Research Article

Proof of concept study to evaluate the safety and effectiveness of a novel Favipiravir dry powder for inhalation formulation in subjects with SARS-COV-2 infection

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ABSTRACT

Aim: The present study is the first hand evidence of the clinical use of dry powder for inhalation (DPI) dosage form of Favipiravir (Favi DPI) in mild to moderate COVID-19 patients. In this study, Favi DPI in the doses of 10 and mg per day was compared to the oral regime of Favipiravir (1600 mg/day) along with the standard of care. **Materials and Methods:** In the current randomized control trial, 64 patients with mild to moderate score of COVID-19 infection were included in three parallel groups (n = 20/group). The main outcome measures included changes in subjects getting reverse transcription polymerase chain reaction (RT-PCR) negative, alleviation of clinical symptoms, SpO_2, inflammatory markers, requirement of hospital stay, and metabolic profiles. **Results:** The Favi DPI groups have shown clinically significant improvement in reduction of viral titer, symptoms, and inflammatory marker levels from the baseline. The biochemical tests, hospital stay, and other safety profile were comparable to oral Favipiravir as a control. There was slightly better efficacy shown by Favi DPI 20 than Favi DPI 10 mg in terms of alleviation of symptoms and RT-PCR negativity. **Conclusion:** All subjects from Favi DPI groups led to clinical cure and no progression of disease as observed with oral Favipiravir regime. The reduced dose can curtail the side effects of Favipiravir which can be achieved through DPI favipiravir formulation requiring many fold less dose than the oral dosage form. This study may provide link to assess superiority of Favi DPI compared to oral, in terms of reduced dosage, toxicity, improved patient compliance, and good prognosis of the disease.

Keywords: Antiviral, COVID-19, dry powder, Favipiravir

Introduction

SARS-CoV-2 has an extremely high transmissibility and a degree of lethality not yet established globally. The incubation period of COVID-19 is comparable to SARS (2–7 days) and MERS (2–14 days). The symptoms of COVID-19 appear after an incubation period of approximately 5 days. The period from the onset of COVID-19 symptoms to development of IgG antibodies range from 14 to 21 days.[1] With the most common symptoms at onset of COVID-19 illness such as generalized weakness, fatigue, fever, cough, loss of taste, and smell,[2] the clinical features reveals a chest CT scan presented as pneumonia, acute respiratory distress syndrome, acute cardiac injury, and incidence of ground-glass opacities leading to lethality. COVID-19 exhibits multimodal pathology including "cytokine storm," that is, secretion of certain chemicals in the body that exaggerate the inflammatory response of the body; reduced body oxygen carrying and viral infection causing pneumonia, infiltration of the lung tissue, respiratory failure and eventually, and lethality.[3]

The current therapies for prevention, management, and treatment of COVID-19 include use of antiviral drugs, immune-boosting supplements, steroids.[4]

The most often used antiviral drugs in COVID-19 therapy is Favipiravir in cases with mild to moderate disease presentation and Remdesivir in patients with moderate to severe disease requiring

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oxygen supplementation.[5] Steroids like Tocilizumab have been used for moderate to severe disease with critically raised inflammatory markers.[6]

For patients with mild to moderate disease, Favipiravir is often recommended as it helps in preventing the multiplication of virus and reduces the viral load that is associated with certain challenges in its current dosage form particularly its dose. The recommended dose of Favipiravir for adults is 1800 mg orally twice daily on day 1, followed by 800 mg orally twice daily for 14 days.[7] The adherence to the therapy by patients is low due to large dose and multiple tablets to be consumed per day. This creates need of administering several tablets of 200 mg multiple times to reach the intended dose.[8] The failure to administer required dose due to inconvenient dosing availability can lead to progression of disease. There are many challenges of the oral administration in attaining systemic effect. To achieve early recovery of patients, it is important to focus on patient compliance and prevent hospitalization and requirement of oxygen and ventilators. This change in the therapy can reduce the load on the health-care system and may prevent the system from frequently collapsing.[9]

In the trials conducted until now, diarrhea, hyperuricemia, and liver toxicity are the typical adverse reactions of oral dose of Favipiravir. All these adverse effects are usually dose-dependent toxicities.[8,10] If any modification and reduction in dosage can reduce these adverse effects of Favipiravir, it will, in turn, improve the therapeutic index of Favipiravir to be used as an antiviral agent/drug in COVID-19.

All these aspects generate a need for development of a novel, safe, and effective formulation with other than oral dosage form of Favipiravir to amplify the outcomes of the current therapy for COVID-19 affected subjects.

In the present study, we are depicting the outcomes of a pilot clinical study of a dry powder inhalation dosage form of Favipiravir, which can be administered by inhalation route in mild to moderate COVID-19 patients who are fit for the oral Favipiravir therapy.

Materials and Methods

Study design

The study was a randomized, parallel arm, controlled, and clinical study comprised patients who were enrolled for the treatment of COVID-19 in a dedicated COVID-19 care setting in India. The study was approved by the Institutional Ethics Committee Lokmanya Medical Research Centre and Royal Pune Independent Ethics Committee. It was registered with Clinical Trial Registry of India with registration number CTRI/2021/05/033750. The study was carried out at three sites, Lokmanya Medical Research Centre, Chinchwad, Pune, Health Nexus Research Centre, Pune and Lifepoint Multispecialty Hospital, Pune. The CONSORT flow of the entire study is depicted in Figure 1.

Inclusion criteria

Adults of age 18–60 of both sexes with positive reverse transcription polymerase chain reaction (RT-PCR) from nasopharyngeal swab test result presenting mild symptomatic or asymptomatic patients having no signs of severe disease (NEWS score ≤6) and no comorbidity at screening and willing to provide consent and follow-up for study duration were included in the study.

Exclusion criteria

Participants with compromised immunity, autoimmune disease or self-reported HIV or syphilis infection and proving to be unfit for the study as per investigator's discretion were excluded from the study. Participants from the vulnerable group such as pregnant or lactating women were also excluded from the study. Participants requiring intensive care unit (ICU) admission and/or artificial ventilation or any other comorbidity, were excluded from the study.

Groups

We screened 64 participants of which all the participants were enrolled into the study. There were no screen failure subjects. They were randomized using a computer generated randomization sheet, either in the standard treatment arm (control) where the patients were provided with conventional care as recommended in clinical management protocol for COVID-19 advocated by Indian Council of Medical Research (ICMR), Ministry of Health and Family Welfare, Government of India[11] or to the treatment arms where the patients were treated with 10 and 20 mg favipiravir dry powder for inhalation (DPI) daily along with the standard protocol (Favi DPI 10 and Favi DPI 20). There were four subjects who got dropped out of the study as not able to follow-up and thus there were 60 evaluable cases for the final analysis of the study. Details are depicted in Figure 1.

Sample size

As an academic trial, we took minimum of sample size of 60 completer patients which were equally divided into three groups control (Oral favipiravir tablets), Favi DPI 10 and Favi DPI 20.

Interventions

As mentioned, the control group received standard of care (SOC) of oral Favipiravir tablets (1600 mg/day/patient) which was provided for 10 days. SOC treatment was continued as per the ICMR protocol. Each patient from the treatment group received SOC and Favipiravir DPI. There was no oral Favipiravir provided in their treatment regime whereas the control group patients received oral Favipiravir as one of the component of SOC. The Favi DPI was manufactured by SAVA Healthcare Limited, Chinchwad, Pune - 411 019 and supplied to the sites. The investigational product was having two components - container with a size 0 capsule and DPI device. Following steps were followed for the use of the device - removal of protective cap, opening mouthpiece to access capsule housing, loading capsule into housing, closing the mouthpiece, and actuating pushbuttons to pierce the capsule and inhale. Inhalation by the patient lifts the capsule from its housing and makes it spin at high speed around its main axis, thus helping powder disaggregation. The powder getting out of the capsule through the two holes created by the piercing system is then inhaled by the patient through the mouthpiece.

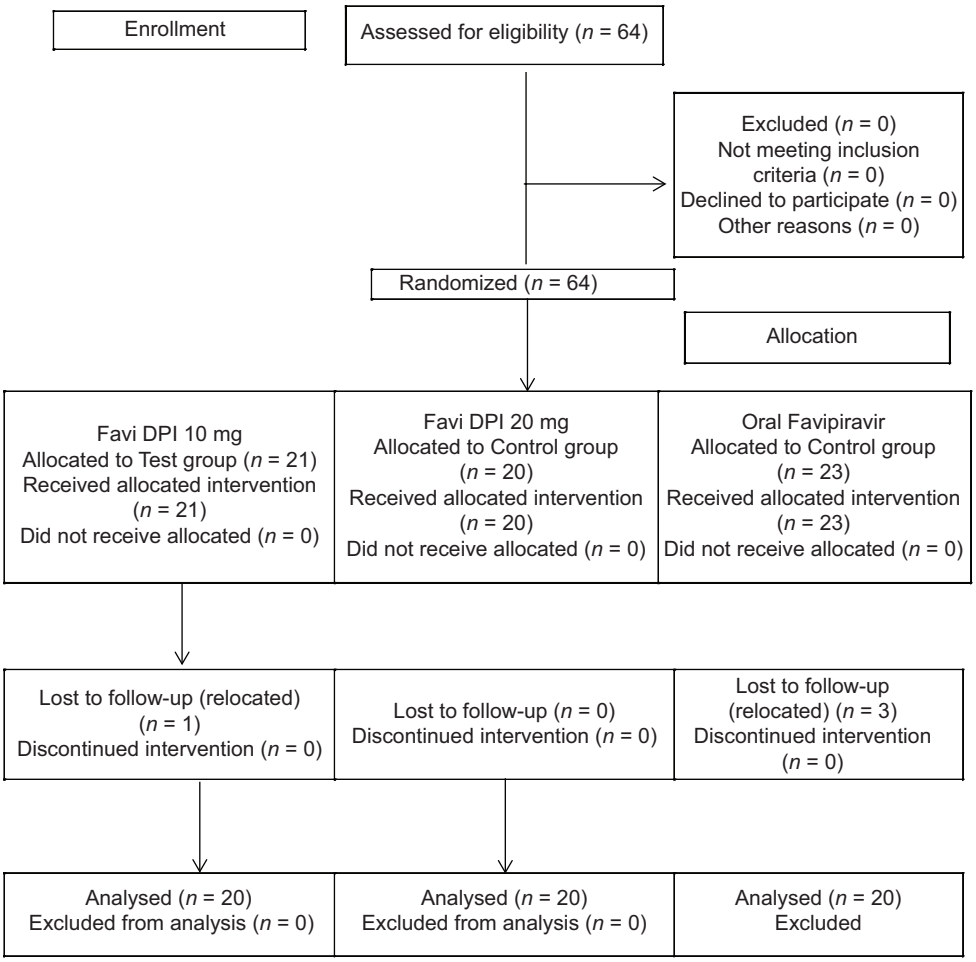


Figure 1: CONSORT Flow chart of the trial events

At the bottom of the mouthpiece, a grid prevents the capsule from reaching the mouthpiece duct. For Favi DPI 10 group patient executed administration of one capsule per day for 10 days and for Favi DPI 20 group, patient executed administration of two capsules of 10 mg each in the interval of 15 min/day for 10 days.

Outcome measures

This study looked at a variety of outcome variables related to the prognosis of COVID-19. The primary outcome measures were, percent population with negative RT-PCR, improvement of clinical symptoms including duration of fever, respiratory distress, cough, sneezing and diarrhea on 5-point ordinal scale, changes in SpO_2 levels, requirements of supplemental oxygen, reduction in elevated levels of inflammatory markers such as CP, LDH, D-dimer, ferritin, and Interleukin 6 on baseline, day 5 and day 10.

In addition, we evaluated, changes in serum electrolyte levels, changes in serum levels of liver enzymes such as serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT), creatinine, requirement of admission to ICU, and duration of hospitalization.

The outcomes of the study were to evaluate safety of Favipiravir DPI by assessing tolerability of intervention by study subjects, changes in biochemical parameters such as liver and renal function test, lipid profile, complete blood count, and adverse events.

Data analysis

The safety population consisted of all subjects enrolled in the study, who have received at least one dose of study products, that is, mITT population. Primary Efficacy and secondary endpoints were analyzed using the PP population.

Demographic and baseline information

Continuous variables that are age and other demographical characteristics were summarized by overall using summary statistics, that is, the number of observations, mean and standard deviation with 95% CI (among normal distribution). Categorical values such as gender and clinical examination were also be summarized using frequencies and percentages.

Analysis of efficacy parameters

In this study, percentage population with negative RT-PCR were compared using Chi-square test. Improvements of clinical symptoms were estimated by Wilcoxon sign rank test for with in group and analysis of variance for between groups. Reductions in elevated levels

of inflammatory markers were assessed using Wilcoxon sign rank test and analysis of variance with Kruskal–Wallis for between groups. Duration of hospitalization, changes in serum electrolyte levels, changes in serum levels of liver enzymes such as SGPT and SGOT, creatinine were analyzed using analysis of variance.

Safety analysis

Mean changes in renal function test, lipid profile, complete blood count, other laboratory variables, and vital signs were assessed using Student's t test. Adverse events were noted as population experiencing events and number of events.

Results

The mean age of participants in the Favi DPI 10 group was 36.05 ± 11.14 years; Favi DPI 20 group was 38.80 ± 09.11 years; and 37.20 ± 11.45 years in Control group with female participation ranging from 25 to 40% and male participation in 60–75% which is in line with the clinical experience of COVID-19 incidence in Indian context.

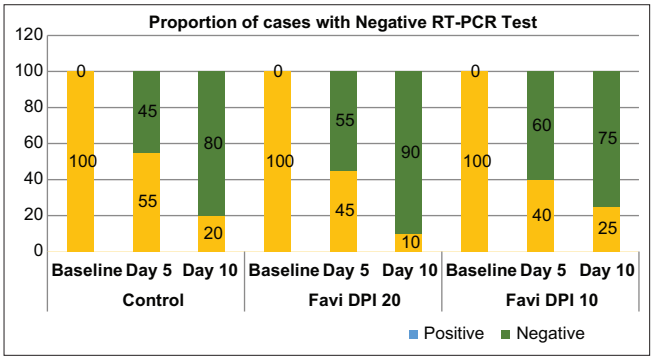
Primary efficacy parameters

Profile of RT-PCR test between the groups

At day 5, Favi DPI 10 and 20 mg showed 45 and 55% subjects becoming RT-PCR negative which was comparable to oral Favipiravir control (60%). Same way on day 10, the cumulative percent population getting RT-PCR negative in Favi DPI 10 and 20 groups were 80% and 90% which was slightly higher to oral Favipiravir group being 75% [Graph 1]. The percentages of subjects getting negative on RT-PCR were significantly increasing in Favi DPI 10 and 20 groups as that of control.

Changes in symptom score between groups

This study results reveal that at baseline mean score of symptoms was comparable, and difference was not statistically significant. This depicts that there was no selection bias and all the subjects in three groups were having comparable status of symptoms such as cough, breathlessness, fatigue, and myalgia. At day 5 and 10 mean scores of symptoms under consideration were significantly reduced from their respective baseline.



Graph 1: Profile of RT-PCR test in the groups

At the end of 5th day, mean score of cough showed a significant fall of 33.3% among Favi DPI 10, 40.0% in Favi DPI 20, and 38.5% in control from baseline. It continued to further decrease till day 10 with a significant fall of 50.0%, 56.0%, and 61.5% in Favi DPI 10, 20, and control groups. [Figure 2] At the end of 5th day, mean score of breathlessness showed a significant fall of 37.2% in Favi DPI 10, 43.2% in Favi DPI 20, and 39.0% in control from baseline which got further reduced in Favi DPI 10, 20, and control group till day 10 to 46.52, 43.25, and 48.79%, respectively [Figure 2]. At the end of 5th day, mean score of fatigue showed a significant fall of 25.6% in Favi DPI 10, 32.6% in Favi DPI 20, and 40.0% in control from baseline which got further reduced in Favi DPI 10, 20, and control group till day to 46.5%, 52.2%, and 47.5%, respectively [Figure 2]. The same trend was observed in mean score of myalgia with a significant fall of mean score to 53.2%, 50.0%, and 52.3 in Favi DPI 10, 20, and control groups at day 10 [Figure 2].

The change in mean score of symptom score was statistically significant within groups compared to their baselines. The potential of alleviation of symptoms by the treatment of Favi DPI in both doses, that is, 10 and 20 were comparable to oral Favipiravir. When compared between Favi DPI 10 and 20 as depicted in Table 1 and Graph 2, there were more subjects getting relieved of the cough and breathlessness in Favi DPI 20 than in Favi DPI 10 group reflecting that the efficacy of the Favi DPI is dependent on the dose administered.

Proportion of cases required supplemental oxygen

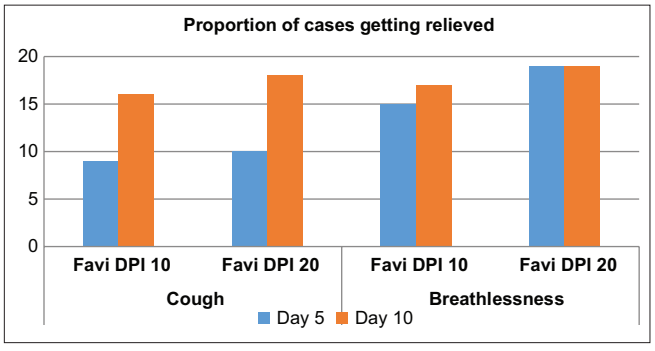
There were no patients in all three groups' required supplemental oxygen. All the subjects were maintaining normal blood oxygen levels being on air.

Changes in SpO_2 levels

In all the three groups, the mean SpO_2 levels were in the range of 96.5–98%. The changes in SpO_2 levels in Favi DPI and oral Favipiravir were comparable from baseline to day 10 [Table 2].

Changes in inflammatory markers

At baseline, the mean D-Dimer was 0.35 µg/ml in Favi DPI 10, 0.32 µg/ml in Favi DPI 20 and 1.04 µg/ml among control which was comparable. At the end of day 10, mean D-Dimer showed fall of 15.6% among Favi



Graph 2: Changes in proportion of cases relieving of cough and breathlessness between groups

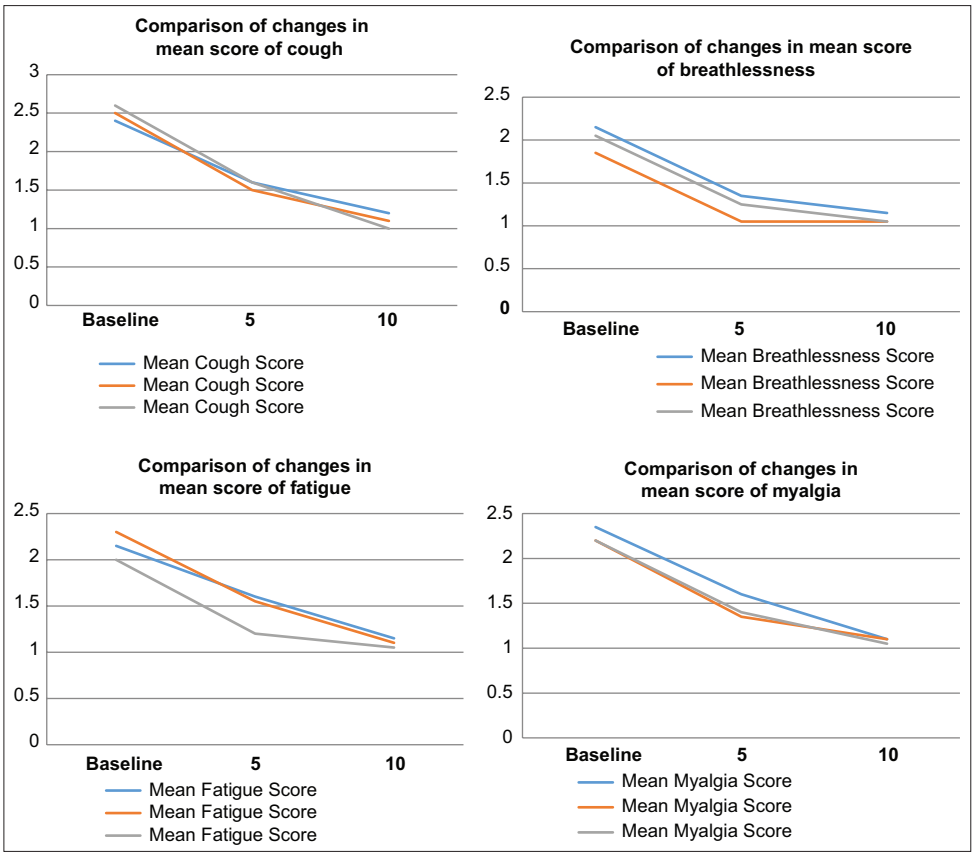


Figure 2: Changes in symptom score between groups

Table 1: Changes in proportion of cases relieving of cough and breathlessness between groups

Duration in days	Percent proportion of cases getting relieved					
	Cough (%)			Breathlessness (%)		
	Favi DPI 10	Favi DPI 20	Control Oral Favipiravir	Favi DPI 10	Favi DPI 20	Control Oral Favipiravir
Day 5	9 (45)	10 (50)	8 (40)	15 (75)	19 (95)	15 (75)
Day 10	16 (80)	18 (90)	20 (100)	17 (85)	19 (95)	19 (95)

DPI 20 and 61.5% among control from baseline. The mean CRP levels at baseline were comparable in all the groups. At day 10, there was significant reduction in CRP levels, that is, 77.8%, 70.0%, and 71.8% in Favi DPI 10, 20, and control from baseline. If compared, the change in CRP levels was similar in Favi DPI and oral Favipiravir groups. At the end of day10, mean Ferritin showed reduction of 19.0%, 27.6%, and 22.6% in Favi DPI 10, 20, and control from baseline. The results were comparable between Favi DPI and oral Favipiravir control group. The mean LDH showed more or less comparable changes in Favi DPI and oral Favipiravir groups. The mean interleukin-6 levels were increased from baseline in Favi DPI 10 and control group whereas there was 59.6% reduction in Interleukin-6 levels in Favi DPI 20 from baseline. The data are showed in Table 3.

Table 2: Changes in SpO_2 levels between groups

Duration in days	Mean SpO_2 (%) (Mean±SD)			*P*-value
	Favi DPI 10	Favi DPI 20	Control	
Baseline	97.45±2.04	97.50±0.95	97.70±1.22	0.852 (NS)
10	96.95±2.93	97.40±2.82	96.65±3.94	-
Mean change (baseline – day 10) (*P*-value)	–0.50±2.06 (0.292) (NS)	–0.10±2.81 (0.875) (NS)	–1.05±4.16 (0.273) (NS)	0.633 (NS)

By Student's *t*-test. By ANOVA - Kruskal–Wallis. NS: Not significant

Secondary outcomes

Changes in serum electrolyte levels was mentioned in Table 4:

The data depict that the serum electrolytes levels such as calcium, sodium, potassium, and chloride were comparable between all the groups at baseline and day 10.

Changes in liver enzyme levels are mentioned in Table 5:

The data depict that the serum levels of liver enzymes such as SGPT and SGOT were comparable between all the groups at baseline and day 10.

Changes in renal function are mentioned in Table 6:

Table 3: Changes in inflammatory markers between groups

Duration	Favi DPI 10	Favi DPI 20	Control	*P*-value
Mean D-Dimer (ug/ml) (Mean±SD)				
Baseline	0.35±0.49	0.32±0.41	1.04±2.27	0.337 (NS)
10	0.44±0.64	0.27±0.26	0.40±0.65	-
Mean change (baseline – day 10) *P*-value	0.09±0.63 0.928 (NS)	–0.05±0.35 0.896 (NS)	–0.64±2.23 0.171 (NS)	0.496 (NS)
Mean CRP (mg/L) (Mean±SD)				
Baseline	11.32±21.86	12.80±25.23	13.90±23.63	0.693(NS)
10	2.51±2.39	3.84±7.36	3.92±6.98	-
Mean change (baseline – day 10) *P*-value	–8.81±21.70 0.012*	–8.95±26.99 0.116 (NS)	–9.98±25.13 0.180 (NS)	0.735 (NS)
Mean Ferritin (ng/ml) (Mean±SD)				
Baseline	113.80±120.70	160.96±158.70	178.45±194.11	0.357 (NS)
10	92.13±96.88	116.55±76.15	138.04±150.17	-
Mean change (baseline – day 10) *P*-value	–21.67±60.57 0.303 (NS)	–44.41±104.39 0.101 (NS)	–40.41±141.50 0.093 (NS)	0.634 (NS)
Mean LDH (U/L) (Mean±SD)				
Baseline	345.23±115.90	297.98±91.95	344.54±121.10	0.313 (NS)
10	380.66±179.28	381.97±144.70	324.30±133.77	-
Mean change (baseline – day 10) *P*-value	35.43±180.26 0.435 (NS)	83.98±182.74 0.052(NS)	-20.24±207.51 0.548 (NS)	0.165 (NS)

By Wilcoxon SIGN rank test. By ANOVA – Kruskal–Wallis. NS: Not significant

The data depict that the serum creatinine levels were comparable between all the groups at baseline and day 10.

Changes proportion of subjects with ICU admission

There were no subjects' required ICU admission at screening and throughout the study period.

Changes hospital stay is mentioned in Table 7:

The requirement of hospital stay was similar and comparable between Favi DPI and oral counterpart.

Changes in biochemical parameters are mentioned in Table 8:

In this data, mean laboratory levels were comparable between three groups at baseline and after treatment for Favi DPI and oral Favipiravir as a control.

Discussion

Favipiravir (T-705; 6-fluoro-3-hydroxy-2-pyrazinecarboxamide) was discovered as an effective antiviral agent through screening chemical library against the influenza virus by Toyama Chemical Co., Ltd. In 2014. Due to its potent anti-viral activities against other RNA viruses such as arenaviruses, bunyaviruses, and filoviruses,[12] it was considered a potentially promising drug for specifically untreatable RNA viral infections especially for COVID-19 which has been declared a pandemic by the WHO.

Despite efforts to contain the virus through measures such as wearing masks, use of hand sanitizers regularly, maintaining social distancing and minimizing travel, and the transmission of this virus are ongoing causing significant spread of the virus all over the globe. The major symptoms of the COVID-19 infection are localized in the respiratory system of human (i.e., characterized by loss of pulmonary function in humans);[11,13] hence, interventions in the lungs would be beneficial for the patient and also for minimizing disease transmission.

Studies identify the viral RNA-dependent RNA polymerase (RdRp) as a potential drug target in COVID-19 treatment due to its crucial role in SARS-CoV-2 replication and transcription. Since Favipiravir is reported to be an effective inhibitor of RdRp and it is reported to cause mutations in the viral genome causing non-lethal phenotype.[14,15] A recent report by Rabie (2021)[16] speaks about modification of Favipiravir molecule to enhance its bioavailability and its potency toward RdRp inactivation.

The present study evaluated the potential clinical benefit of favipiravir DPI in patients with mild symptomatic COVID-19 compared to its oral administration. Favipiravir has established use for novel influenza viruses, activity against SARS-CoV-2, and promising candidate in treating COVID-19 as reported from other countries, including China, Russia, Turkey, and Japan.[11,17]

Table 4: Changes in mean electrolyte levels between groups

Duration	Favi DPI 10	Favi DPI 20	Control	*P*-value
Mean calcium (mg/dl) (Mean±SD)				
Baseline	9.30±0.56	8.88±1.38	9.13±0.70	0.409 (NS)
10	9.13±0.69	9.33±0.75	9.49±0.56	
Mean change (baseline – day 10) (*P*-value)	–0.18±0.65 (0.249) (NS)	0.45±1.61 (0.268) (NS)	0.35±0.62 (0.051) (NS)	0.181 (NS)
Mean Sodium (mmol/l) (Mean±SD)				
Baseline	142.25±2.81	141.84±7.54	139.17±4.82	0.157 (NS)
10	140.57±2.98	141.17±3.35	140.26±3.81	
Mean change (baseline – day 10) *P*-value	–1.68±3.94 0.072 (NS)	–0.67±6.95 0.671 (NS)	1.10±6.03 0.427 (NS)	0.314 (NS)
Mean potassium (mmol/l) (Mean±SD)				
Baseline	4.35±1.02	4.44±0.99	4.30±0.60	0.887 (NS)
10	5.09±1.45	4.80±0.97	4.57±0.85	
Mean change (baseline – day 10) *P*-value	0.74±1.24* 0.016	0.36±1.58 0.326 (NS)	0.26±0.90 0.207 (NS)	0.467 (NS)
Mean chloride (mmol/l)				
Baseline	103.80±4.49	103.14±4.62	101.83±5.65	0.445 (NS)
10	101.59±5.56	100.98±4.44	101.68±3.95	
Mean change (baseline – day 10) *P*-value	–2.21±7.80 0.220(NS)	–2.16±6.58 0.159 (NS)	–0.145±6.99 0.927 (NS)	0.585 (NS)

By student *t*-test. By ANOVA - Kruskal–Wallis. NS: Not significant

Table 5: Changes in liver enzyme levels between groups

Duration	Favi DPI 10	Favi DPI 20	Control	*P*-value
Mean SGPT (U/l) (Mean±SD)				
Baseline	25.37±11.05	38.53±32.44	60.46±92.15	0.01*
10	29.82±15.60	35.51±26.05	40.60±26.24	-
Mean change (baseline – day 10) *P*-value	4.45±10.84 0.136 (NS)	–3.02±22.65 0.764 (NS)	–19.86±100.14 0.81 (NS)	0.722 (NS)
Mean SGOT (U/l) (Mean±SD)				
Baseline	31.60±10.80	36.96±17.72	48.30±53.62	0.436 (NS)
10	31.38±14.85	33.67±16.33	29.86±8.33	-
Mean change (baseline – day 10) *P*-value	–0.22±13.23 0.603 (NS)	–3.29±20.92 0.515 (NS)	–18.43±54.97 0.04*	0.247 (NS)

By Wilcoxon sign rank test. By ANOVA – Kruskal–Wallis. *Significant. NS: Not significant

Table 6: Changes in creatinine levels between groups

Duration	Favi DPI 10	Favi DPI 20	Control	*P*-value
Mean creatinine (mg/dl) (Mean±SD)				
Baseline	0.71±0.17	0.69±0.13	0.73±0.15	0.692 (NS)
10	0.65±0.11	0.70±0.15	0.66±0.12	-
Mean change (baseline – day 10) *P*-value	–0.06±0.13 0.061 (NS)	0.01±0.15 0.780 (NS)	-0.07±0.16 0.071 (NS)	0.199 (NS)

By student *t*-test. By ANOVA. NS: Not significant

In the present study, a pilot clinical study involving 60 subjects was performed. Subjects were equally divided in Favipiravir DPI 10 and 20 mg/day and compared to control group, that is, oral administration of Favipiravir as per standard protocol for 10 days. The standard treatment involving antihistaminic, antipyretic, antibiotic, and multivitamin composition was provided to subjects from three interventional groups.

It is associated with certain challenges in its current dosage form particularly its dose. The recommended dose of Favipiravir for adults is

1800 mg orally twice daily on day 1, followed by 800 mg orally twice daily for 14 days. The adherence to the therapy by patient's low due to large dose and multiple tablets to be consumed. This creates need of administering several tablets of 200 mg multiple times to reach the intended dose.[18] The failure to administer required dose due to inconvenient dosing availability can lead to progression of disease. In the present study, we have used a novel route of Favipiravir as a DPI to present therapeutic advantage over reduced dosage and dosing regimen along with expiring safety and efficacy of the same in comparison with oral Favipiravir.

Table 7: Changes hospital stay between groups

Groups	Mean hospital stay (days) (mean±SD)
Favi DPI 10	2.32±2.63
Favi DPI 20	2.05±2.86
Control	1.58±2.01

By ANOVA. P=0.672, not significant

It was observed from the data obtained from the study that there was comparable efficacy of Favi DPI 20 group as that of oral Favipiravir as a control. The Favi DPI in 20 mg/day dose shows around 90% subjects getting negative RT-PCR Vs. About 80% in oral control group on day 10. There was significant reduction in symptoms such as cough, breathlessness, and fatigue form baseline to day 10 in Favi DPI 20 group; which also was comparable to oral control group.

There was consistent improvement in blood SPO_2 levels from baseline to day 10 in all three interventional groups.

There were raised inflammatory parameters witnessed at baseline in all three interventional groups. There was reduction in elevated levels of inflammatory markers in all three groups indicative of clinical cure and good prognosis of disease. It affirms that Favipiravir in DPI format provides same effectiveness in improving prognosis of disease by reducing the inflammatory markers and cytokine storm as that of oral Favipiravir. At the end of day 10, mean HS-CRP showed an insignificant

Table 8: Changes biochemical parameters between groups

Laboratory investigation	(Mean±SD)					
	Favi DPI 10 (n=20)		Favi DPI 20 (n=20)		Control (Oral Favi tablets) (n=20)	
	Baseline	Day 10	Baseline	Day 10	Baseline	Day 10
Total cholesterol	148.84±45.96	169.32±44.32	158.00±39.96	185.84±35.15	162.58±37.69	183.68±34.97
HDL cholesterol	36.58±9.25	40.74±9.83	36.58±9.39	40.26±9.91	33.74±9.68	37.95±7.83
LDL cholesterol	87.84±38.89	94.79±37.08	94.53±31.98	111.37±38.53	99.84±32.48	115.16±35.97
Triglycerides	161.63±89.74	225.26±128.74	161.53±116.35	248.11±174.07	170.11±78.86	211.58±115.53
VLDL cholesterol	32.33±17.95	45.04±25.75	32.30±23.27	49.61±34.80	34.02±15.77	50.05±35.47
Alkaline phosphate	88.91±42.70	86.65±42.15	77.60±23.23	88.79±36.21	78.12±15.72	88.34±22.70
Bilirubin total	0.54±0.16	0.72±0.55	0.64±0.32	0.73±0.60	0.80±0.89	0.71±0.46
Bilirubin direct	0.17±0.06	0.21±0.14	0.18±0.07	0.20±0.17	0.25±0.27	0.21±0.12
Bilirubin indirect	0.37±0.11	0.92±1.95	0.41±0.13	0.52±0.44	0.55±0.62	0.49±0.34
GGT	25.84±10.57	27.78±12.99	35.46±22.48	31.05±17.30	33.33±16.36	35.19±21.21
Total protein	7.23±0.58	7.17±0.41	7.05±0.66	7.33±0.44	7.00±0.58	7.15±0.36
Albumin g/dl	4.51±0.45	4.41±0.31	4.36±0.44	4.44±0.34	4.40±0.40	4.42±0.32
Serum ALB/globulin ratio	1.76±0.34	1.65±0.26	1.66±0.30	1.56±0.22	1.72±0.15	1.67±0.21
Blood urea nitrogen	11.88±3.30	13.46±4.04	12.21±3.60	13.70±4.26	11.21±2.49	11.98±2.36
Uric acid	4.93±1.46	4.98±1.44	4.26±2.21	5.30±1.82	4.64±2.04	5.05±1.49
Total leucocytes count	5.88±1.44	8.38±3.08	7.03±2.88	13.52±20.26	7.36±2.23	8.34±3.25
Neutrophils	69.62±11.05	64.47±9.89	66.59±15.92	65.67±9.09	73.88±12.17	65.72±12.02
Lymphocyte percentage	24.49±9.01	29.56±8.71	27.93±14.67	28.97±8.24	21.49±11.51	29.20±11.06
Monocytes	2.96±1.09	3.29±0.80	3.15±1.91	2.92±1.18	3.07±1.52	2.85±0.85
Eosinophils	2.45±2.74	2.16±2.39	1.88±1.71	1.84±1.41	1.06±0.46	1.70±2.33
Basophils	0.20±0.08	0.23±0.12	0.20±0.08	0.30±0.25	0.18±0.05	0.21±0.07
TotaL Rbc	4.84±0.88	4.77±0.78	4.57±0.80	4.91±0.71	4.72±1.08	4.98±0.59
Hemoglobin	13.31±1.79	13.13±1.99	13.07±2.10	14.08±1.89	13.97±2.07	13.99±1.76
Hematocrit (PCV)	42.74±4.67	41.15±8.36	41.91±4.98	44.96±3.03	43.22±4.41	44.99±4.29
Platelet distribution width	12.19±2.12	12.21±2.16	12.01±2.62	12.02±1.86	12.21±1.69	11.59±1.67
Mean platelet volume	10.43±0.88	10.69±1.20	10.35±1.15	10.39±0.90	10.42±0.72	10.17±0.76
Platelet count×10^3/μL	214.61±50.55	321.00±84.21	244.88±88.50	310.18±97.76	240.65±68.99	332.71±97.57

By Student t-test. Not significant

fall of 77.8% in control, 70.0% in Favi DPI 20, and 71.8% in Favi DPI 10 from baseline. At the end of day 10, mean Ferritin showed an insignificant fall of 19.0% in Control, 27.6% in Favi DPI 20, and 22.6% in Favi DPI 10 from baseline. There was reduction in interleukin six levels in Favi DPI groups but not significant statistically.

Serum electrolyte levels such as calcium, sodium, potassium, and chlorine were not altered significantly after treatment with oral as well as Favi DPI. Liver enzymes such as SGPT and SGOT in Favi DPI 10 and 20 mg groups were lower than the baseline levels and the values were comparable to what was seen in Favipiravir oral groups.

At baseline, all subjects were not requiring the ICU admission, eventually, in oral Favipiravir treated group single subject, that is, 5% required ICU admission. There was no mortality. In Favi DPI 10 and 20, there was no single requirement of ICU admission.

According to above table, mean hospital stay was 2.32 days among control, 2.05 days among Favi DPI 20, and 1.58 days among Favi DPI 10. If compared change was comparable in all the groups and difference was not statistically significant.

Above table states that, 40.0% of the total cases among control required supplemental oxygen which was more as compared to 10.0% among Favi DPI 20 and 15.0% among Favi DPI 10 and difference was not statistically significant.

In all intervention groups, 100% subjects got discharged from hospital in 10 days. At baseline 50.0–55.0% of the cases did not require hospitalization in all the groups which was comparable, and the difference was not significant. At the end of 5th day, 90.0% of the cases in Favi DPI 20 and 95.0% in Favi DPI 10 were not hospitalized which was comparable to 90% of cases in control and the difference was not significant. The biochemical parameters such as renal, liver function test, and lipid profile along with hematological parameters were assessed on baseline and day 10. There were no significant changes post treatments indicating safety of oral as well as Favipiravir DPI.

From the data obtained from this study, it can be interpreted that the Favipiravir DPI in dose 20 mg once a day was not clinically inferior to oral Favipiravir in standard dosing regimen. The comparable safety and efficacy related parameters suggest equivalent effectiveness of Favipiravir DPI compared to oral dosage form. This study provided first hand evidence of safety of Favipiravir DPI in two dosage forms, that is, 10 and 20 mg/day in COVID-19 patients for 10 days. All subjects led to clinical cure and there were no adverse events in the study from all interventional groups. Favipiravir DPI possesses potential to elicit clinical cure in very less dose as compared to oral regime of Favipiravir. This fact may provide link to assess superiority of Favi DPI compared to oral, in terms of reduced dosage, toxicity, improved patient compliance, and thus good prognosis of the disease. There was no progression and need to stop medication or terminate subject prematurely in Favi DPI groups, indicating therapeutic benefits.

Comparison between Favi DPI 10 and Favi DPI 20

When compared between Favipiravir DPI in 10 and 20 doses, it is observed that there were 10% more subjects became RT-PCR negative on day 5 as well as day 10 with Favi 20 in comparison to Favi 10 [Table 1].

We have assessed the reduction in scores of symptoms to judge the relief from symptoms in both groups. It was observed from the data that there was more reduction in scores of cough, fatigue, myalgia, headache, and persistent pain in chest from baseline in Favi DPI 20 than Favi DPI 10 group. There were more subjects getting relieved of cough and breathlessness in Favi DPI 20 than Favi DPI 10.

Overall, Favi DPI appears as a promising alternative to the oral dosage form of Favipiravir with uncompromised safety and efficacy in COVID-19. The Favi DPI 20 is marginally better than Favi DPI 10 in relieving COVID associated symptoms as well as viral clearance. The limitations of the study are sample size and to further validate the safety and efficacy of Favi DPI a RCT (randomized controlled trial) with large cohort is warranted.

Advantage of DPI as a novel dosage form of Favipiravir in terms of its efficacy

The antiviral drug Favipiravir, is a structural analogue of guanosine, undergoes chemical transformation in infected cells by cellular enzymes into a nucleotide form — Favipiravir ribose triphosphate (FVP-RTP). FVP-RTP is able to bind to viral RNA-dependent RdRp and integrate into the viral RNA chain, causing a significant mutagenic effect in the viral RNA genome. Besides the virus inhibiting effect, the increased synthesis of mutant virions under the action of Favipiravir possess a threat of the emergence of novel threatening viral strains with high pathogenicity for humans and animals and acquired resistance to chemotherapeutic compound.[19]

K229R mutation in motif F of the PB1 subunit of the influenza virus RNA-dependent RdRP confers resistance to Favipiravir *in vitro* and in cell culture. K229R also conferred Favipiravir resistance to RdRp of other influenza-A virus strains, and its location within a highly conserved structural feature of the RdRP suggests that other RNA viruses might also acquire resistance through mutations in motif F.[20]

Reducing the viral titer at efficacious and lowest possible dosage could be crucial to lower chances of resistant mutations as a side effect of Favipiravir. Favipiravir in a DPI dosage form can ascertain very low dose 20 mg (DPI) compared to 1600 mg (oral) per day which is almost 99% less dose with DPI and can thought to be producing less drug resistant mutations being as effective in inhibiting virus (SARS-CoV-2, indicated by equal efficacy in population getting negative RT-PCR results in the present study).

Favipiravir DPI biological availability

Driouich *et al.* (2020)[21] report that Favipiravir is not detected in the lungs with a low dose of 18.75 mg/day dose to animals through i.p.

route. With the preclinical studies of Favipiravir DPI, we have detected a~100 ng/g of favipiravir in the lungs at 1.92 mg/kg/day DPI dose. In Hamster model we have observed the effectiveness of Favipiravir DPI in reducing the viral load. This demonstrated that the inhalation drug delivery of Favipiravir is efficacious to produce the antiviral effect (The data of the antiviral activity in Hamster model is not depicted here).

Favipiravir was confirmed with a mean lung to plasma ratio ranging from 0.35 to 0.44, when given intraperitoneally[21] in female hamsters with elimination half-life of around 2–5.5 h.[22] In research carried out by SAVA Healthcare Ltd., Favipiravir DPI shows a mean lung to plasma ratio of 0.49 in female rats which is in line with the literature reports indicative of DPI Favipiravir formulation possessing the capacity to reach the deep lungs is similar to what is achieved when given intraperitoneally. It can be thought as an advantage of DPI over the oral dosage form of Favipiravir.

Plasma concentration of Favipiravir in patients in the United States is found to be 50% of that in Japanese patients, suggesting a possible ethnic or regional difference in its pharmacokinetics (PK).[23] Since inhalation is known to bypass the PK variability attributed to irregular gastrointestinal absorption and first-pass hepatic metabolism of drugs,[24] it is tempting to speculate that such ethnic variability in stability of Favipiravir in plasma of COVID-19 patients will be minimum with Favipiravir DPI.

Conclusion

There were 90% subjects getting RT-PCR negative in 10 days in Favi DPI 20 treated group suggesting comparable antiviral activity and viral clearance as that of the oral Favipiravir. There was reduction in symptoms such as cough, breathlessness, and fatigue in Favi DPI 20 treated group comparable to oral Favipiravir as control. From the data obtained from this study, it can be interpreted that the Favipiravir DPI in dose 20 mg once a day was not clinically inferior to oral Favipiravir in standard dosing regimen. There was reduction in elevated inflammatory markers such as CRP, Ferritin, and interleukin in Favi DPI 10 and 20 treated group from baseline to day 90 which was comparable to oral Favipiravir. This study provided first hand evidence of safety of Favipiravir DPI in two dosage forms, that is, 10 and 20 mg/day in COVID-19 patients for 10 days. The assessment of effectiveness of Favi DPI 10Vs. Favi DPI 20, it is indicated through data that Favi DPI 20 was slightly better than Favi DPI 10 in viral load reduction and relieving cough, fatigue, myalgia, and from baseline to day 10. All subjects from Favi DPI groups led to clinical cure and there was no progression of disease. The reduced dose can curtail side effects of Favipiravir which can be achieved through DPI Favipiravir formulation as it requires many fold less dose than the oral counterpart. This study may provide link to assess superiority of Favi DPI compared to oral, in terms of reduced dosage, toxicity, improved patient compliance, and good prognosis of the disease.

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