Serum-Derived Bovine Immunoglobulin Inhibits Proteolysis of COVID-19 Spike Protein

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Objective

Determine if serum-derived bovine immunoglobulin (SBI) can inhibit proteolytic cleavage of the SARS-CoV-2 spike protein S2' domain by TMPRSS2.

Introduction

- Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) viral entry into the gastrointestinal tract can increase gut permeability for both viral and bacterial infections. Translocation of infection leads to increased inflammatory cytokines and increase systemic inflammation.[5]
- Viral entry (SARS-CoV2) into a cell is mediated by the virus's surface spike protein binding to the angiotensin-converting enzyme 2 (ACE2) cell receptor, followed by the proteolytic cleavage of spike S1' and S2' domains by Furin and Transmembrane Serine Protease 2 (TMPRSS2).[1]
- Proteolytic cleavage of the S2' domain by TMPRSS2 is required for fusion of the viral and host membranes and the release of viral RNA into the cell. Inhibiting the cleavage of the S2' domain by TMPRSS2 has been shown to reduce viral entry into the cell.[7]
- Serum derived bovine immunoglobulin/protein isolate (SBI) is an oral supplement composed mostly of immunoglobulins with broad specificity for microbial and viral components.
- SBI has the potential to mitigate proteolytic cleavage of the S2' by a couple of mechanisms.
    1. Finding the S2' domain and sequestering it from TMPRSS2. Bovine CoV and SARS-CoV-2 spike protein epitopes have high homology[8] which could result in cross reactivity.
    2. SBI could contain some residual protease inhibitors as it is derived from fractioning edible grade bovine plasma which contains protease inhibitors.

Figure 1

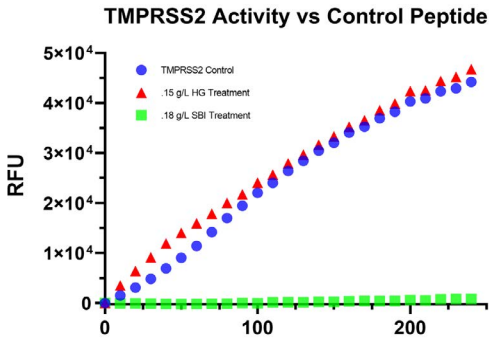

Figure 2

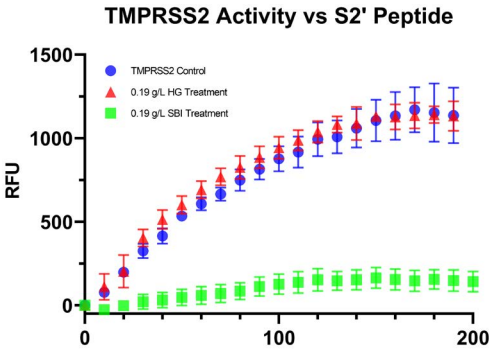

Figure 3

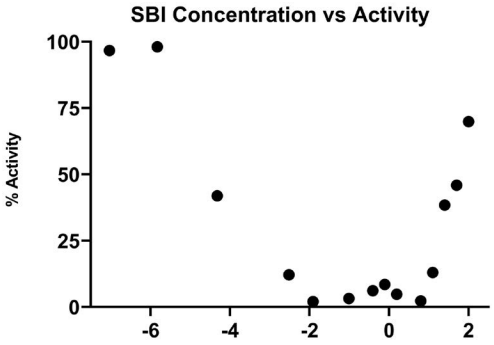

Figure 1 - TMPRSS2 cleavage reaction of control peptide measured by relative fluoresence units (RFU) shows complete inhibition by SBI but not HG. TMPRSS2/SBI, SBI/peptide, TMPRSS2 only and peptide only control data not shown.

Figure 2 - TMPRSS2 cleavage reaction of S2' peptide measured by relative fluoresence units (RFU) shows effective inhibition by SBI but not HG. TMPRSS2/SBI, SBI/peptide, TMPRSS2 only and peptide only control data not shown. Error bars represent +/- 1 standard deviation.

Figure 3 - SBI significantly reduces TMPRSS2 hydrolysis at low concentrations. TMPRSS2 cleavage of S2' peptide activity normalized from TMPRSS2 control activity versus SBI concentration.

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Discussion

- SBI strongly inhibits TMPRSS2. Initial reaction velocity was inhibited by 90% at 0.19 g/L SBI inclusion and extent of reaction was decreased by 95%. (Figure 2)
- The hydrolyzed gelatin negative control protein treatment showed no inhibition of TMPRSS2 at 0.19 g/L inclusion.
- SBI inhibits over 50% of TMPRSS2 activity at 50µg/L and inhibits over 90% of activity between 10 mg/L and 10g/L inclusions.
- This is comparable to therapeutically approved serine protease inhibitors such as gabexate, IC50 41 µg/L.[2]
- As expected, at very dilute concentrations of SBI, TMPRSS activity was not inhibited as the SBI is too dilute.
- Unexpectedly, as SBI concentrations increased above 10 g/L the inhibition of TMPRSS lessened though inhibition was still observed up too 100 g/L.
- The cause of the lessened inhibition that these higher concentrations is not fully understood.
- Controls show that SBI at 10-100g/L + S2' peptide does not show significant signal, indicating the high concentrations of SBI are not hydrolyzing the S2' peptide.
- Additionally controls of TMPRSS2 + SBI at 10-100 g/L showed no significant signal, indicating signal is not simply background fluorescence from the SBI and the enzyme.

Conclusion

- SBI is an effective inhibitor of TMPRSS2 activity against a SARS-CoV-2 S2' fluorogenic peptide.
- SBI shows TMPRSS2 inhibition levels comparable to serine protease inhibitors approved for therapeutic use.
- Future work is needed to understand the mechanisms of TMPRSS2 inhibition by SBI.
- While SBI can inhibit a key mechanism in SARS-CoV-2 cell entry, further in vitro work is require to determine if SBI can reduce SARS-CoV-2 infection in enterocytes.

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