LOVELACE BIOMEDICAL

SARS-CoV-2 Efficacy of Calcium Glycerophosphate (CGP) in a Syrian hamster model

AkPharma Inc

Proposal 2021-236

IACUC FY20-117

Study FY21-155

Lovelace Test Article L0880

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Rationale

This study is the final in a series of eight studies* performed by Lovelace Biomedical and associated facilities on respiratory uses of calcium glycerophosphate (CGP) in animals and humans, underwritten by AkPharma Inc. Prior studies in this sequence, as well as by others, have demonstrated potential utility of a CGP nasal spray in nasal passage opening in animals and humans similar to that of extant OTC decongestants. Earlier work by other investigators dating to 1996 has suggested cellular activity by CGP in disparate body realms including urinary bladder relief from interstitial cystitis symptoms (oral), non-irritative epidermal skin renewal acceleration (topical) and accelerated surgical wound healing (topical). A single in vitro study in by Datta and Weis, Texas Tech University, 2014, demonstrated preservation of CGP-treated human cell wall integrity vs. a cytomix challenge. Theory for CGP activity has centered on probable CGP insertion into the sphingosineàsphingosine-1-phosphate (S1P) sequence, with CGP acting both as a phosphorylation agonist and dephosphorylation inhibitor conferring greater longevity on the upregulated bioactive S1P.

In 2019 the Datta and Weis observation became timely following disclosure of the colonizing mechanics of the epidemic SARS-CoV2 virus, which was theorized to more readily implant following a host cell hyper-responsive cytokine storm. It was felt that in vivo animal studies were mandated to determine whether this conjectured mechanism suggested practicable real-life implications of Datta and Weis in down-modification of viral spike embedment. The study presently reported on, along with its immediate predecessor indicate that while there are some post-mortem histological effects that may merit further study, that they are not significant, and more importantly, there were no gross effects differences post inoculation between the virus-infected CGP-treated and untreated animals. There were no adverse histological effects observable on the animals from the CGP inhalation treatment.

Executive Summary

The objective of this study was to test whether aerosolized calcium glycerophosphate (CGP) has a therapeutic effect against SARS-CoV-2 in a Syrian hamster model. CGP was dosed by aerosol exposure twice daily, starting two days prior to viral inoculation and continuing until animal sacrifice (i.e. Days -2 through Day 4; terminal on the morning of Day 5). The average daily dose over the course of the study was 1.5 mg/kg deposited in the lungs. Groups of animals were inoculated with 3 different doses of virus that may be similar to environmental exposures (e.g. 10-1,000 $TCID_{50}$/animal). Control groups received the same viral challenge but were treated with sterile water replacing CGP.

In-life data collection and metrics of efficacy included twice daily observations, daily body weights, and nasal swabs for detection of both genomic and subgenomic viral RNA by RT-qPCR on Day 3, and 5 post-infection. Terminal collections to gauge efficacy included lung weights, reported as a percent of body weight, and pulmonary tissue collection for detection of genomic and subgenomic RNA by RT-qPCR. Pulmonary tissue was also collected for live virus quantification (i.e. $TCID_{50}$). Lung tissue and nasal tissue were also fixed in 10% neutral buffered formalin and stored for potential future analysis by histopathology. Histopathology is not included in this summary.

Twice daily observations: No animals had clinical observation calls.

Body Weight: There was a dose dependent loss in body weight driven by the viral challenge dose and ranging up to a 10% group average loss. There was no significant effect of CGP treatment.

Nasal Swab RT-qPCR: There was a significant decrease in genomic and subgenomic RNA in Group 6 (High virus + CGP treatment) compared to Group 3 (High virus + water control) on Day 3 but did not remain significant on day 5. There was no difference in amplification of genomic or subgenomic RNA from nasal swabs between any other groups on any of the days of collection.

Lung Weight: Lung weights, expressed as a percent of body weight, suggested a dose dependent mild to severe inflammation based on viral challenge dose. There was no effect of CPG-treatment when compared to controls.

Lung Gross pathology: There was a dose dependent increase in gross pathology scores driven by the viral challenge. There was no significant effect of CGP treatment.

Executive Summary

Pulmonary tissue RT-qPCR: Genomic and subgenomic RNA levels in the lungs of all groups were similar. There was no significant effect of CGP treatment.

Pulmonary tissue $TCID_{50}$: Levels in the lungs of all groups were similar. There was no significant effect of CGP treatment.

Overall, CGP administered by inhalation twice daily did not appear to offer substantial advantages over water dosed in the same way. One of the goals of the current study was to use lower doses of the viral challenge (10, 100 and 1000 $TCID_{50}$/animal) compared to past studies ($8x10^4$ $TCID_{50}$/animal) to determine if the effects of CGP may be more visible in a model with more environmental level exposure paradigm. While the different doses of virus did result in a more mild – moderate dose dependent phenotype the effects of inhaled CGP were not significant. There may have been a local effect in the nasal cavity as evidenced by reduction in genomic and subgenomic viral RNA at Day 3 in the highest viral challenge group. Subgenomic RNA is thought to represent a subset of viral RNA that is more representative of replicating virus as opposed to non-replicating virus or virus that was administered at inoculation. Data here may suggest that CGP could have a slight, local, viral replication inhibitory effect where it was administered, the nasal cavity. There were no adverse effects of delivering CGP by inhalation to the animals.

Study Design

EXPERIMENTAL DESIGN

Table 1: Experimental Design

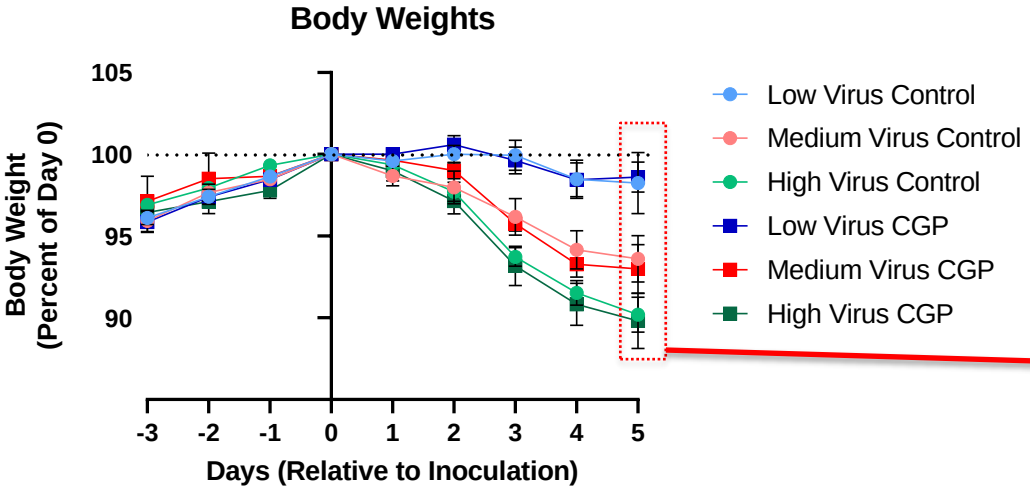
Group	Group Description[1]	Inoculation Titer (TCID50/animal)2	Test Article[3]	Clinical Readouts	Endpoints and readouts
1	Group 4 Control	10	Aerosolized water: twice daily Days -2 through 4; 14 total exposures	Starting at Day -3: Daily body weights; twice daily observations; nasal swabs for RT-qPCR on Days 3, and 5 (hold, processing to be added by amendment only).	Lung: One genomic RT-qPCR and one TCID50 per animal. Half lung infused with formalin and fixed (hold, histopathology to be added by amendment only).
2	Group 5 Control	100			
3	Group 6 Control	1000			
4	CGP 1	10	Aerosolized 1.5% CGP: twice daily Days -2 through 4; 14 total exposures		
5	CGP 2	100			
6	CGP 3	1000			

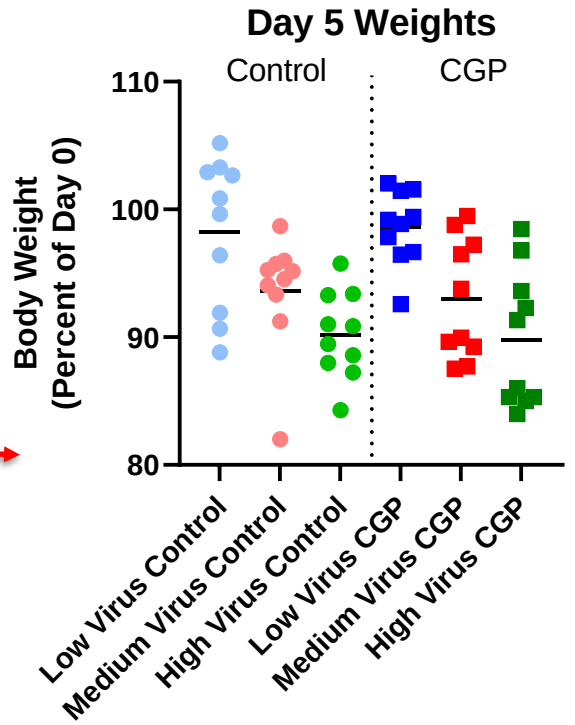
[1] Each group will have 10 animals.

[2] Inoculation will be given intranasally (IN) at 200µL/animal. Dosing will be intranasal (IN)

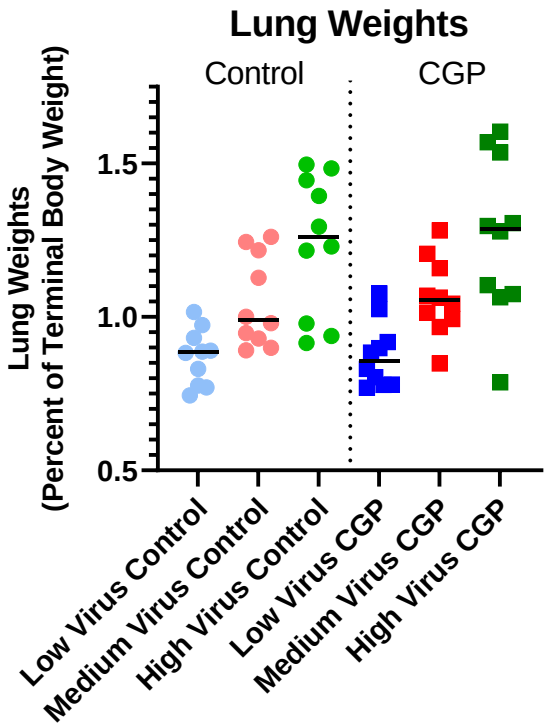
[3] Delivered by full body exposure in a chamber. To occur twice daily between approximately 7:30a.m. and 9:30 a.m. and 3:00p.m. and 5:00p.m each day from Day -2 through Day 4. On the day of inoculation (Day 0), viral inoculation will occur AFTER TA or vehicle exposure.

Body Weights





Lung Weights



Quantification of Dosing (Aerosol)

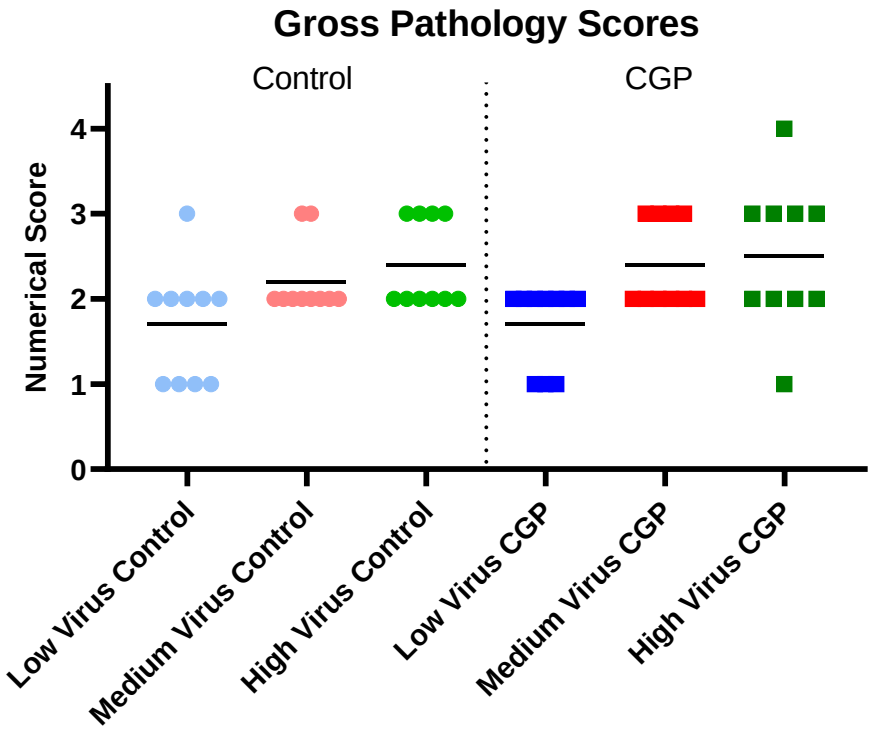
FY21-155 Total Aerosl **Presented** Dose By Exposure (mg/kg)				
Group	Day	A.M.	P.M.	Day
Vehicle	-2	0.2	0.1	0.4
	-1	0.1	0.1	0.3
	0	0.3	0.2	0.5
	1	0.3	0.6	0.9
	2	0.5	0.2	0.7
	3	0.1	0.1	0.3
	4	0.1	0.1	0.2
TA	-2	8.1	7.9	16.0
	-1	6.8	7.2	13.9
	0	7.6	8.3	15.9
	1	7.2	7.9	15.1
	2	5.7	6.9	12.6
	3	7.6	7.6	15.2
	4	6.5	7.0	13.5

FY21-155 Total Aerosl **Deposited** Dose By Exposure (mg/kg)				
Group	Day	A.M.	P.M.	Day
Vehicle	-2	0.0	0.0	0.0
	-1	0.0	0.0	0.0
	0	0.0	0.0	0.1
	1	0.0	0.1	0.1
	2	0.0	0.0	0.1
	3	0.0	0.0	0.0
	4	0.0	0.0	0.0
TA	-2	0.8	0.8	1.6
	-1	0.7	0.7	1.6
	0	0.8	0.8	1.6
	1	0.7	0.8	1.5
	2	0.6	0.7	1.3
	3	0.8	0.8	1.5
	4	0.7	0.7	1.4

Clinical Observations

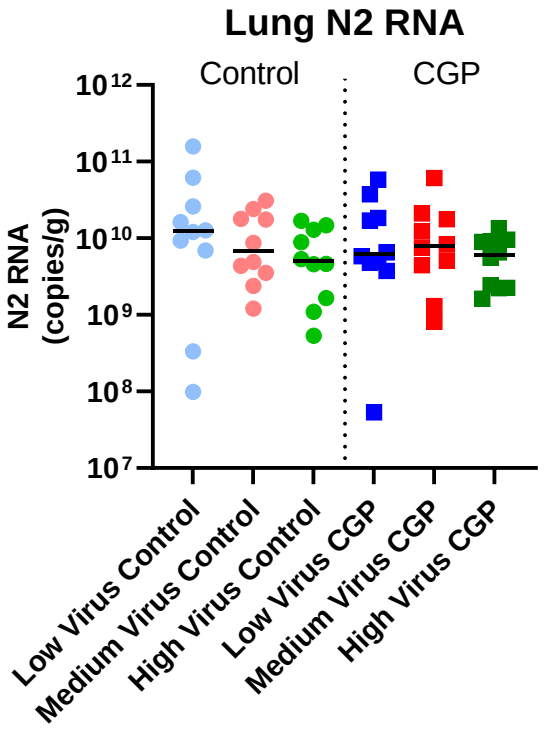
- No clinical calls

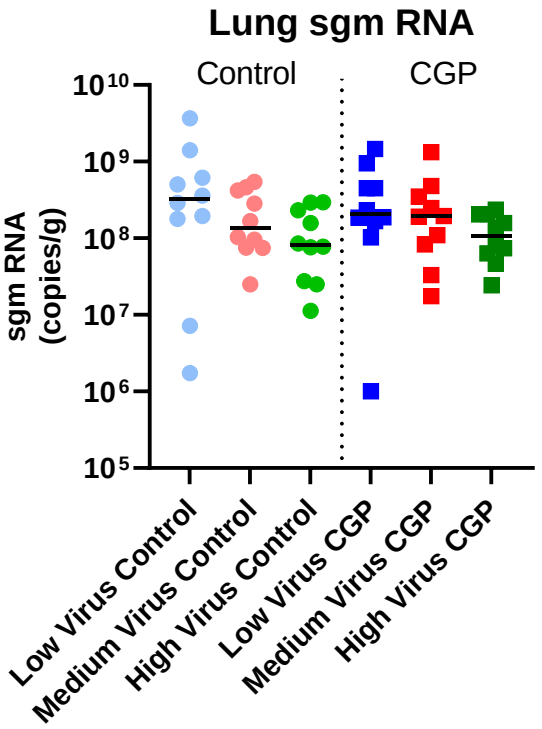
Goss Pathology Scores



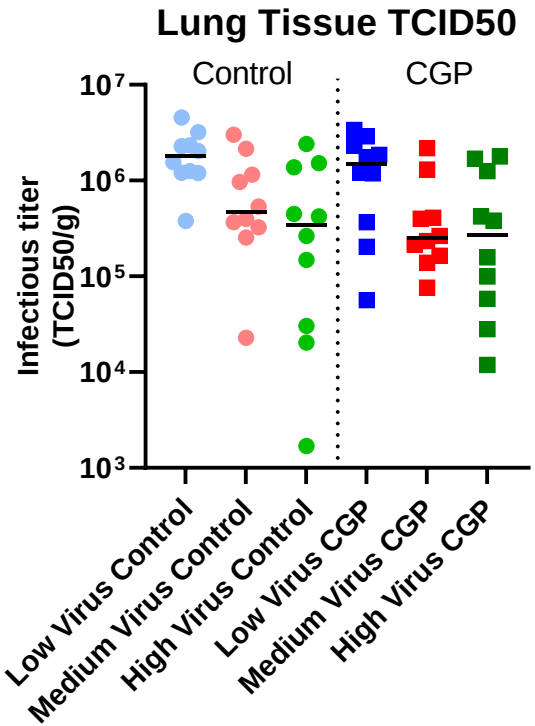
	Low Virus Control	Medium Virus Control	High Virus Control	Low Virus CGP	Medium Virus CGP	High Virus CGP
Number of values	10	10	10	10	10	10
Minimum	1.000	2.000	2.000	1.000	2.000	1.000
Maximum	3.000	3.000	3.000	2.000	3.000	4.000
Range	2.000	1.000	1.000	1.000	1.000	3.000
Mean	1.700	2.200	2.400	1.700	2.400	2.500
Std. Deviation	0.6749	0.4216	0.5164	0.4830	0.5164	0.8498
Std. Error of Mean	0.2134	0.1333	0.1633	0.1528	0.1633	0.2687

Pulmonary RT-qPCR

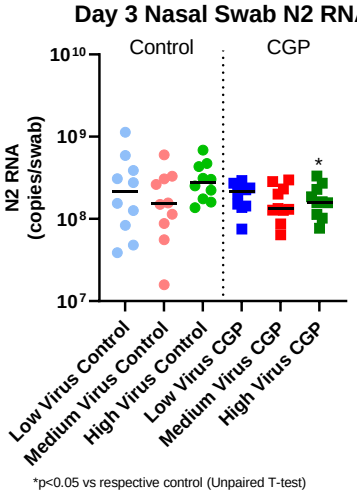




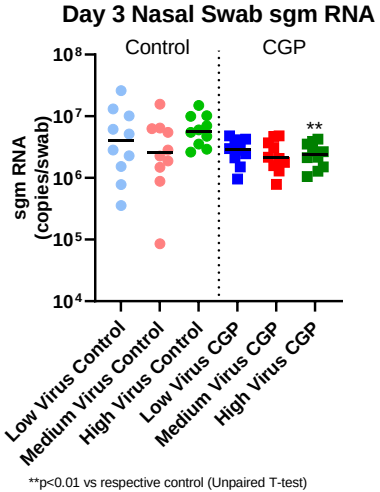
Pulmonary TCID50



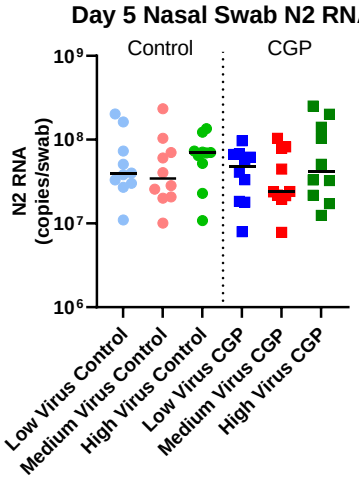
Day 3 and 5 Swab RT-qPCR

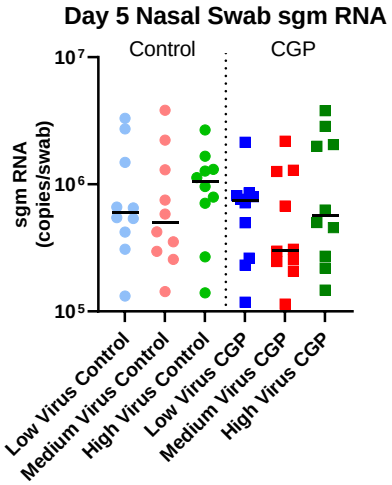


*p<0.05 vs respective control (Unpaired T-test)



**p<0.01 vs respective control (Unpaired T-test)





Other

- Lungs and skulls (nasal turbinates) are fixing in 10% NBF and will be held at Lovelace. Further processing by amendment only.