Novel antiviral activity of PADs inhibitors against human beta-coronaviruses SARS-CoV-2 and HCoV-OC43

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INTRODUCTION. Emerging zoonotic RNA viruses have repeatedly attracted the attention of researchers over the past few decades. Novel coronaviruses (CoVs) in particular need special attention, due to the high mortality rates, the lack of effective therapies, the potential to spillover from a large reservoir of animal hosts, and the high rate of transmissibility that allows them to cause epidemics. To date, seven human CoVs (HCoVs) have been identified: among them, HCoV-OC43 and SARS-CoV-2, the causative agent of the ongoing epidemic of atypical pneumonia (COVID-19), belong to beta genus. A very recent study described the putative roles of a family of cellular enzymes called peptidylarginine deiminases (PADs) in COVID-19 disease. PADs are a family of calcium dependent enzymes that catalyze the post-translational modification citrullination, a process in which the guanidinium group of a peptidyl-arginine is hydrolyzed to form peptidyl-citrulline, a non-genetically coded aminoacid. PADs dysregulation leads to an aberrant citrullination which is a characteristic biomarker of several inflammatory conditions. Moreover, a correlation has recently emerged between PADs dysregulation and other viral infections, including human rhinovirus and cytomegalovirus. Based on these evidences, the aim of this work was to evaluate whether PAD inhibitors were a reliable new class of host-targeted antivirals against coronaviruses.
MATERIAL AND METHODS. By using the HCoV-OC43 and SARS-CoV2 strains as models of infection in human lung fibroblasts (MRC-5) and monkey kidney cells (Vero-E6), we tested the antiviral activity of well characterized PAD inhibitors. We used real time quantitative PCR to quantify copies of the viral genomes, Western blot analysis to evaluate the expression of viral proteins, and plaque assay to evaluate the production of new virions. Furthermore, we assessed the pattern of citrullination upon infection by using a citrulline-specific rhodamine phenylglyoxal (RhPG)-based probe.
RESULTS. HCoV-OC43 and SARS-CoV-2 infections were significantly associated to PAD-mediated citrullination *in vitro* and to an increase of PAD expression, both at mRNA and protein levels. Moreover, the pharmacological inhibition of PAD enzymes led to a significant reduction of viral replication, suggesting that PAD4 isoform in particular might play a major role in OC43 replication.
DISCUSSION AND CONCLUSION. Our results suggested that i) citrullination is a process that can be induced by RNA viruses, such as HCoV-OC43 and SARS-CoV-2, as a mechanism to foster their replication, and 2) that increase of PADs activity is central for beta-coronavirus replication. Taken together, we provide evidence that PADs inhibitors deserve consideration against human beta-coronaviruses infection.