Acta Pharmacologica Sinica

CORRESPONDENCE

Ceftazidime exhibits a broad inhibition to the infection of SARS-CoV-2 prototype and Omicron variant in vitro by blocking spike protein-ACE2 interaction

Acta Pharmacologica Sinica (2023) 44:1932–1934; https://doi.org/10.1038/s41401-023-01071-0

Dear Editor,

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has coexisted with human beings over 3 years. It has undergone considerable evolution and several variants of concern (VOCs), including Alpha, Beta, Gamma, Delta and Omicron, have been classified by the World Health Organization (WHO). Especially, the Omicron variant of SARS-CoV-2 exhibits striking transmissibility and immune evasion, and has been spreading rapidly worldwide since it was first reported in South Africa in November 2021 [1]. Omicron variant contains over 30 mutations in its spike protein (S) and 15 of them are located in the receptor binding domain (RBD). S-RBD mediates the binding of SARS-CoV-2 to angiotensin-converting enzyme 2 (ACE2), which is the main viral entry receptor for SARS-CoV-2 on host cells and also the major target for neutralizing antibodies. These mutations in S-RBD lead to the extensive neutralization escape from most therapeutic SARS-CoV-2 monoclonal antibodies (mAbs). Moreover, the effectiveness of all current vaccines was also reduced on Omicron variant for its neutralization escape [2]. A drug that can inhibit the binding of Omicron variant S-RBD to ACE2 should be useful for controlling SARS-CoV-2 Omicron variant infection.

Our previous work has identified that ceftazidime inhibits SARS-CoV-2 infection in vitro by binding to S-RBD and consequently blocking S-RBD interaction with ACE2 [3]. A single center report from Egypt reported that ceftazidime exhibits a good efficacy in the management of COVID-19 patients infected with SARS-CoV-2 prototype, Alpha, and Beta variants. Compared with the patients treated according to the WHO guidelines (dexamethasone and antiviral drugs, including favipiravir, remdesivir, hydroxyl chloroquine, et al.), the mean recovery time of patients treated with dexamethasone and ceftazidime was reduced from 18.66 ± 0.56 days to 13.29 ± 0.62 days [4]. Although ceftazidime has shown efficacy in previous SARS-CoV-2 strains, whether ceftazidime is able to inhibit the infection of the currently widespreading Omicron variant needs further investigation.

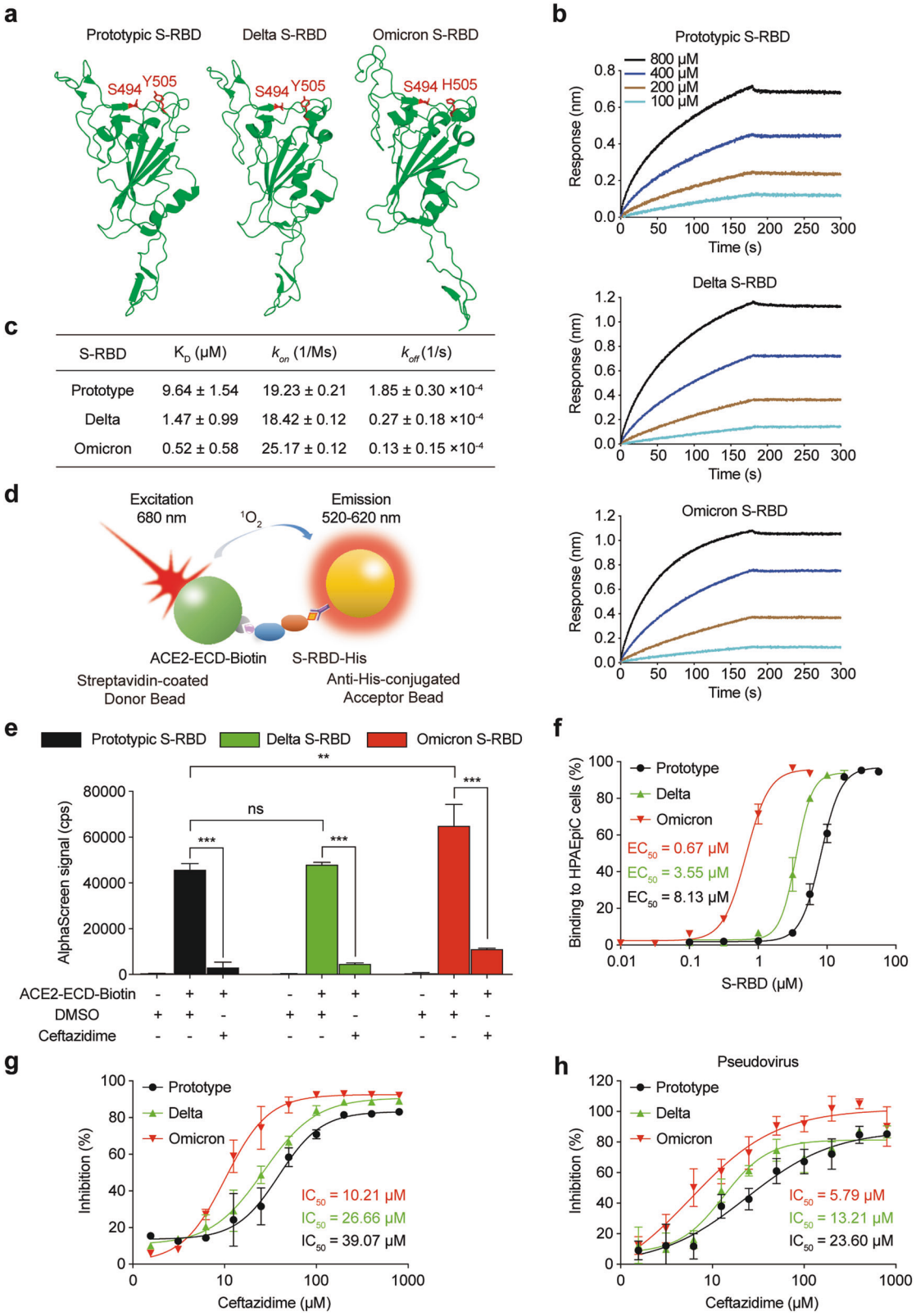
Firstly, we compared the molecular structures of prototypic, Delta and Omicron S-RBDs. Although Omicron variant contains 15 mutations in RBD (Supplementary Information, Fig. S1), the overall structure remains very similar among all three S-RBDs (Fig. 1a). Our previous work has demonstrated that residues Ser494 (S494) and Tyr505 (Y505) in prototypic S-RBD at the ACE2 binding interface play key roles in stabilizing ceftazidime binding [3]. Y505 was mutated to His505 (H505) in Omicron S-RBD (Fig. 1a), whether the substitution from phenyl ring to imidazole affects the binding to ceftazidime needs further investigation. To answer this question, we applied a bio-layer interferometry (BLI) experiment to examine the binding affinity between ceftazidime and S-RBD. Along with the elevated concentrations of ceftazidime, it showed a rapid association rate and a slow dissociation rate for all three S-RBDs, indicating a strong and stable interaction between ceftazidime and S-RBDs (Fig. 1b). Moreover, the statistic results revealed that k_{on} value of Omicron S-RBD was significantly higher than those of prototypic and Delta S-RBDs. Meanwhile, k_{off} value of Omicron S-RBD was lower. K_D value of Omicron S-RBD binding to ceftazidime was only 0.52 μM, which was much lower than those of prototypic and Delta S-RBDs (Fig. 1c), indicating an enhanced interaction between Omicron S-RBD and ceftazidime.

To investigate whether ceftazidime is able to block the binding of Delta and Omicron S-RBDs to ACE2, we established an AlphaScreen system to detect the interaction between S-RBD and the extracellular domain (ECD) of ACE2 (Fig. 1d). Anti-His-conjugated acceptor beads bind to prototypic, Delta or Omicron His-tagged S-RBD (S-RBD-His), respectively. The biotinylated ACE2-ECD (ACE2-ECD-Biotin) binds to streptavidin-coated donor beads. The interaction between S-RBD and ACE2-ECD brings the donor and acceptor beads into close proximity, resulting in light emission at 520–620 nm upon illumination at 680 nm. Compared with prototypic S-RBD–ACE2, Delta S-RBD–ACE2 showed the comparable AlphaScreen signal. Whereas, Omicron S-RBD–ACE2 exhibited a significantly higher signal (Fig. 1e), which was consistent with the results that Omicron spike protein has a markedly higher affinity for ACE2 [2]. Notably, 100 μM ceftazidime showed a strong inhibition on the binding of ACE2 to S-RBDs of prototype, Delta and Omicron variants (Fig. 1e). Thus, ceftazidime could be a broad inhibitor for prototype, Delta and Omicron variants of SARS-CoV-2.

We next examined the inhibitory effect of ceftazidime on the binding of S-RBD protein to ACE2-expressing human pulmonary alveolar epithelial cells (HPAEpiC). Similar to the AlphaScreen results, Omicron S-RBD bound to HPAEpiC cells with the highest capacity compared with prototypic and Delta S-RBD proteins. The half maximal effective concentration (EC_{50}) for prototypic, Delta or Omicron S-RBD binding to HPAEpiC cells was 8.13, 3.55 or 0.67 μM, respectively (Fig. 1f). Treatment of cells with ceftazidime at increasing concentrations led to a significant decrease in S-RBD binding signal (Fig. 1g), indicating the efficient inhibition on the binding of all three S-RBDs to HPAEpiC cells by ceftazidime. The half maximal inhibitory concentration (IC_{50}) for prototypic, Delta or Omicron S-RBD was 39.07, 26.66 or 10.21 μM, respectively (Fig. 1g).

Finally, we evaluated the inhibitory effect of ceftazidime on the entry of SARS-CoV-2 pseudovirus into 293T cells overexpressing human ACE2 (293T-ACE2). Prototypic, Delta and Omicron SARS-CoV-2 pseudoviruses were pre-mixed with DMSO or ceftazidime at a series of concentrations at 37 °C for 1 h, and then cocultured with 293T-ACE2 cells. At 48 h post infection, pseudovirus cell entry of prototype, Delta or Omicron variant was inhibited by ceftazidime with an IC_{50} of 23.60, 13.21 or 5.79 μM, respectively (Fig. 1h). Ceftazidime has been clinically used as a drug for the treatment of bacterial pneumonia and the blood concentration of ceftazidime can reach over 300 μM. At this concentration, ceftazidime showed more than 80% inhibition of all three SARS-

Received: 16 January 2023 Accepted: 23 February 2023
Published online: 21 March 2023



CoV-2 pseudoviral infection in vitro, suggesting its broad usage and strong potency to inhibit cell entry of SARS-CoV-2 variants.

Ceftazidime is an FDA-approved β-lactam antibiotic. Whereas, our previous work has shown that other β-lactam antibiotics had little inhibitory effect on S-RBD–ACE2 interaction. We further demonstrated the binding site for ceftazidime on S-RBD overlaps with that of ACE2. Especially, S494 and Y505 in S-RBD are two critical residues for ceftazidime binding [3]. In this work, our results showed that the substitution of H505 for Y505 in Omicron S-RBD even enhanced the interaction between S-RBD and ceftazidime

Fig. 1 Ceftazidime exhibits a broad inhibition to the infection of SARS-CoV-2 prototype and Omicron variant in vitro. a Molecular structures of prototypic/Delta/Omicron S-RBD (Arg319-Phe541, PDB: 7dk3/7w92/7wpd). Residues 494 and 505 were shown as sticks and colored in red. **b** Binding of ceftazidime to prototypic/Delta/Omicron S-RBD was measured by BLI experiments in an Octet RED96 instrument. The biotin-conjugated S-RBD was captured by streptavidin that was immobilized on a biosensor and tested for the binding with ceftazidime at gradient concentrations. **c** Binding capacity of ceftazidime to prototypic/Delta/Omicron S-RBD. The values of K_D, k_{on} and k_{off} for the binding of ceftazidime to prototypic/Delta/Omicron S-RBD were obtained by BLI experiments ($K_D = k_{off}/k_{on}$). **d** Schematic diagram of AlphaScreen system to detect the interaction between prototypic/Delta/Omicron S-RBD-His and ACE2-ECD-Biotin, adapted from PerkinElmer application notes. **e** Effect of ceftazidime (100 μM) on the AlphaScreen signal generated by prototypic/Delta/Omicron S-RBD-His (0.1 μM) and ACE2-ECD-Biotin (0.1 μM). DMSO was used as a vehicle control. Absence of ACE2-ECD-Biotin was used as a negative control of the AlphaScreen system. **f** Soluble prototypic/Delta/Omicron S-RBD binding to HPAEpiC cells was examined by flow cytometry analysis. EC_{50} was indicated in the graph. **g** The inhibitory effect of ceftazidime on the binding of S-RBD protein to HPAEpiC cells. Cells were treated with ceftazidime at different concentrations. The inhibition rate was calculated by the decrease of the proportion of S-RBD-His-APC^+ cells of each group compared with that of DMSO vehicle control group. IC_{50} was indicated in the graph. **h** ACE2-expressing 293T cells were treated with DMSO or serially diluted ceftazidime. Inhibition of GFP-encoding prototypic/Delta/Omicron SARS-CoV-2 typed pseudovirus entry into cells by ceftazidime. IC_{50} was indicated in the graph. One representative result of three independent experiments is shown in **b**. Data represent the mean ± SEM ($n \geq 2$) in **c**, **e**–**h**. ***$P < 0.001$; **$P < 0.01$; ns not significant (unpaired two-tailed Student's t test).

(Fig. 1b, c). The Omicron variant of SARS-CoV-2 has been reported to have multiple sublineages including BA.1, BA.2, BA.3, BA.4, BA.5 and descendent lineages (including newly emerged BQ.1, BQ.1.1, BF.7, BA.4.6 and XBB). It is noteworthy that all of these sublineages of Omicron variant contain S494 and H505 in S-RBD. Thus, it is tempting to speculate that ceftazidime has the capacity to inhibit the interaction between S-RBDs of all known sublineages of SARS-CoV-2 Omicron variant and ACE2, and subsequently blocks the viral infection. Whereas, ceftazidime is not suggested to be used for the prevention of COVID-19 to avoid the induction of bacterial drug resistance.

In summary, our findings demonstrate that ceftazidime exhibits a broad inhibition on the infection of SARS-CoV-2 prototype, Delta and Omicron variants in vitro by blocking spike protein-ACE2 interaction. Ceftazidime is usually used to treat a variety of bacterial infections, such as pneumonia. Besides, ceftazidime showed a good inhibitory activity toward Mpro to hinder the replication of the virus [5]. Clinically, ceftazidime was proved to be efficient for the management of moderate and severe cases of COVID-19 [4]. Considering the affordable price and minimal side effects of ceftazidime compared with other antiviral drugs, ceftazidime should be considered as one of the first-line antibiotics used for the treatment of COVID-19.

ACKNOWLEDGEMENTS

This work was supported by grants from National Key Research and Development Program of China (2020YFA0509000), National Natural Science Foundation of China (31830112, 32030024, 31525016 to JFC, 32170769, 31970702 to CDL), Shanghai Rising-Star Program (21QA1409700), Program of Shanghai Academic Research Leader (19XD1404200), Personalized Medicines-Molecular Signature-based Drug Discovery and Development, the Strategic Priority Research Program of the Chinese Academy of Sciences (XDA12010101), the Youth Innovation Promotion Association of the Chinese Academy of Sciences (2020266), the Young Elite Scientist Sponsorship Program by CAST (2019QNRC001), National Ten Thousand Talents Program, and the Fundamental Research Funds for the Central Universities (22120220378, 22120220499). The authors gratefully acknowledge the support of SA-SIBS scholarship program.

AUTHOR CONTRIBUTIONS

CDL and JFC conceptualized the project and designed the experiments. CDL, YZZ and ZYL performed the experiments and data analysis. YL, XYL and YW purified the recombinant proteins of S-RBD and ACE2. MWH and XCP prepared SARS-CoV-2 pseudovirus. CDL and JFC interpreted the results. The manuscript was drafted by CDL and edited by JFC.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41401-023-01071-0.

Competing interests: The authors have filed a patent (202110081412.2) for the application of ceftazidime and its derivatives in inhibiting SARS-CoV-2 infection.

Yun-zhe Zheng[1], Zhao-yuan Liu[1], Yue Li[1], Xiao-ying Lv[2], Yi Wu[2], Meng-wen Huang[1], Xing-chao Pan[1], Jian-feng Chen[1,3]✉ and Chang-dong Lin[2,4]✉

[1]State Key Laboratory of Cell Biology, Center for Excellence in Molecular Cell Science, Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, University of Chinese Academy of Sciences, Shanghai 200031, China; [2]Fundamental Research Center, Shanghai YangZhi Rehabilitation Hospital (Shanghai Sunshine Rehabilitation Center), School of Life Sciences and Technology, Tongji University, Shanghai 200092, China; [3]School of Life Science, Hangzhou Institute for Advanced Study, University of Chinese Academy of Sciences, Hangzhou 310024, China and [4]Frontier Science Center for Stem Cell Research, Tongji University, Shanghai 200092, China

These authors contributed equally: Yun-zhe Zheng, Zhao-yuan Liu

Correspondence: Jian-feng Chen (jfchen@sibcb.ac.cn) or Chang-dong Lin (linchangdong@tongji.edu.cn)

REFERENCES

1. Hong Q, Han W, Li J, Xu S, Wang Y, Xu C, et al. Molecular basis of receptor binding and antibody neutralization of Omicron. Nature. 2022;604:546–52.
2. Flemming A. Omicron, the great escape artist. Nat Rev Immunol. 2022;22:75.
3. Lin C, Li Y, Zhang Y, Liu Z, Mu X, Gu C, et al. Ceftazidime is a potential drug to inhibit SARS-CoV-2 infection in vitro by blocking spike protein-ACE2 interaction. Signal Transduct Target Ther. 2021;6:198.
4. Eid RA, Elgendy MO, El-Gendy AO, Elgendy SO, Belbahri L, Sayed AM, et al. Efficacy of ceftazidime and cefepime in the management of COVID-19 patients: single center report from Egypt. Antibiotics. 2021;10:1278.
5. Mostafa A, Kandeil A, Elshaier YAMM, Kutkat O, Moatasim Y, Rashad AA, et al. FDA-approved drugs with potent in vitro antiviral activity against severe acute respiratory syndrome coronavirus 2. Pharmaceuticals. 2020;13:443.