Bromodomain and extraterminal (BET) protein inhibitor, apabetalone, reduces ACE2 expression and attenuates SARS-CoV-2 infection in vitro

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Abstract

Background / Introduction: SARS-CoV-2 causes life threatening COVID-19 complications including acute coronary syndrome, venous thromboembolism, hyperinflammation and damage in multiple tissues. The SARS-CoV-2 "spike protein" binds cell surface receptors including angiotensin-converting enzyme 2 (ACE2) for entry into host cells to initiate infection. Host cell dipeptidyl peptidase-4 (DPP4 / CD26) is implicated as a cofactor in uptake. Recent evidence indicates expression of factors involved in SARS-CoV-2 uptake into host cells is regulated by BET proteins, epigenetic readers modulating gene expression. Apabetalone, the most clinically advanced BET inhibitor (BETi), is in phase 3 trials for cardiovascular disease (CVD) (1,2). In cultured human cardiomyocytes, apabetalone suppressed infection with SARS-CoV-2 and prevented dysfunction of cardiac organoids induced by the cytokine-storm that arises in patients with severe symptoms (3). However, anti-viral properties of apabetalone in other cell types are not known.

Purpose: To examine effects of apabetalone on SARS-CoV-2 infection in cell culture via downregulated expression of cell surface receptors involved in viral entry. Cell systems used mimic initial sites of infection in the lung as well as cell types contributing to complications in late stages of infection.

Methods: Gene expression was measured by real-time PCR, protein levels by immunoblot or flow cytometry, and binding of recombinant SARS-CoV-2 spike protein by flow cytometry. Infection with SARS-CoV-2 was determined in a BSL3 facility. Infectivity was quantified by determining levels of viral spike protein amongst total cells via imaging on an Operetta CLS.

Results: In Calu-3, a human bronchial epithelial cell line, apabetalone dose-dependently downregulated ACE2 gene expression (up to 98%), reduced ACE2 protein levels (up to 84%) and diminished binding of SARS-CoV-2 spike protein (up to 77%, p<0.001 for all parameters). Further, apabetalone abolished infection of Calu-3 cells with live SARS-CoV-2, which was comparable to other antiviral agents. Apabetalone-driven ACE2 downregulation was also observed in extrapulmonary cell types including HepG2, Huh-7 or primary hepatocytes (up to 90%, p<0.001 for all cell types), and Vero E6, a monkey kidney epithelial cell line (up to 38%, p<0.05). DPP4/CD26, a potential cofactor for SARS-CoV-2 uptake, was also downregulated by apabetalone in Calu-3 cells (mRNA ~65% and protein ~40%, p<0.001), which may be synergistic with ACE2 reductions to impede SARS-CoV-2 infection.

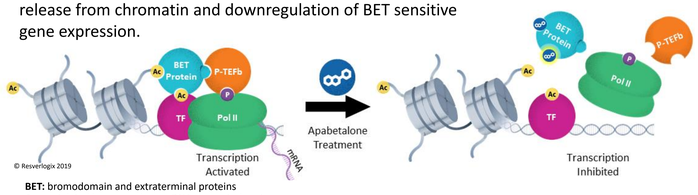
References:

1: doi:10.1001/jama.2020.3308 **2:** doi:10.1186/s12933-020-01199-x

3: 10.1016/j.cell.2021.03.026

Apabetalone Mechanism of Action

BET proteins control gene transcription through interactions with acetylated histones and transcription factors that promote recruitment of RNA polymerase II. Apabetalone, an orally available small molecule, binds to bromodomains in BET proteins, causing their release from chromatin and downregulation of BET sensitive gene expression.



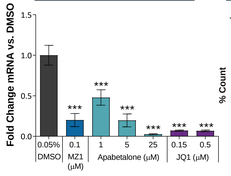
BET: bromodomain and extraterminal proteins
Ac: acetylated lysine residue on DNA associated proteins
BD: bromodomain
TF: transcription factor
Yellow halo indicates selectivity of apabetalone for bromodomain 2 within BET proteins

Haarhaus et. al 2019 Curr Opin Nephol Hyperten

Results

Apabetalone downregulates ACE2 expression and diminishes binding of SARS-CoV-2 spike protein in human Calu-3 lung cells

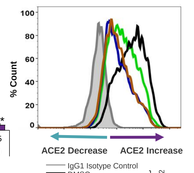
ACE2 Gene Expression
Real-time PCR



***p<0.001, ANOVA followed by Dunnett's

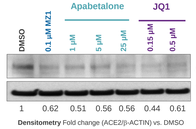
Compound	IC50 ACE2 mRNA
Apabetalone	1.4 μM
MZ1	0.055 μM
JQ1	0.068 μM

Cell Surface ACE2 Protein
Flow cytometry

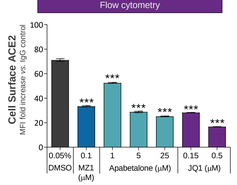


Compound	IC50 surface ACE2
Apabetalone	1.05 μM
JQ1	0.017 μM

ACE2 Protein Levels
Western blot



Binding of SARS-CoV-2 Spike Protein to Calu-3 Cells
Flow cytometry



Positive controls:
JQ1: a pan-BETi with equal affinity for BET bromodomains BD1 and BD2.
MZ1: a proteolysis targeting chimera (PROTAC) that directs BET proteins for degradation.

These results are from 48 hours of treatment.

ACE2 is the receptor on host cells responsible for SARS-CoV-2 uptake and infection

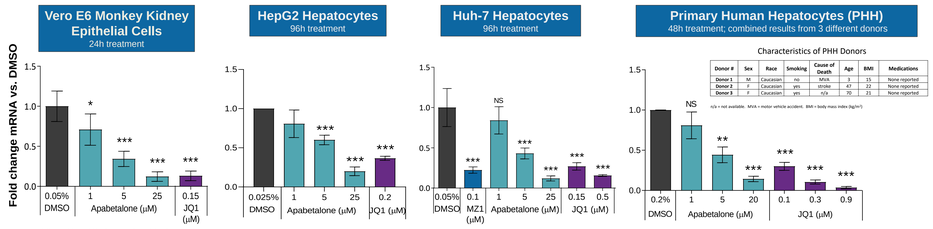
Apabetalone downregulates ACE2 gene expression in extrapulmonary cell types

Vero E6 Monkey Kidney Epithelial Cells
24h treatment

HepG2 Hepatocytes
96h treatment

Huh-7 Hepatocytes
96h treatment

Primary Human Hepatocytes (PHH)
48h treatment; combined results from 3 different donors



Characteristics of PHH Donors

Donor #	Sex	Race	Smoking	Cause of Death	Age	BMI	Medications
Donor 1	M	Caucasian	no	MVA	3	15	None reported
Donor 2	F	Caucasian	yes	stroke	47	22	None reported
Donor 3	F	Caucasian	yes	n/a	70	21	None reported

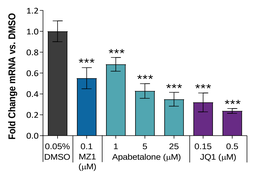
n/a = not available. MVA = motor-vehicle accident. BMI = body mass index (kg/m²)

***p<0.001, **p<0.01, *p<0.05, NS = not significant ANOVA followed by Dunnett's

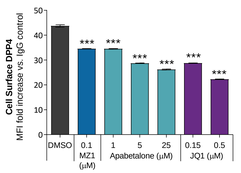
Downregulation of ACE2 in multiple cell types may reduce SARS-CoV-2 toxicity in different organs and may reduce "long COVID"

Apabetalone downregulates DPP4, a potential cofactor for SARS-CoV-2 uptake

DPP4 Gene Expression
Real-time PCR



Cell Surface DPP4 Protein Levels
Flow cytometry

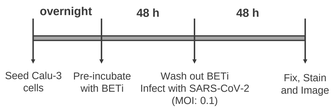


***p<0.001, **p<0.01, *p<0.05, NS = not significant ANOVA followed by Dunnett's

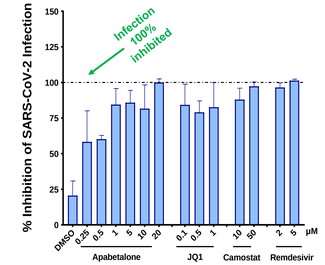
Downregulation of DPP4 may be synergistic with ACE2 reductions to block SARS-CoV-2 uptake and infection

Apabetalone abolishes SARS-CoV-2 infection of Calu-3 cells, comparable to antiviral agents

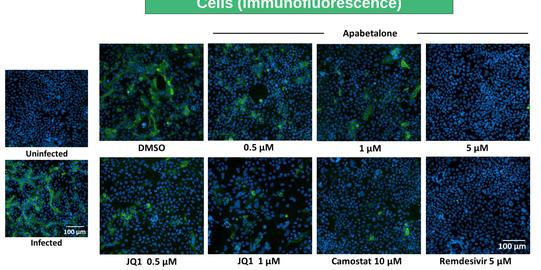
Experimental Design



SARS-CoV-2 Viral Infection of Calu-3 Cells



SARS-CoV-2 Viral Infection of Calu-3 Cells (immunofluorescence)



SARS-CoV-2 spike protein in green Calu-3 nuclei in blue.

SARS-CoV-2 was strain BEI_USA-WA1/2020 obtained BEI Resources and was isolated from a person who'd travelled to Wuhan, China.

Antiviral Agents:
Remdesivir: a ribonucleotide analogue that inhibits viral RNA polymerase. FDA approved for COVID-19 treatment.
Camostat: Protease inhibitor that prevents priming of SARS-CoV-2 spike protein required to initiate infection. Currently in clinical trials to treat COVID-19.

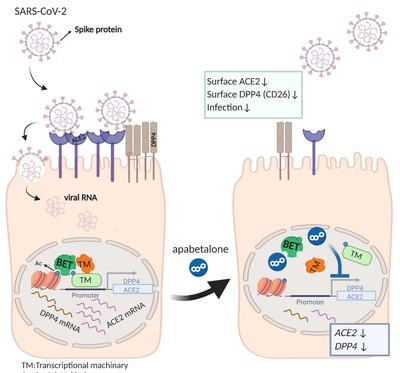
Summary and Conclusions

Apabetalone is an orally available inhibitor of BET proteins in phase 3 clinical trials for cardiovascular indications with an established, favorable safety profile.

Apabetalone downregulates ACE2 gene expression, protein levels and cellular binding of SARS-CoV-2 spike protein in cell culture, and strikingly reduces infection of human Calu-3 lung cells with SARS-CoV-2.

DPP4 / CD26, a potential cofactor for SARS-CoV-2 infection is also downregulated by apabetalone, with possible synergistic effects with ACE2 reductions to block cellular SARS-CoV-2 uptake and infection.

Health Canada approved a clinical trial to evaluate apabetalone to treat COVID-19 (ClinicalTrials.gov Identifier: NCT04894266).



TM:Transcriptional machinary
Ac: Acetylated lysine

Select data are published in *Biomedicines* **2021**, *9*(4), 437; doi.org/10.3390/biomedicines9040437