partially conserved in related *coronaviridae:* SARS-COV and MERS. We also screened and identified drugs which increase cellular MG concentration to virucidal levels and found two antitumor drugs with historical antiviral activity, doxorubicin and paclitaxel, were the best candidate for repurposing. Our findings provide evidence of potential vulnerability of SARS-CoV-2 to inactivation by MG and a scientific rationale for repurposing of doxorubicin and paclitaxel for treatment of COVID-19 disease, providing efficacy and adequate therapeutic index may be established.

INTRODUCTION

Pandemic dataset collected 29th Sept 2020

Cases
33 423 469
Deaths
1 002,678

A global pandemic of COVID-19 disease caused by infection with the SARS-CoV-2 coronavirus has developed from January 2020. It has produced a global public health emergency with currently (29th Sept 2020) over 33 million infections and over 1 million deaths, with both rapidly increasing. New treatments are urgently required for COVID-19 disease until effective vaccines are developed. A rapid route to achieve this is repurposing of existing drugs with previously undisclosed activity against coronavirus infection.



Aim: To explore if reactive metabolites of the human host can be exploited to produce a virucidal response against SARS-CoV-2 and if clinically approved drugs may be found that reactive metabolites to virucidal levels repurposed for treatment of COVID-19 disease

METHODS

Sequences of SARS-CoV-2, SARS-CoV and MERs and human host proteins.

Reference sequences of the 29 proteins of the SARS-CoV-2 proteome (Table S1) and sequences of analogous proteins of SARS-CoV was obtained from the NBCI reference sequence database (www.ncbi.nlm.nih.gov). Sequences of reviewed proteins of the human proteome, 18,821 were obtained from the UniProtKB database (www.uniprot.org).

Receptor binding domain analysis and Prediction of arginine residues activated for methylglyoxal modification.

Receptor binding domain (RBD) analysis is a protein primary sequenced based informatics method to deduce amino acid residues in functional domains of proteins. Arginine residues of proteins are activated towards reaction with MG by decrease of pK_a of the guanidino side chain which facilitates formation of the MG-guanidino sidechain encounter (Rabbani and Thornalley, 2012;Rabbani et al., 2016a). To identify similar arginines residues in SARS-CoV proteins, we used the Clustal Omega software on-line.

Culture of HEK293 cells *in vitro*.

The HEK293 cell line was cultured in Dulbecco's Modified Eagles Medium (DMEM) pIRES2-GLO1-EGFP plasmid (Glo1+ vector) and pIRES-EGFP plasmid (empty vector) were prepared as described (Ahmed et al., 2008). HEK293 cells were stably transfected with Glo1+ and empty vector using Lipofectamine 2000, according to the manufacturer's instructions. Transfected colonies with GFP fluorescence were selected. Assessment of Glo1 activity and protein, as described (Arai et al., 2014;Xue et al., 2014), indicated a 4 - 5 fold increase in Glo1 activity and protein. HEK293 cells stably tranfected with empty and GLO1+ vectors were incubated with and without cell permeable Glo1 inhibitor, S-p-bromobenzylglutathione cyclopentyl diester (BBGD) (Thornalley et al., 1996), doxorubucin and paclitaxel at the concentrations indicated for 2 days and effect on cell growth assessed by viable cell number counts, and median growth inhibitory concentrations GC_{50} deduced. Cellular MG concentration and flux of formation of D-lactate, a surrogate measure of flux of formation of MG, was assayed.

RESULTS

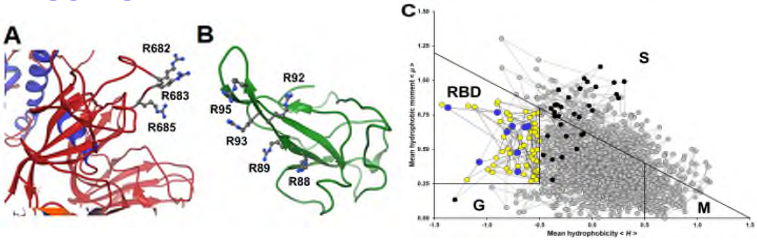


Figure 1 | Activation of functional arginine residues towards modification by methylglyoxal in the SARS-CoV-2 proteome. **(A)** Activated arginine residues triad of Spike protein, $R_{682}R_{683}AR_{685}$. (**B**) Activated arginine residue pentad of nucleoprotein, $R_{88}R_{89}ATR_{92}R_{93}IR_{95}$. **(C)** Receptor binding domain (RBD) plot for SARS-CoV-2 Spike protein. The RBD is the area bound by the trapezium in the upper left-side region of the chart. Key: ●, arginine residue in the RBD;●, arginine residues outside the RBD; ●, other amino acid residues in the RBD other amino acid residues in the RBD; and ●, other amino acid residues outside the RBD. Other predicted domains: surface (S), globular (G) and membrane (M). **(D)** Arginine enrichment in individual proteins of SARS-CoV-2 proteins.

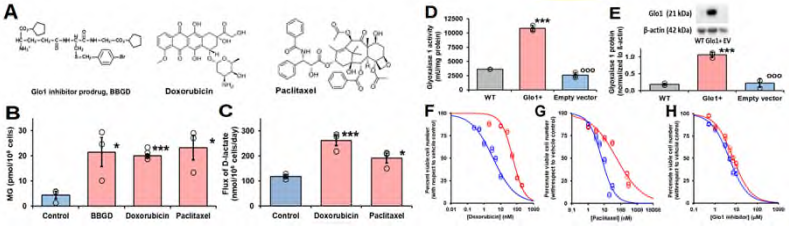
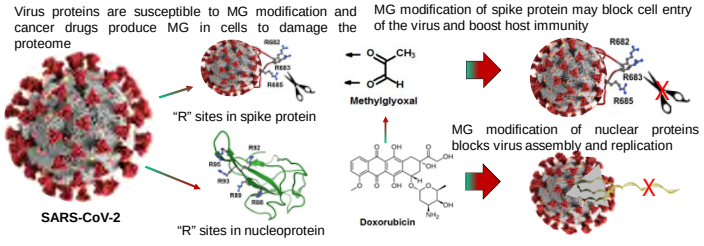


Figure 2 | Glyoxalase 1 inhibitor prodrug, doxorubicin and paclitaxel increase cellular concentration of methylglyoxal to virucidal levels. **A)** Molecular structures of drugs. Glyoxalase 1 inhibitor prodrug, S-p-bromobenzylglutathione cyclopentyl diester (BBGD), Doxorubicin and Paclitaxel. **(B)** and **(C)** Increase in cellular MG in HEK293 cells and flux of formation of D-lactate (surrogate for flux of MG), respectively. **(D)** and **(E)** Activity and protein of Glo1, respectively, in HEK293 cells: wild-type (WT) and cells stably transfected to overexpress Glo1 (Glo1+) and empty vector (EV). Glo1 activity and protein were increased 4 – 5 fold. This was maintained for > 10 passages. **(F)** – **(H).** Effect of Glo1 expression on anti-proliferative activity. HEK293 cells were incubated with and without treatment for 48 h. Data (6 drug concentrations in triplicate) were fitted by non-linear regression to the dose-response equation, $V = 100 \times GC_{50}{}^{n}/(GC_{50}{}^{n} + [Drug]^{n})$, solving for GC_{50} and, n (logistic regression coefficient). **(F)** Doxorubicin, (**G**) Paclitaxel and **(H)** BBGD.

Table: SARS-Cov-2 proteins with putative activated arginine residues in functional domains

Protein	Activated arginine in RBD SARS-Cov-2
M-protein	R105 (RLFARTRSM)
nsp1	R43 (LSEARQHLK), R73 (VFIKRSDAR), R124 (KVLLRKNGN)
nsp2	R64 (WYTERSEKS), R107 (TIQPRVEKK)
nsp3	R30 (ELDERIDKV), R586 (STIQRKYKG), R712 (EFLKRGDKS)
Nsp8 and nsp12	R75 (YKQARSEDK) and R555 (KNRARTVAG)
nsp15	R61 (LWAKRNIKP), R138 (FRNARNGVL)
Spike protein	R682 (TNSPRRAR), R685 (PRRARSVAS)
NC	R36 (RSGARSKQR), R40 (RSKQRRPQG), R41 (KQRRPQGL), R88 (IGYYRRATR), R89 (GYYRRATRR,), R93 (RATRRIRGG), R95 (TRRIRGGDG); R185 (QASSRSSSR), R191 (SSRSRNSSR), R195 (RNSSRNSTP), R262 (PRQKRTATK)
ORF8 and ORF10	R52 (RVGARKSAP) and R234 (RMNSRNYIA)

Graphical illustration of the hypothesis



DISCUSSION

We provide evidence of vulnerability of SARS-CoV-2 to modification and inactivation by MG. We also reveal, for the first time, increase in cellular concentration of MG in the antiproliferative activity of doxorubicin and paclitaxel – thereby providing a mechanistic rationale for repurposing of these drugs against SARS-CoV-2 and treatment of COVID-19 disease. There are now 37 clinical trials of anticancer drugs treatment of COVID-19, including some inducing proteotoxicity.

CONCLUSION

Doxorubicin and paclitaxel may potentially have application for treatment of COVID-19 and are worthy of evaluation in SARS-CoV-2 live virus cultures and animal models. Relatively low dose of Doxorubicin and paclitaxel, short duration treatment may reduce the side effects associated with the long-term use of these drugs. Experimental studies to validate our predictions are now in progress at QU.