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PERSPECTIVE

The newly emerged SARS-Like coronavirus HCoV-EMC also has an "Achilles' heel": current effective inhibitor targeting a 3C-like protease

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From the global outbreak of SARS-CoV caused infection disease in 2003, coronaviruses (CoVs) are known to be a great threat to the human health. Recently, a new SARS-like coronavirus, human betacoronavirus 2c EMC/2012 (HCoV-EMC), has been identified and the appearance of this new CoV raises concerns that a new spread of CoV may occurs in the future. By solving the crystal structure of HCoV-EMC main protease with a wide-spectrum anti-CoV inhibitor N3, we confirmed that that N3 blocks the function of HCoV-EMC main protease through a similar mechanism to other CoVs. Together with the good pharmaceutical features, N3 is conceivable to be effective to HCoV-EMC and other CoVs appearing in the future. These findings make it convincing that CoVs will not be a threat to human health.

In the year of 2012, a new respiratory illness similar to severe acute respiratory syndrome (SARS) that spread globally in 2003 and infected over 8,000 people with more than 800 fatalities (Li et al., 2010), was identified in Europe, the Middle East and Hong Kong. The infection was reported to manifest clinically with fever, cough and breathing difficulties. Some patients also developed acute renal failure. Sequence analysis suggested that the causative agent of this newly emerged SARS-like illness is a new coronavirus (CoV) which has been named as the human betacoronavirus 2c EMC/2012 (HCoV-EMC). No clinically approved treatment is available for CoV infection since the outbreak of SARS in 2003, the appearance of this new CoV raises concerns that a new epidemic of CoV infection may occur in future.

CoVs are positive-sense, single-stranded RNA viruses and are featured by the largest viral RNA genomes known to date (Yang et al., 2003). Replication of coronavirus requires correct proteolytic processing of the replicase polyprotein by viral proteases, in particular a chymotrypsin-like protease ($3CL^{pro}$, also known as main protease M^{pro}). Since $3CL^{pro}$ is unique in the virus but not found in the host cell, this protein is a prominent target for the development antivirals against CoV infections (Yang et al., 2005), and a number of inhibitors have been discovered that prohibit the infection of CoV through their action on $3CL^{pro}$ (Yang et al., 2005).

To elucidate the inhibitory mechanism and provide information to aid the discovery of new candidate compounds against CoV infections, we present the crystal structure of HCoV-EMC $3CL^{pro}$ complexed with the broad-spectrum anti-CoV inhibitor N3. HCoV-EMC $3CL^{pro}$ processes a conserved molecular fold and the dimeric architecture of CoV main proteases (Fig. 1A), which is consistent with that was observed in SARS-CoV $3CL^{pro}$ (Yang et al., 2003). A protomer of HCoV-EMC $3CL^{pro}$ dimer is featured by three domains: Domains I (residues Ser1–Pro102) and II (residues Ala103–Asp200) process an anti-paralleled β-barrel structure and shares high structural similarity with trypsin-like serine proteases. Domain III (residues Lys201–Gln306) contains five α-helices to form a compact globular structure.

The catalytic dyad Cys148–His41 and the substrate binding site of HCoV-EMC $3CL^{pro}$ are located on the interface of Domain I and II, which is identified by the bound inhibitor N3 (Fig. 1B) and

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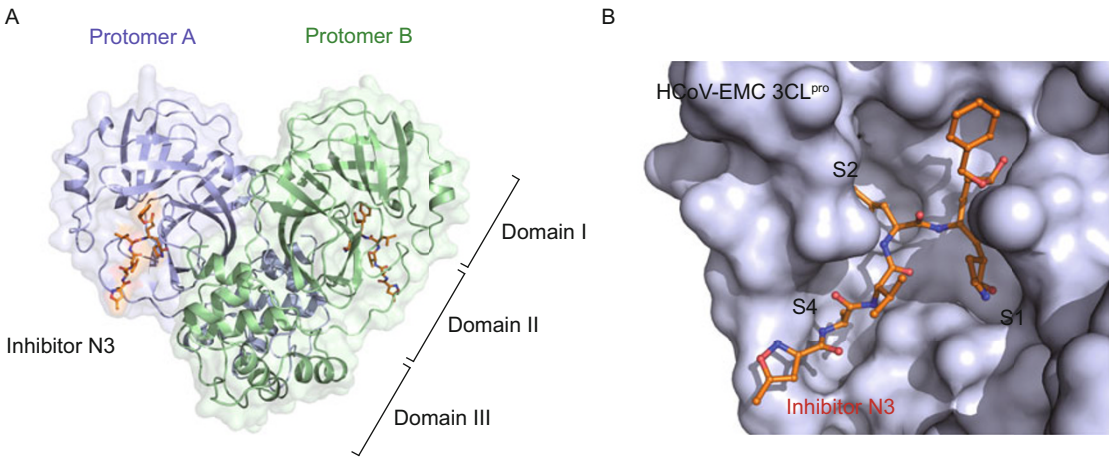

Figure 1. Structure of HCoV-EMC 3CLpro complexed with the wide-spectrum anti-CoV inhibitor N3. (A) The overall structure of HCoV-EMC 3CL^{pro} dimer. The polypeptide of HCoV-EMC 3CL^{pro} are shown as a cartoon representation. The bound inhibitor N3 molecules are highlighted as colored sticks. The conserved Domain I, II and III are labeled. (B) A surface representation of inhibitor N3 binding to HCoV-EMC 3CL^{pro}. HCoV-EMC 3CL^{pro} is covered with a blue molecular surface and the bound N3 molecule is shown as colored sticks. Key subsites are labeled out.

presents a conserved architecture with those are in other CoVs (Anand et al., 2002; Anand et al., 2003; Yang et al., 2003, 2008; Zhao et al., 2008). The S^{γ} atom of the catalytic residue, Cys148, and the $N^{\varepsilon 2}$ atom of the general base, His41, directly contribute to the catalytic reaction. The S1 subsite of the substrate-binding site, which confers absolute specificity for the Gln-P1 substrate residue on CoV 3CL^{pro}s (Ziebuhr et al., 2000), is mainly formed by Leu144–Cys147, His166 and Glu169 (Fig. 2A). Comparing with other CoV 3CL^{pro}s, S1 subsite shows high conservations. Moreover, subsites S2 and S4, which are crucial for substrate and inhibitor binding, also show high structural and sequence similarities, indicating HCoV-EMC 3CL^{pro} functions through a conserved mechanism like other CoVs.

In our previous studies, a peptide mimic inhibitor N3 (Fig. 2B) was found to prohibit the replication of CoVs in different groups by attenuating the function of CoV 3CL^{pro} (Yang et al., 2005). To be consistent with that the high similarities between the key residues of CoV 3CL^{pro}s for substrate recognition, we found that N3 can effectively inhibit the proteolytic activity of HCoV-EMC 3CL^{pro} with an IC_{50} of 0.28 ± 0.02 μmol/L (Fig. 2C). The S^{γ} atom of Cys148 forms a covalent bond with the C^{β} atom of the vinyl group of N3, which indicates a Michael addition reaction with nucleophilic attack by S^{γ} on the N3 C_{β}. The lactam group of N3 occupies the S1 subsite and forms two stable hydrogen bonds with His166 and His175 of 3CL^{pro}. The side chains of leucine and alanine at the P2 and P4 sites respectively in N3 bind to the S2 and S4 subsites of 3CL^{pro} with excellent complementarity. All these structural information indicate that N3 blocks the function of HCoV-EMC 3CL^{pro} through a similar mechanism as in other CoVs.

AG7088 is a potent inhibitor of rhinovirus 3C^{pro} but failed to inhibit 3CL^{pro} of SARS-CoV (Shie et al., 2005) and a number of AG7088 analogues were discovered to combat CoVs by targeting 3CL^{pro} (Liang, 2006; Lee et al., 2009). Although the inhibitory effect of inhibitor N3 to HCoV-EMC 3CL^{pro} is not extremely good, it presents good pharmaceutical properties with comparable inhibitory activity (Fig. S2). The β-phase half-life ($t_{1/2}$) of inhibitor N3 was 70 min and all rats survive with the injection of 140 mg/kg N3. These results indicate the great potential to make inhibitor N3 as the wide-spectrum anti-CoV drug in the future.

Besides HCoV-EMC, a number of coronaviruses, e.g. alphacoronavirus HKL10, have been isolated from bat (Lau et al., 2012). Although these newly identified CoVs display distinct epidemic and pathologic features, they present high sequence and structure similarity for virally encoded proteins, in particular the replicase protein, with those of SARS-CoV and other well-known CoVs. It is conceivable that current inhibitors, which were discovered based on SARS-CoV, are also effective against HCoV-EMC and other CoVs that may appear in future. If this "Achilles' heel" hypothesis proves to be correct, CoVs will not be a threat to human health.

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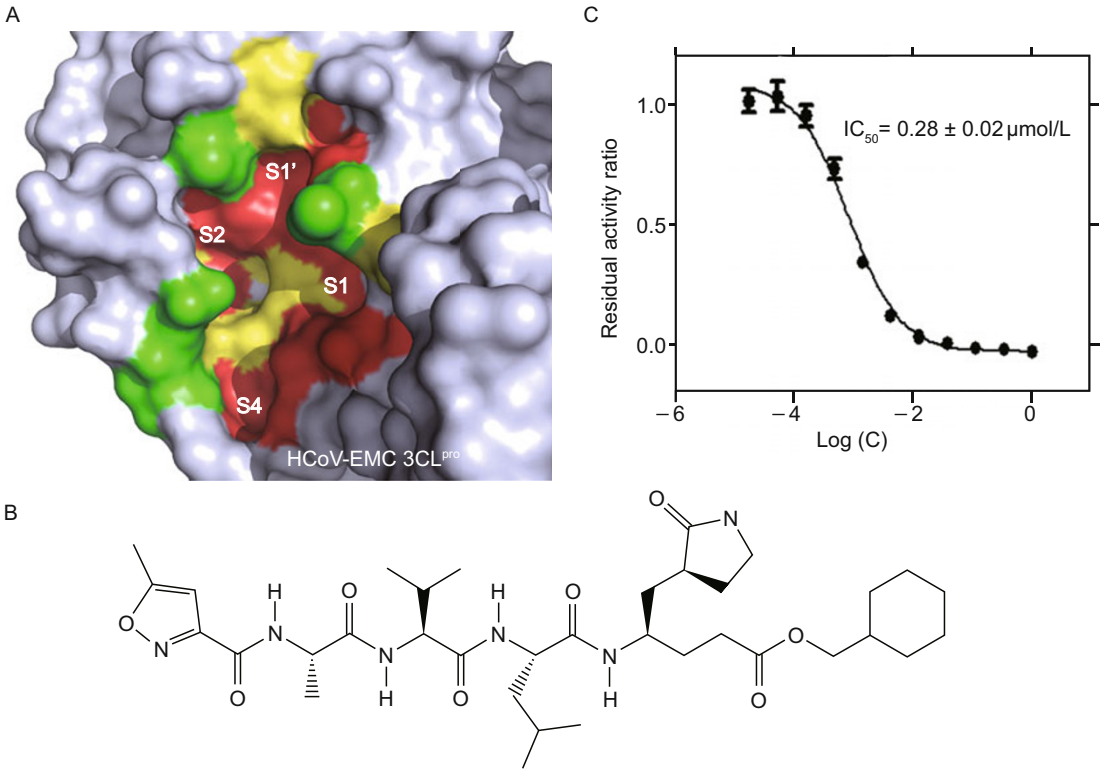

Figure 2. The wide-spectrum anti-CoV inhibitor N3 inhibits the activity of HCoV-EMC 3CL^{pro} through a conserved mechanism. (A) The substrate binding site of CoVs 3CL^{pro}. The strickly conserved, conserved and not conserved residues located at the substrate binding site are colored as red, yellow and green, respectively. (B) The structural formula of inhibitor N3. (C) N3 inhibits the proteolytic activity of HCoV-EMC 3CL^{pro} with an IC_{50} of 0.28 ± 0.02 μmol/L. The result was obtained from three independent experiments performed in duplicate.

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