Parasitic Connection between COVID-19 and Diarylamidines

Subjects: Infectious Diseases

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As emerging severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants (Omicron) continue to outpace and negate combinatorial vaccines and monoclonal antibody therapies targeting the spike protein (S) receptor binding domain (RBD), the appetite for developing similar COVID-19 treatments has significantly diminished, with the attention of the scientific community switching to long COVID treatments.

long COVID serine protease diarylamidines

1. Introduction

As of 8 March 2023 (accessed: https://coronavirus.jhu.edu/map.html), more than 676 million COVID-19 cases and 6.8 million deaths had been reported globally. During that time, SARS-CoV-2 underwent significant mutations [1], resulting in Alpha (B.1.1.7), Beta (B.1.351), Delta (B.1.617.2), Gamma (P.1), and Omicron variants of concern (VOC). The most recent of these is the Omicron subvariant XBB.1.5, which is expected to be the aetiology of the majority of USA COVID-19 infections by early 2023 [2][3][4][5]. Unlike the original Wuhan strain, Omicron variants induce clinical symptoms associated with the upper respiratory tract and larynx, where the airway temperature is significantly lower [6], the viral replication rate is considerably higher [7], and the hosts' immune response is reduced. Such conditions contribute to Omicron's high transmissibility and ability to evade the hosts' immune response.

The high transmissibility of Omicron has not only proved its infection and reinfection potential in humans and semi-domesticated species [8][9], but recent reports suggest the variant is prevalent in wild species [10]. Moreover, studies suggest that the requirement for P3 arginine in S1/S2 for furin cleavage common to different hosts provides a window for zoonotic transfer [11]. Accordingly, the World Health Organization has stressed the importance of veterinary and wastewater surveillance of *de novo* mutations and antigenic shifts in other species that could later appear in humans [12]. Water surveillance in cities and provinces has provided some unique insights into the emergence of the Wuhan and new Omicron strains and the transition from inter-pandemic to pandemic periods. In addition, previous studies have shown that increasing wastewater temperatures from 4 °C to 10 °C can more than double the decay rate of free-form SARS-CoV-2 RNA, measured by detecting the N1 and N2 genes [12]. However, water surveillance relates to viral identification and not the exact origins of the Wuhan and Omicron strains, the latter first being identified in South Africa on 24 November 2021 [13][14].

2. Bats, Pangolins, Parasites, and Diarylamidines: Making the Connection

An attempt to address the critical issue of the intermediate hosts of SARS-CoV-2 was recently presented by Tang et al. [15] in the general context of human coronaviruses but was left unresolved with additional vectors missing. Currently, the vectors associated with potential intermediate hosts (bats and pangolins) [16] include bat flies (*Diptera*: *Nycteribiidae* and *Streblidae*), bugs (*Hemiptera: Cimicidae* and *Polyctenidae*) that transmit blood ectoparasites (protozoans *Trypanosoma* spp. and *Plasmodium* spp.) [17][18], Wolkberg and Kaeng Khoi RNA viruses (*Bunyavirales*: *Bunyaviridae* and *Peribunyaviridae*), and dengue virus (*Flaviviridae*) [19]. A plethora of divergent viruses and different vectors found on live bats collected from Yunnan Province, China, between 2012 and 2015 was recently examined by Xu et al. [20]. Researchers encountered mainly arthropod-specific viruses and three possible arboviruses, suggesting that viral spillage between bats and common parasitic arthropods rarely occurs; whether this is the case for other parasite arthropods such as *Hemiptera* (triatomine bugs) could not be addressed. *Hemiptera* is renowned for its ability to transmit *Trypanosoma* spp., which are found in approximately 100 bat species worldwide [21]. The potential vectors for *T. cruzi* involve > 130 species of triatomine insects, five of which are epidemiologically more significant: *Triatoma infestans*, *Triatoma brasiliensis*, *Triatoma dimidiata*, *Rhodnius prolixus*, and *Panstrongylus megistus.* Currently, there are no documented cases of *Hemiptera* transmitting *Trypanosoma* species from bats to humans. However, just before the start of the pandemic, a related *Trypanosoma cruzi* genotype, specifically *T. cruzi* (TcBat), was reported for the first time in the brains of several bat species sourced from Yunnan Province (*Noctilio* spp., *Myotis* spp., and *Artibeus* spp.) [22]; unfortunately, the researchers did not test the blood or visit the animals to test for arthropods during the time of capture (2010–2015). TcBat was initially discovered in South America and has been detected in a Colombian child [23] and human mummies. Several bat families house the genotype, and phylogenic studies suggest that TcBat is a monophyletic lineage predominant in Colombia, Brazil, and Panama. In addition to TcBat, another bat trypanosome species reported to infect mammals and a handful of humans in Central America and north-western South America is *Trypanosoma rangeli* (*T. rangeli*) [24].

Before 2000, the origins of bat trypanosomes remained elusive until extensive evolutionary and taxonomic studies relating to the genus *Trypanosoma* resulted in the “bat-seeding hypothesis” proposed by Hamilton et al. in 2012. The hypothesis suggested that *T. cruzi* was the first bat trypanosome due to its expansive host range and employment of alternative vectors (triatomine bugs) permitting its transmission among New and Old-World terrestrial mammals. Additional multilocus phylogenetic analyses on multiple bat trypanosomal species sourced from Europe, South America, Africa, China, and Japan confirmed their placement within the *T. cruzi* clade, validating the hypothesis [24]. Hamilton further postulated that said clade probably arose from an ancestral group of isolated trypanosomes exclusively evolving in bats with multiple spill-over events into terrestrial mammals. The *T. cruzi* population is classified into six discrete typing units (DTUs): TcI to TcVI, with the recently named TcBat as the seventh. Trypanosomes within the *T. cruzi* clade are distributed into three main phylogenetic lineages.

Trypanosoma spp. are extracellular and intravascular blood parasites that cause debilitating acute or chronic disease in camels, cattle, humans, and other domestic animals. There are two phases of infection, acute and

chronic, with the former associated with bone marrow hypoplasia anaemia, thrombocytopenia, and leukopenia in mammalian hosts [21]. Cardiomyopathy is the most common clinical outcome, and, until 2019, *Trypanosoma* infections and the associated disease (*Trypanosomiasis*) were endemic in 36 sub-Saharan African countries [25]. Strategies employed by trypanosomes to evade the host immune system include antigenic variation, induction of suppressor macrophages, myeloid-derived suppressor cells (MDSCs), and regulatory T cells [26].

Another parasitic disease [26] endemic on many continents [27] is babesiosis, a tickborne infection transmitted to humans by the bite of *Ixodes scapularis*, harbouring the parasite *Babesia microti (B. microti)* [28]. Prevalent in vertebrates, *B. microti* was recently found in 53 confiscated rare Sunda pangolins native to Thailand [26]. Moreover, additional reports found evidence of SARS-CoV-2-related coronaviruses circulating in Sunda pangolins [27]. Currently, there are no studies investigating *B. microti* and SARS-CoV-2 co-infections in humans or pangolins and the transfer of immunosuppressive factors, primarily due to the lack of testing and suitably available animal models. Furthermore, other reports show that co-infections involving *Babesia.* spp. [29] and *Trypanosoma.* spp. [30] are common with other parasitic species. In addition to blood parasites, several bacteria, including *Borrelia burgdorferi sensu lato* (*B. burgdorferi*), benefit from co-infection with *Babesia*, which can prolong severe Lyme arthritis in mice when compared to controls [30][31].

However, exceptions do occur; for example, *Plasmodium* spp. infection prior to SARS-CoV-2 infection was reported to contribute to the disease's poor prognosis and was seen to reduce the incidence of malaria in endemic regions [32][33][34]. Furthermore, additional investigations suggested that high exposure to malaria compared to low exposure prior to SARS-CoV-2 infection offered better protection against severe or critical COVID-19 [35].

Diarylamidines, diminazene aceturate (DIZE), and pentamidine have been used to treat *Trypanosomiasis* (*T. cruzi and T. brucei*—Chagas disease and African Sleeping. sickness), Leishmania, and Babesiosis in humans and cattle for decades [36][37][38][39]. DIZE and pentamidine are transported into trypanosomes, exerting their protozoalcidal abilities by targeting the adenine–thymine (A-T)-rich hairpin loops in circular and kinetoplast DNA resulting in the complete and irreparable nucleic acid loss. In addition, diarylamidines can also bind to mitochondrial II topoisomerase and transfer RNA [40]. Outside of their interactions with parasites, numerous studies have demonstrated that DIZE and pentamidine can act as bacterial growth inhibitors and adjuvants to common antibiotics [41][42][43][44][45][46][47][48]. Moreover, DIZE was shown to inhibit the production of lipopolysaccharide (LPS) endotoxins [49][50], whilst pentamidine interacts with LPS via lipid A [51][52] (**Table 1**).

Table 1. Antiparasitic and synergistic capabilities of diminazene aceturate and pentamidine when used separately or in conjunction with antibiotics.

Diarylamidines & Combinations	Pathogen/Disease	Hosts/Experimental	Findings	References
DIZE & AZI	*T. brucei*	Albino Rats	↓ Parasitaemia	[44]

Diarylamidines & Combinations	Pathogen/Disease	Hosts/Experimental	Findings	References
			↓ Leukocytosis	
DIZE	*T. congolense*	BALB/c mice C57BL/6 mice	↓ LPS-induced production ↓ IL-6, IL-12, TNF and IFN-γ	[41]
DIZE	*S. mansoniex vivo*	mice	↓ Aminotransferase levels ↓ Immature and adult parasites	[53]
DIZE	*T. congolense*	Pre-treated macrophages	↓ Phosphorylation of mitogen-activated protein kinase (p38), STAT1 and STAT3	[49]
CLD, DIZE, ACE, IMD	*C. babesiosis*	Dogs	↓ Malaise, fatigue, chills, fever	[39]
DIZE, CHL, STP	Antibiotic-resistant *K. pneumoniae*, *S. aureus* and *E. coli*	Minimal inhibitory concentrations (MICs) assay	↑ Gram-negative and positive bacterial sensitivity to antibiotics ↓ in MIC	[43]
PENT, colistin	*T. brucei rhodesiense*	66-year-old female	↓ Parasitaemia by 75% (trypomastigotes)	[38]
PENT	*A. baumannii*	Murine Infection Model	↑ sensitivity of colistin-resistant *A. baumannii* to gram-positive antibiotics	[46]
PENT analogue	*A. baumannii* & *K. pneumoniae*.	Murine model	↑ sensitivity of colistin-resistant *A. baumannii* to gram-positive	[47]

Diminazene aceturate (DIZE), Pentamidine (PENT), Chloramphenicol (CHL), Streptomycin (STP), Imidocarb dipropionate (IMD), Clindamycin (CLD), Azithromycin (AZI), Aceturate (ACE). *Trypanosoma brucei* (*T. brucei*), *Trypanosoma congolense* (*T. congolense*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Schistosoma mansoniex vivo*

Diarylamidines & Combinations	Pathogen/Disease	Hosts/Experimental	Findings	References
(P35)			antibiotics	

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of animal model studies, including (i) amelioration of neurological pathologies (Alzheimer's) [54]; (ii) cardiac (atherosclerotic plaque stabilization, cardiac fibrosis) [55][56][57]; (iii) pulmonary (hypertension) [58]; (iv) liver (injury and biliary fibrosis) [59]; (v) diabetic (type 1) [60]; (vi) acute radiation exposure/damaged tissue (ARE) [61] (**Table 2**).

Table 2. Amelioration of cardiac, neurological, kidney, and systemic experimental models by diminazene aceturate (DIZE).

Diarylamidine	Disease/Pathology	Hosts/Experimental	Findings	References
DIZE	AD	SAMP8 mice	↓ Neuropathology	[54]
DIZE	Liver injury & BF	MDR gene-2 knockout mice	↓ NOX enzyme assembly and ROS generation ↑ myofibroblasts & tissue repair	[59]
DIZE	ATP & HPS	ApoE-Knockout mice	Modulating macrophage response & taurine biosynthesis	[57]
DIZE	CF & DAD	W rats	↑ Protective effect on the heart under the pathological condition of kidney injury	[55]
DIZE	PHY	SD male rats	↑ Vasoprotective axis of the LRAS, ↑ pulmonary vasoreactivity, ↑ enhanced cardiac function, ↓ inflammatory cytokines	[56]
DIZE	NPP	W diabetic male rats	↑ Glomerular ACE2 & AT2 receptor expression	[60]

Diarylamidine	Disease/Pathology	Hosts/Experimental	Findings	References
			↓ fibrosis and apoptosis	
DIZE	MORI	WAG/RijCmcr rats	↑ Survivability in rat models of H-ARS and DEARE	[61]
DIZE	CAR	W rats	↑ Acute antiarrhythmic-mic potential *in vivo* modulation of cardiomyocytes contraction and excitability properties	[62]

Alzheimer's disease (AD), Senescence Accelerated Mouse (SAMP8), Biliary fibrosis (BF), Multiple Drug Resistant (MDR), Atherosclerotic Plaques (ATP), Hepatic Steatosis (HPS), Cardiac Fibrosis (CF), Diastolic Dysfunction (DAD), Pulmonary Hypertension (PHY), Nephropathy (NPR), Multiorgan Radiation Injury (MORI), Ischemia (IC), Myocardial Infarction (MI), Wistar Rats (W rats), Sprague-Dawley Rats (SD rats), Cardiac Arrhythmia (CAR), Hematologic Acute Radiation Syndrome (H-ARS), Multiorgan Delayed Effects of Acute Radiation Exposure (DEARE), Lung Renin-Angiotensin System (LRAS).

3. Diarylamidines, Serine Proteases

The cationic amidine groups are thought to be responsible for their interactions with single-stranded, double-stranded, and supercoiled DNA [63]. These trypsin/mesotrypsin [64] substrate mimics are charged at neutral pH (poor oral bioavailability) and are part of a group of diarylamidines (diminazene, pentamidine, hydroxysitlbamidine, and 4’,6-diamidino-2-phenylindole, (DAPI)) which inhibit the activity of all ASIC subtypes, including ASIC1a [65], whose blockade has been shown to be beneficial in animal models of Huntington’s and Parkinson’s diseases [66][67], ischemic stroke [68], and multiple sclerosis [69][70].

Due to its nanomolar potency for subtype ASIC1 and its inability (apart from stroke) to cross the blood–brain barrier (free form), DIZE is often limited to usage as a standard drug for in vitro modelling of new ASIC inhibitors [71]. Like many ASIC drugs (hydroxysitlbamidine and pentamidine) [72], diminazene, as well as its metabolite para-amino benzamidine (pABA), are serine protease inhibitors [73]. More recently, a cell-based proteolytic assay investigating the repurposing potential of DIZE and the clinically approved ASIC drugs camostat, nafamostat, and gabexate [74][75][76] for the treatment of mild COVID-19 further demonstrated DIZE’s broad spectrum ability to inhibit the catalytic triad of TMPRSS2 (HIS296, ASP345, and SER441), as well as furin’s active sites (ASN192, LEU227, SER253, ASP258, ASP306, THR367, and SER36). In an attempt to further understand DIZE’s ability to inhibit both proteases, additional modelling studies conducted by the authors revealed that the drug adopted a similar binding

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