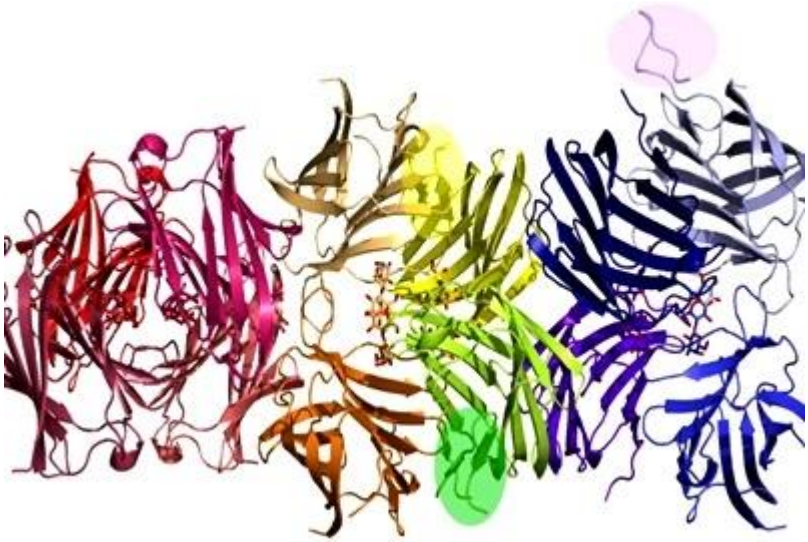
COVID-19 is treated with antiviral drugs, certain antibiotics, corticosteroids, and supportive care; however, these treatments are not clinically effective in combating this disease. The authors from India conducted this randomized, double-blind, placebo-controlled clinical trial to investigate the efficacy of the carbohydrate ProLectin M (PL-M) in outpatients diagnosed with mild to severe COVID-19. At the molecular level, the researchers used nuclear magnetic resonance (NMR) spectroscopy to demonstrate that galectin-3 (Gal-3) binds strongly to PL-M and the S1 subunit of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike (S) protein.

The biological functions of galectins, which are not yet fully understood, encompass roles in development, cell proliferation, apoptosis regulation, inflammation, fibrosis, tissue regeneration, and host defense. Mammalian galectins have one or two highly conserved carbohydrate recognition domains (CRDs). Gal-3 is expressed in the nucleus, cytoplasm, mitochondrion, cell surface, and extracellular space. Gal-3 plays an important role in cell-to-cell or cell-to-matrix interactions, cell growth and differentiation, macrophage activation, antimicrobial activity, angiogenesis, and apoptosis. (Hara A, et al. Galectin-3 as a Next-Generation Biomarker for Detecting Early Stage of Various Diseases. Biomolecules. 2020; 3:10:389)



The S protein is a glycosylated homotrimer with each monomer composed of subunits S1

and S2, separated by host cell proteases. The S1 domain comprises the N-terminal domain (NTD), the receptor binding domain (RBD) with a receptor binding motif (RBM), and two C-terminal domains. Viruses frequently utilize glycans on the host cell surface to enter the cells. Lenza et al. demonstrated that the CRD of Gal-3 binds strongly to the NTD of the SARS-CoV-2 S1 subunit to facilitate viral entry (Lenza MP, et al. Characterization of N-linked glycans in the receptor binding domain of the SARS-CoV-2 spike protein and their interactions with human lectins. Angew Chem Int Ed. 2020;59:23763-23771). Gal-3 was also shown to promote the release of proinflammatory cytokines such as interleukin-6 and tumor necrosis factor-α. According to the authors, targeting viral NTD or Gal-3 could inhibit SARS-CoV2 cell entry and associated inflammatory responses. They also stated that the continual emergence of new SARS-CoV-2 variants threatens the ability to control infections. Therefore, antivirals may be the only viable solution to prevent transmission by inhibiting virus entry into cells or viral replication.

To antagonize Gal-3, scientists developed "ProLectin M" or PL-M, a guar gum-derived carbohydrate composed of α-(1-6)-D-mannopyranose. In previous studies they used two different approaches to investigate the effects of PL-M on Vero cells infected with SARS-CoV-2: firstly, cells were cultured with different concentrations of PL-M prior to SARS-CoV-2 infection, and secondly, the cells were infected with SARS-CoV-2 and then treated with various concentrations of PL-M. The results showed that a PL-M concentration of more than 7 mg/mL reduced the viral RNA copy number by almost 99% compared to the control. In the second approach, PL-M treatment of infected cells at a concentration of more than 7 mg/mL reduced viral load by about 40%.

In this study, outpatients diagnosed with mild to severe COVID-19 were treated orally with PL-M.

About the study

The initial clinical trial with PL-M included 20 outpatients (10 treated with placebo and 10 treated with PLM) diagnosed with mild to severe COVID-19, with an average age of 36.6 years in the placebo group and 41.6 years in the PL-M group. The exclusion criteria included oxygen saturation levels (SpO2) ≤ 94%, pregnancy or breastfeeding, active cancer or chemotherapy, and allergies to any component in the study. Participants treated with any investigational COVID-19 treatment within 30 days before screening were also excluded from this study.

Patients were given one tablet (1400 mg of PL-M or matching placebo) every hour for 7 days, with a maximum dose of 10 tablets *per* day. On days 1, 3, and 7, nasopharyngeal/oropharyngeal swab samples were taken from each patient to evaluate COVID-19 positivity using the RT-PCR method. Changes in clinical symptoms, adverse events, clinical biochemistry or hematology, blood markers of inflammation, and COVID-19 antibody levels were recorded at each visit. Patients were monitored for 28 days after randomization.

The researchers used NMR spectroscopy to examine interactions between the full-length Gal-3 or its truncated CRD form and the SARS-CoV-2 S1 subunit.

Results

The first COVID-19 clinical trial that enrolled 20 COVID-19 outpatients (10 treated with placebo and 10 treated with PLM) showed that treatment with PL-M significantly increased RT-PCR cycle counts for the nucleocapsid (N) and ORF genes on days 3 and 7 compared to placebo. From day 3 after treatment, all PLM-treated subjects were RT-PCR negative for both genes, while none of the participants in the placebo group were RT-PCR negative until day 7. When this first clinical trial was extended to 34 COVID-19 patients (17 placebo and 17 PLM-treated subjects) the results were similar to those of the first trial mentioned above. On day 7, all participants in the PL-M treated group had cycle counts above the target cut-off value of 29. Most of the participants (94%) in the placebo group had cycle counts below the target cut-off value.

NMR spectroscopy showed that PL-M bound relatively strongly to Gal-3 and that Gal-3 bound strongly to sugar-binding sites on the SARS-CoV2 S1 domain. The authors concluded that PL-M competes with the S1 protein for binding to Gal-3, compromising the SARS-CoV-2 entry into susceptible target cells.

Conclusion

This research group has demonstrated that the carbohydrate PL-M can reduce the viral load in Vero cells. In clinical studies with COVID-19 patients, PL-M treatment resulted in a rapid reduction of viral load and increased viral clearance, with no adverse effects.

In addition, NMR spectroscopy showed that Gal-3 has a high affinity for PL-M and the S1

subunit of the SARS-CoV-2 S protein. From this, the authors concluded that PL-M inhibits the cell entry of SARS-CoV-2 by binding to Gal-3. This antagonizes the binding of Gal-3 to the viral S1 protein.

The authors suggested that PL-M could be used for COVID-19 treatment and prevention due to its significant efficacy and tolerability.

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