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Original article

The efficacy of Zafirlukast as a SARS-CoV-2 helicase inhibitor in adult patients with moderate COVID-19 Pneumonia (pilot randomized clinical trial)

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Abstract

Objective: To assess the efficacy of Zafirlukast as a SARS-CoV-2 Helicase Inhibitor in adult patients with moderate COVID-19 symptoms (hospitalized patients with COVID-19 pneumonia who were not admitted to an intensive care unit).

Methods: We conducted a randomized, double blind, placebo-controlled, pilot trial with adult patients with moderate COVID-19 pneumonia. The sample was randomized to Zafirlukast 10 mg BD for 10 days plus standard care vs placebo plus standard care. The primary outcome was the complete resolution of all symptoms. The secondary outcomes were the duration of oxygen therapy, and length of hospital stay (LOS).

Results: In total, 40 patients were randomized (20 to Zafirlukast and 20 to the control). The time to the resolution of clinical symptoms in both groups was not significantly different. Regarding the fever, 0.3 days [95% CI, −1.45, 1.95], p = 0.76, for shortness of breath, the difference was 0.4 days [95% CI, −2.67, 3.46], p = 0.68, for cough the difference was 0.2 days [95% CI, −1.45, 1.95], p = 0.98, for sputum the difference was 0.5 days [95% CI, −0.75, 1.85], p = 0.57, for vomiting the difference was 0.1 days [95% CI, −0.50, 0.30], p = 0.93, for fatigue the difference was 0.3 days [95% CI, −3.2, 3.62], p = 0.64. The LOS per day for the two groups was not significantly different, 0.9 days [95% CI, −2.03, 4.28], p = 0.64. The LOS per day for the two groups was not significantly different, 1.1 days [95% CI, −2.03, 4.28], p = 0.94, nor was the duration of oxygen therapy per day, 13 days [95% CI, −1.79, 4.49], p = 0.49. Regarding the 7 category ordinal scale, there was no significant difference between the two groups at day 7 (p-value = 0.02), day 14 (p-value = 0.60) and day 28 (p-value = 0.48).

Conclusion: Among adult patients hospitalized with COVID-19 pneumonia, the treatment with Zafirlukast, compared to placebo, did not significantly improve symptoms resolution.

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Introduction

SARS-CoV-2, a highly contagious human coronavirus, was first reported in Wuhan, China in late 2019. Since then, the virus spread globally and became a pandemic affecting all aspects of life. The disease is usually self-limiting with mild respiratory symptoms, but can progress to moderate or severe symptoms including, but not limited to fever, cough, sputum, shortness of breath (SOB), fatigue, desaturation and evidence of pneumonia by chest x-ray which can progress to respiratory failure, acute respiratory distress syndrome and death [1,2].

Despite extensive clinical studies conducted over the last two years, there is still no drug of choice as first line therapy with enough supporting evidence to treat SARS-COV-2. Many clinical trials have been conducted with multiple medications, but with a variable degree of success and mixed results. There is a continued need for clinical trials and effort to discover new antiviral drugs to treat COVID-19. Drug repurposing is an efficient strategy to discover effective medication against this virus. Antiviral medication which
Patients were randomly assigned by a computer-generated randomization procedure to receive Zafirlukast plus standard care or placebo plus standard care. The Zafirlukast dose was 20 mg twice daily for 10 days, dispensed by the pharmacy in each study site.

The current standard care for COVID-19 was based on the protocol for the management of COVID-19 pneumonia, which included the use of an antibiotic, dexamethasone, supplemental oxygen and an anticoagulant (enoxaparin). Data were collected daily, which included the vital signs, daily symptoms, comorbidities, medication, and complications until day 10, then on days 12, 14, 21 and 28 days in an electronic case-report form. The discharged patients were contacted and interviewed telephonically on day 10 then on day 12, 14, 21 and 28 days by an interviewer unaware of the assigned trial group, to assess the clinical symptom resolution and return to routine activities. On day 14, the patients were asked to provide a new nasopharyngeal sample for PCR.

The primary outcome measures were complete resolution of all symptoms, and the time to symptom improvement, defined as the time the symptom resolved (fever, cough, sputum, shortness of breath (SOB), fatigue, and vomiting). The secondary outcome measures were the duration of oxygen therapy, LOS, transfer to an ICU and PCR test conversion from positive to negative at day 14.

The clinical status at 7, 14 and 28 days was evaluated with the use of a seven-level ordinal scale. The scores on the scale were defined as follows: a score of 1 indicated not hospitalized with no limitations on activities; 2 - not hospitalized but with limitations on activities; 3 - hospitalized and not receiving supplemental oxygen; 4 - hospitalized and receiving supplemental oxygen; 5 - hospitalized and receiving oxygen supplementation administered by a high-flow nasal cannula or noninvasive ventilation; 6 - hospitalized and receiving mechanical ventilation; and 7, death [8]. The trial was conducted in accordance with the International Conference on Harmonization Guidelines for Good Clinical Practice and the Declaration of Helsinki. The trial was approved by Institutional Review Board of King Abdullah International Medical Research Center and the Saudi Food and Drug Authority.(RC20/206/R) June 2020.

Statistical analysis

The continuous variables are expressed as mean ± standard deviation (SD), and the categorical variables as count and percentage (%). We applied an unpaired or paired t-test to the variables with continuous scales. For the categorical responses, we used a Pearson chi-square or Fisher exact test. We used the generalized estimating equation to assess the drug effect over repeated times for the WHO severity scales by regrouping the scales into a binary scale ‘0’ and ‘1’ (patients those who did not require oxygen as ‘0’, and required oxygen as ‘1’), and the risk rate (RR) with a 95 % confidence interval. A p-value less than 0.05 was considered as statistically significant. All statistical analyses were done using SAS software version 9.4 from SAS Institute, Cary, NC, USA.

Results

All the patients who participated in the study were admitted to the hospital with a diagnosis of moderate COVID-19 pneumonia, based on the inclusion and exclusion criteria. The sample size was 40 patients. All the 40 patients completed the 28 days of follow-up. Exclusions after the enrollment were due to non-adherence to study drug or withdrawal of consent or loss of contact.

Those who were excluded were not included in the final data analysis because they did not completed the study so we do not know their final outcomes. Five patients were withdrawn because they have been transferred to ICU in less than 48 h of admission to the ward. They were too sick to the extent of requirement of ICU
transfer, therefore, they are not included in the study as per our inclusion and exclusion criteria.

In terms of the baseline characteristics, there were no statistically significant difference between the two groups, including age, gender, nationality, comorbidities, and symptoms at presentation, standard of care and the 7 category ordinal scale at baseline (Table 1). The mean age was 51 (± 12.5) years with an equal gender distribution. The majority (60 %, n = 24) were Saudi nationality, and 37 (92 %) patients received supplemental oxygen at the baseline. The standard of care was similar between the two groups; no patients received any specific antiviral drug as part of the standard of care.

The LOS per day in the two groups was not significantly different, the mean was 8.3 days for Zafirlukast vs 7.2 days for the placebo [95 % CI, − 2.03, 4.28], p = 0.94. The duration of supplemental oxygen per days for the two groups was not significantly different, the mean was 6.6 days for Zafirlukast vs 5.3 days for the placebo; [95 % CI, − 1.79, 4.49], p = 0.49.

The time to the resolution of clinical symptoms in the two groups was not significantly different: for the fever, the mean was 2.2 days for Zafirlukast vs 2.5 days for placebo [95 % CI, − 1.19, 0.69]; p = 0.76, for SOB, the mean was 6.5 days for Zafirlukast vs 6.1 days for placebo [95 % CI, − 2.67, 3.46], p = 0.68, for cough, the mean was 4.9 days for Zafirlukast vs 4.7 days for placebo [95 % CI, − 1.45, 1.195], p = 0.98, for the sputum, the mean was 1.3 days for Zafirlukast vs 0.8 days for placebo [95 % CI, − 0.50, 0.30], p = 0.93, for fatigue, the mean was 5.5 days for Zafirlukast vs 5.8 days for placebo [95 % CI, − 4.32, 3.62], p = 0.64 (Table 2).

For the 7 category ordinal scale, there was no significant difference between the two groups. At baseline (day 1), the scale was 4.2 for Zafirlukast vs 3.9 for the placebo (p-value = 1), at day 7, the scale was 2.1 for Zafirlukast vs 2.4 for the Placebo (p-value = 0.62), at day 14, the scale was 1.4 for Zafirlukast vs 1.2 for the placebo (p-value = 0.80) and at day 28, the scale was 1.2 for Zafirlukast vs 1.1 for the placebo (p-value = 0.48) (Table 3). Three patients required transfer to an ICU, 2 in the Zafirlukast group and 1 in the placebo group, with no significant difference (p-value = 0.50) (Table 2).

In total, 31 (77 %) patients (15 on Zafirlukast and 16 on the placebo arm) did the COVID-19 swap on day 14, 11 patients had a negative conversion at day 14 (4 negative in Zafirlukast and 7 negative in placebo arm) with no significant difference between the two arms (p-value = 0.70). The remaining 20 (27 %) patients remained positive (Table 3).

Regarding the safety profile, there was no worsening of liver enzymes and hepatotoxicity between the two groups. During the follow-up period to 28 days, no deaths were recorded.

Discussion

The current study was a pilot randomized clinical trial aiming to evaluate the potential clinical efficacy of Zafirlukast in patients hospitalized with moderate COVID-19 pneumonia. The Zafirlukast treatment added to the standard care was not associated with clinical improvement in patients with moderate COVID-19 pneumonia. Symptoms recovery (fever, cough, sputum, SOB, fatigue) did not differ between Zafirlukast and placebo after 10 days of treatment. There was no significant difference between Zafirlukast and placebo in term of requiring oxygen, and LOS. The Zafirlukast did not reduce the conversion of the viral PCR from positive to negative at day 14, compared with the standard care alone. Our patients were similar with regard to baseline characteristics, severity of illness at enrollment and type of standard care they received.

Zafirlukast is an oral leukotriene receptor antagonist used for maintenance treatment of asthma in combination with an inhaled steroid and long-acting beta-2 agonist. However, its ability to inhibit the COVID-19 helicase is a new discovery [3]. To our knowledge, this is the first and only clinical trial evaluating the efficacy of Zafirlukast as a potential treatment for COVID-19. There is a few reports about

### Table 1
Baseline characteristics of the sample.

| Variables                        | All N = 40 | Zafirlukast N = 20 | Placebo N = 20 | p-value |
|----------------------------------|-----------|-------------------|---------------|---------|
| Age (y), mean ( ± SD)            | 51 (12.5) | 52 (10.3)         | 51 (14.8)     |         |
| Sex                              |           |                   |               |         |
| Male, N (%)                      | 20 (50 %) | 13 (65 %)         | 7 (35 %)      | 0.1128  |
| Female, N (%)                    | 20 (50 %) | 7 (35 %)          | 13 (65 %)     |         |
| Contact with patients with COVID-19 | 22 (55 %) | 11 (55 %)        | 11 (55 %)     |         |
| Saudi nationality N (%)          | 24 (60 %) | 12 (60 %)         | 12 (60 %)     |         |
| Comorbidities                    |           |                   |               |         |
| Hypertension, N (%)              | 12 (30 %) | 5 (25 %)          | 7 (35 %)      | 0.490   |
| Diabetes, N (%)                  | 20 (50 %) | 10 (50 %)         | 10 (50 %)     | 1.0000  |
| Obesity N (%)                    | 17 (42 %) | 7 (35 %)          | 10 (50 %)     | 0.5231  |
| Cardiac N (%)                    | 3 (7.5 %) | 2 (66 %)          | 1 (33 %)      | 1.0000  |
| Renal N (%)                      | 1 (2.5 %) | 1 (5 %)           | 0 (95 %)      | 1.0000  |
| Smoking N (%)                    | 2 (5 %)   | 1 (5 %)           | 1 (5 %)       | 1.0000  |
| Other comorbidities N (%)        | 0 (0 %)   | 0 (0 %)           | 0 (0 %)       |         |
| Symptoms:                        |           |                   |               |         |
| Fever                            | 40 (100 %)| 20 (100 %)        | 20 (100 %)    |         |
| Cough                            | 39 (97 %) | 20 (100 %)        | 19 (95 %)     | 1.0000  |
| Short of breath                  | 40 (100 %)| 20 (100 %)        | 20 (100 %)    |         |
| Sputum                           | 12 (30 %) | 9 (45 %)          | 3 (15 %)      | 0.0824  |
| Fatigue                          | 34 (85 %) | 17 (85 %)         | 17 (85 %)     | 1.0000  |
| Vomiting                         | 12 (30 %) | 6 (30 %)          | 6 (30 %)      | 1.0000  |
| Oxygen requirement               | 37 (92 %) | 19 (95 %)         | 18 (90 %)     | 1.0000  |
| Abnormal CXR                     | 40 (100 %)| 20 (100 %)        | 20 (100 %)    |         |
| Medications:                     |           |                   |               |         |
| Antiviral agents                 | 0 (0 %)   | 0 (0 %)           | 0 (0 %)       |         |
| Antibiotics                      | 40 (100 %)| 20 (100 %)        | 20 (100 %)    |         |
| Dexamethasone                    | 40 (100 %)| 20 (100 %)        | 20 (100 %)    |         |
| Enoxaparin                       | 40 (100 %)| 20 (100 %)        | 20 (100 %)    |         |
| The 7 category ordinal scale N(%)|          |                   |               |         |
| At baseline = 4 (day 1):         | 36 (90 %) | 18 (90 %)         | 18 (90 %)     | 1.0000  |

### Table 2
Efficacy outcomes.

| Variable (per day) | Zafirlukast | Placebo | Absolute difference | 95 % CI | p-value |
|--------------------|-------------|---------|---------------------|--------|---------|
| Length of stay     | 8.3         | 7.2     | 1.1                 | (−2.03, 4.28) | 0.94    |
| Oxygen-duration    | 6.6         | 5.3     | 1.3                 | (−1.79, 4.49) | 0.49    |
| Cough-Duration     | 4.9         | 4.7     | 0.2                 | (−1.45, 1.05) | 0.98    |
| SOB-duration       | 6.5         | 6.1     | 0.4                 | (−2.67, 3.46) | 0.68    |
| Fever-duration     | 2.2         | 2.5     | 0.3                 | (−1.19, 0.69) | 0.76    |
| Sputum-duration    | 1.35        | 0.80    | 0.55                | (−0.75, 1.85) | 0.09    |
| Vomiting           | 0.30        | 0.4     | 0.1                 | (−0.50, 0.30) | 0.93    |
| Fatigue-duratation | 5.5         | 5.8     | 0.3                 | (−4.32, 3.62) | 0.64    |
helicase inhibitors in the SARS coronavirus helicase but not with Zafirlukast [9,10].

With the current COVID-19 epidemic, and the urgency for a specific therapy for the disease and the large number of clinical trials globally, negative or mixed results occurs frequently. For example, no clinical benefit was observed in randomized, controlled trials evaluating hydroxychloroquine for the treatment of COVID-19, and the addition of azithromycin did not improve outcomes [11]. Similar studies showed no beneficial effect of hydroxychloroquine for adult hospitalized with COVID-19 [12,13]. Only a few high quality clinical trials with solid evidence indicated a significant positive efficacy and ultimately changed the practice and the guidelines for the management of COVID-19.

Zafirlukast was able to inhibit the enzyme activity in vitro at an IC50 of 16.3 µM [3] but it is not known if this value is an adequate threshold and whether the Zafirlukast level in human plasma (in vivo) was sufficient for the inhibition of SARS-CoV-2. In our study, we used the dose of Zafirlukast 10 mg PO BD which is the usual dose for the maintenance treatment of asthma. It is not known if the asthma dose can be used for COVID-19 pneumonia or if higher doses are required. We did not increase the dose because of potential hepatotoxicity. The ideal dose required to achieve viral suppression needs to be tested in vitro through pharmacological studies.

It is not surprising the Zafirlukast did not reduce the conversion of viral PCR from positive to negative at day 14. A report showed that the median duration of viral shedding in COVID-19 was 20 days in patients with moderate and severe disease and could be as long as 37 days [14]. Another study indicated that the viral PCR conversion from positive to negative lagged behind the symptom resolution in COVID-19 [15].

Another explanation of the lack of efficacy of Zafirlukast on symptom recovery is the time of the drug delivery in relation to the duration of symptoms. All our patients had a symptom duration ranging from one to ten days prior to admission. The earlier the initiation of treatment, the better efficacy of the drug, since the peak SARS-CoV-2 viral load usually occurs before symptom onset [17]. When the patient develops COVID-19 pneumonia, the systemic inflammatory cascades usually dominate, rather than the viral pathogenicity [17].

Non-adherence and lack of self-report of symptoms is possible in our patients. However, telephonic interviews were conducted by an interviewer to assess the clinical symptoms and return to normal activities or development of new symptoms. The adherence to the medication was emphasized.

Our study has the advantages to be the first clinical study in literature evaluating the efficacy of Zafirlukast with hospitalized COVID-19 pneumonia patient. The study had a blinded, placebo-controlled design with a rigorous study protocol and monitoring. For the two groups, all the baseline characteristics at baseline were similar.

Our trial has several limitations. It is pilot study with a small sample size. During the study period, the rate of admission for COVID-19 pneumonia in our hospital was low, of the admitted patients, a limited number were eligible for the study protocol and willing to participate. A larger sample size may have been required to have sufficient power to detect statistically significant differences between the treatment groups. Mild and outpatient cases of COVID-19 were not included. Mild cases usually self-limited and most of cases recovered with symptomatic treatment only and they do not usually require specific anti - SARS-CoV-2 therapy as per guidelines at the time of conducting the trial. The follow-up was limited to 28 days after randomization because our objectives were the evaluation of recovery from acute illness, rather than long-term complications. Absence of long-term follow-up remains limitation of this study. Our assessment depended on symptom recovery (fever, cough, sputum, SOB, fatigue) and are subject to potential recall bias.

Combination therapy has been tested in SARS [18,19] and in MERS-CoV [20]. A combination might enhance the antiviral efficacy and improve the clinical outcomes. Hung et al. reported the importance of combining antiviral therapies in patients with severe COVID-19, aiming to reduce the viral load and mitigate symptoms [21].

In conclusion, for adult patients hospitalized with moderate COVID-19 pneumonia, treatment with Zafirlukast, compared to placebo, did not significantly improve symptom resolution. However, the study was a pilot study with a small sample size.

Ethical approval

The trial was approved by Institutional Review Board of King Abdullah International Medical Research Center and the Saudi Food and Drug Authority.

Competing interests

None declared

References

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