REVIEW

Nanotechnology-based therapeutic formulations in the battle against animal coronaviruses: an update

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Abstract Outbreak of infectious diseases imposes a serious threat to human population and also causes a catastrophic impact on global economy. Animal coronaviruses remain as one of the intriguing problems, known to cause deadly viral diseases on economically important animal population, and also these infections may spread to other animals and humans. Through isolation of the infected animals from others and providing appropriate treatment using antiviral drugs, it is possible to prevent the virus transmission from animals to other species. In recent times, antiviral drug-resistant strains are being emerged as a deadly virus which are known to cause pandemic. To overcome this, nanoparticles-based formulations are developed as antiviral agent which attacks the animal coronaviruses at multiple sites in the virus replication cycle. Nanovaccines are also being formulated to protect the animals from coronaviruses. Nanoformulations contain particles of one or more dimensions in nano-scale (few nanometers to 1000 nm), which

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could be inorganic or organic in nature. This review presents the comprehensive outline of the nanotechnology-based therapeutics formulated against animal coronaviruses, which includes the nanoparticles-based antiviral formulations and nanoparticles-based adjuvant vaccines. The mechanism of action of these nanoparticles-based antivirals against animal coronavirus is also discussed using relevant examples. In addition, the scope of repurposing the existing nano-enabled antivirals and vaccines to combat the coronavirus infections in animals is elaborated.

Introduction

Social and global sustainable development depends on economically important animal population which is mainly affected due to the emergence of viral diseases and it eventually leads to the decline in animal capital, animal product, and associated international trade (Astudillo and Rosenberg 1983; Khamassi Khbou et al. 2020). Among the livestock population, farm animals contribute directly or indirectly to agricultural sector and food production, which influence the world's economy (Thornton 2010). Developing countries like India are well known for their world's largest livestock population (Birthal and Rao 2002).

Outbreak of zoonotic diseases causes a crippling effect on human population with nearly one billion infections and kills millions of people worldwide every year (Karesh et al. 2012; Espinosa et al. 2020). Moreover, it is believed that these infectious diseases are emerged due to the human civilization and the consequences of anthropogenic changes (Lindahl and Grace 2015). Zoonotic diseases caused by animal viruses are deadly and contagious which gets transmitted to humans through cross-host switching events (Parrish et al. 2008). In case the animal viruses are left uncontrolled at initial stage, these viruses could gradually evolve and infect humans in vast numbers (Murcia et al. 2009). In most cases, before identifying the etiologic cause of the viral disease, it gets transmitted to other species. It is important to contain the spread of animal viruses to humans by treating them at the first place. To meet this, researchers worldwide put several efforts to develop drugs and vaccines which act efficiently against animal viruses. Failure to contain the spread of zoonotic viral diseases would leave a devastating impact on global economy and public health (Madhav et al. 2017).

Coronaviruses of animals

Coronavirus (CoV) is an enveloped virus containing single-stranded, positive-sense RNA as genetic material whose structure is composed of proteins such as glycoprotein S present in the viral envelope, transmembrane glycoprotein M, nucleocapsid protein N, and transmembrane protein E (Burrell et al. 2016). Coronaviruses belong to the Nidovirales order, under which three families, namely, Coronaviridae, Arteriviridae, and Roniviridae are present. Coronaviridae consist of two subfamilies, Coronavirinae and Torovirinae. Coronavirinae are classified into four genera, namely, alphacoronavirus, betacoronavirus, deltacoronavirus, and gammacoronavirus. The alphacoronavirus includes human and animal virus (pig, cat, dog), examples of betacoronavirus are human and mouse hepatitis virus (MHV) and other animal viruses (cows), deltacoronavirus comprise of viruses isolated from pigs and birds, and gammacoronavirus includes viruses of birds and cetaceans (Burrell et al. 2016).

Coronavirus causes severe respiratory and enteric diseases in a wide range of animal's hosts (Woo et al. 2009; Chan et al. 2012). These coronaviruses carry the zoonotic potential and could easily spread to other mammals including humans (Zhang et al. 2021; Tiwari et al. 2020). Table 1 shows the list of animal coronavirus and its prevalence with suitable examples.

Transmissible gastroenteritis virus (TGEV) is quite similar to porcine epidemic diarrhea virus (PEDV) and belongs to alphacoronavirus genera, which affects gastrointestinal tract and causes severe diarrhea and high mortality in neonatal piglets (Alonso et al. 2014). PEDV is an airborne infectious disease that causes severe diarrhea and also kills nursing pigs, transmit through fecal–nasal route from pig-to-pig or farm-to-farm (Alonso et al. 2014). Although the source of this PEDV was unknown in 1980s, later, it is listed as endemic swine virus which possesses close genetic

Table 1 Different genera of animal coronaviruses, prevalence, and their examples

Genus	Prevalence	Animal hosts	Animal viruses	Reference
Alphacoronavirus	Mammals	Pig	Transmissible gastroenteritis virus	Alonso et al. 2014)
			Porcine epidemic diarrhea virus, swine acute diarrhea syndrome coronavirus	
		Cat	Feline infectious peritonitis virus	Tekes and Thiel 2016)
			Feline enteric coronavirus	
		Dog	Canine coronavirus	Licitra et al. 2014)
Betacoronavirus	Mammals	Bat	severe acute respiratory syndrome coronavirus-2	Gorbalenya et al. 2020)
		Mice	Murine hepatitis virus	Kuo and Masters 2013)
		Cow	Bovine coronavirus	Suzuki et al. 2020)
Deltacoronavirus	Pigs and birds	Pig	Porcine deltacoronavirus	Wang et al. 2019)
Gammacoronavirus	Whales and birds	Chicken	Infectious bronchitis virus	Colvero et al. 2015)
		Turkey	Turkey coronavirus	Dea and Tijssen 1989)

similarity to human CoV 229E (Duarte et al. 1994). Feline coronavirus (FCoV) is a single-stranded, positive sense, and lipid enveloped RNA virus, which belongs to alphacoronaviridae family and causes feline infectious peritonitis and fatal disease in cats. FCoV affects the gastrointestinal tract, respiratory tract, and central nervous system of cats and virus transmission to other cats occurs via fecal–oral route (Tekes and Thiel 2016). Canine enteric coronavirus and canine respiratory coronavirus (CCoV) affect the enteric and respiratory tract of dogs and transmit through fecal–oral route (Licitra et al. 2014). Bovine coronavirus (BCoV) causes enteric and respiratory disease in neonatal calves and adults and thus leads to high calf mortality, deterioration of milk production, and health of adult cattle. BCoV has zoonotic transmission to other species including wild ruminants and BCoV like human enteric coronavirus are isolated from a child with acute diarrhea (Kuo and Masters 2013). MHV is the most ubiquitous coronavirus that affects the central nervous system of mice (Compton et al. 2004). Avian infectious bronchitis virus (IBV) is an acute and highly contagious virus for all ages of chickens and causes respiratory illness and high mortality rate in juvenile birds (Dhama et al. 2014). IBV, belonging to the gamma coronavirus, is a positive sense, non-segmented, single-stranded RNA virus. Infectious bronchitis causes respiratory disease and reproductive system failure and also affects the renal system and causes nephritis defects in chickens (Colvero et al. 2015).

The rise of new CoVs and variants with the prominent difference in tissue or host tropism and virulence properties from existing strains is highly probable (Saif 2004). For instance, porcine respiratory coronavirus (PRCV) evolved from TGEV through a deletion mutation in the spike gene which is otherwise responsible for high virulence of TGEV. Highly virulent feline infectious peritonitis virus (FIPV) is considered as the systemic viral variant of less virulent FCoV. A targeted gene recombination event between the mouse S protein and the feline CoV empowers the FCoV to infect mice (Saif 2004).

In the year 2003, severe acute respiratory syndrome coronavirus (SARS-CoV) identified in bats, gets transmitted to humans through direct contact with fecal-aerosols and other body fluids of infected bats, and causes respiratory infections in humans. Through respiratory droplets of infected people, these viruses easily spread to other humans and thus become a major threat to the human race (Wang et al. 2005). Besides humans, SARS-CoV like similar coronavirus are isolated from animals such as civet cats and raccoon dogs, which are highly contagious and thus spread to new host species (Guan et al. 2003). Zhou et al. identified a novel coronavirus, swine acute diarrhea syndrome (SADS-CoV), which is similar to HKU2-coronavirus and its genome sequence is 98.48% identical to that of the bat coronavirus (Zhou et al. 2018). SADS-CoV is most likely originated from the reservoir hosts of SARS-related coronaviruses species, horseshoe bats (Rhinolophus).

Conventional therapeutic strategies to target animal coronaviruses

Various therapeutic approaches are established to treat animal coronavirus-induced infections in natural hosts and closely related species. 3-Chymotrypsin-like protease (3CLpro) is a coronavirus protease enzyme that plays an important role in the replication of coronavirus (Prajapat et al. 2020). Dipeptidyl and tripeptidyl (GC373-GC376) protease inhibitors displayed potent inhibitory action against a wide range of animal coronaviruses such as FIPV, BCV, TGEV, and MHV (Kim et al. 2013, 2012). In addition, peptide compounds such as GC523, GC543, GC551, GC583, GC587, GC591, GC597, GC772, and GC774 are tested against 3CLpro of ferret, feline, and mink coronavirus. Among, GC587 peptide is effective with high 3CLpro inhibitory effect in all coronaviruses studied (Perera et al. 2018). The Griffithsin, a protein isolated from red algae *Griffithsia* sp., acts as an anti-CoV drug which inhibits the S protein of coronavirus (O'Keefe et al. 2010). The TP29 peptide ligand targets the non-structural protein 10 (nsp10) of MHV CoVs with a noticeable decrease in the viral load in the liver after administrating the MHV infected mice with this peptide (Wang et al. 2015). Remdesivir (GS-5734) is a nucleotide prodrug, which exhibits excellent antiviral activity by reducing the viral replication of infectious bat CoV in human airway epithelial cells (Sheahan et al. 2017). Lopinavir and Ritonavir demonstrate anti-MERS-CoV activity in animal models by protecting the cells from viral infections and decrease the overall viral load in animals (Wilde et al. 2014; Sheahan et al. 2020). Ivermectin, a parasitic drug, has been documented for broad-spectrum virucidal activity against RNA viruses such as Avian Influenza A, Zika, Porcine Reproductive and Respiratory, dengue, and severe acute respiratory syndrome coronavirus 2 (Heidary and Gharebaghi 2020). In vivo studies revealed that ivermectin reduces the IL-6/IL-10 ratio in the lung tissue of SARS-CoV-2-infected animals but it requires systematic clinical trials in humans to prove its efficacy (Melo et al. 2020).

Similarly, vaccines for animal coronavirus diseases have also been developed. Currently, animal coronavirus vaccines, i.e., live-attenuated or inactivated vaccines are developed using different bacterial expression systems. Hou and team developed an oral and intranasal recombinant vaccine (live attenuated) with the nucleocapsid protein of PDEV using *Lactobacillus casei* as expression vector and as a result, high levels of IgA and IgG are seen (Hou et al. 2007). Zhang et al. produced an oral bivalent DNA vaccine containing S genes of PEDV and TGEV which is delivered by attenuated *Salmonella typhimurium* (Zhang et al. 2016). Takamura et al. established a vaccine containing BCoV infected cell extract with oil as adjuvant and upon immunization, high levels of hemagglutination-inhibition antibody are seen in vaccinated cattle (Takamura et al. 2020). Similarly, an IBV vaccine consists of multiepitope peptide where two N and one S genes are fused with plasmid and expressed using *E. coli* (Yang et al. 2009).

Advantages of nanotechnology in virus research

Nanotechnology has revolutionized the field of biomedical science, with immense potential in the design of point-of-care diagnostics to therapeutics for viral diseases (Ramos et al. 2017; Krishnan et al. 2021). Emergence of antiviral-resistant pandemic viruses is most likely due to exposing the animals to conventional antiviral drugs (Kumar et al. 2020). With the advent of nanotechnology-based approach, the characteristics of nanomaterials such as high surface area, surface charge, morphology, and size are utilized in developing the potent antiviral nanoformulations for better therapeutic efficacy (Ramos et al. 2017). Incorporation of nanoscale materials in drug formulations imparts superior advantages such as improved drug stability, drug solubility, controlled release of drugs with increased bioavailability, and also amount of drug and associated toxicity aspects are reduced (Jampílek and Kráľová 2019; Chakravarty and Vora 2020). Nanostructured materials can be directly applied as an antiviral agent or alternatively used a carrier of antiviral drug. For instance, metallic nanoparticles such as silver nanoparticles act as effective antiviral agents against many types of viruses (Galdiero et al. 2011). Solid-lipid nanoparticles (SLN)-based antiviral drug formulations have already proved its effectiveness in multiple instances. Using various types of lipids, SLN-based formulations with different antiviral drugs, namely, zidovudine, maraviroc, ritonavir, and few other drugs, are prepared for effective cellular uptake and targeted drug delivery

(Patra et al. 2018). For instance, the in vitro cellular uptake of the zidovudine drug in murine neuro-2a and HeLa cells for acquired immunodeficiency syndrome (AIDS) therapy is improved due to the introduction of nanoformulation which contains the drug loaded into lipid-polymer-based core–shell nanoparticles as compared to nascent drug (Joshy et al. 2018a, 2018b). Injectable nanoformulations of anti-HIV drugs, tenofovir, and elvitegravir have shown a long-lasting drug exposure and reduced elimination half-time in mice model (Gao et al. 2018; Prathipati et al. 2017). Nanoformulations are also known for non-viral gene therapy where multifunctional nanoparticles are used to improve the delivery of specific genes, protect the cargo from nucleases mediated degradation, and also enhance the targeting mechanism (Lin et al. 2018; Ariza-Saenz et al. 2018).

Nanoformulations are advantageous over conventional antiviral drugs as it acts through multiple ways to arrest the virus replication in mammals (Jampílek and Kráľová 2019). Nanoparticles have been shown to interact with virus either inside or outside host cells and subsequently block the viral replication through more than one way. Some of the common approaches include (a) nanoparticles interact with surface proteins of virus and thereby prevent cellular entry, (b) nanoparticles inactivate virus particles before it comes in contact with host cells, and

(c) nanoparticles interact with viral genome and/or during the subsequent steps involved in the formation of mature virions (Jampílek and Kráľová 2019). Metal nanoparticles such as gold, silver, and copper exert antiviral activity by inhibiting the viral entry to host cells. These nanoparticles inhibit virus through (a) direct attachment to virus and inactivate them and (b) interfere with virus-host cell binding. Zinc nanoparticles displayed virucidal activity through its interference with viral DNA polymerase activity and thus, virus replication inside host cells is inhibited (Aderibigbe 2017; Rai et al. 2016).

On the other hand, nanostructured materials are used in the preparation of adjuvant-based nanovaccines for animals to prevent the infections caused by animal coronavirus. Incorporation of virus entity in the preparation of nanovaccine with adjuvants has gained more attention in recent years. Nanovaccines offer certain advantages such as controlled immune response and elicited B cell immunity as a result of improved antigen processing pathways (Singh 2021). However, conventional vaccines suffer from limitations such as weak immunogenicity and poorly protective.

Having witnessed the recent coronavirus disease-19 (COVID-19) pandemic caused due to deadly coronavirus, SARS-CoV-2 which could have possibly originated from bats (primary reservoir), it is

Fig. 1 Nanoformulations to combat the coronavirus infections common in animal hosts and its influence on virus transmission to humans
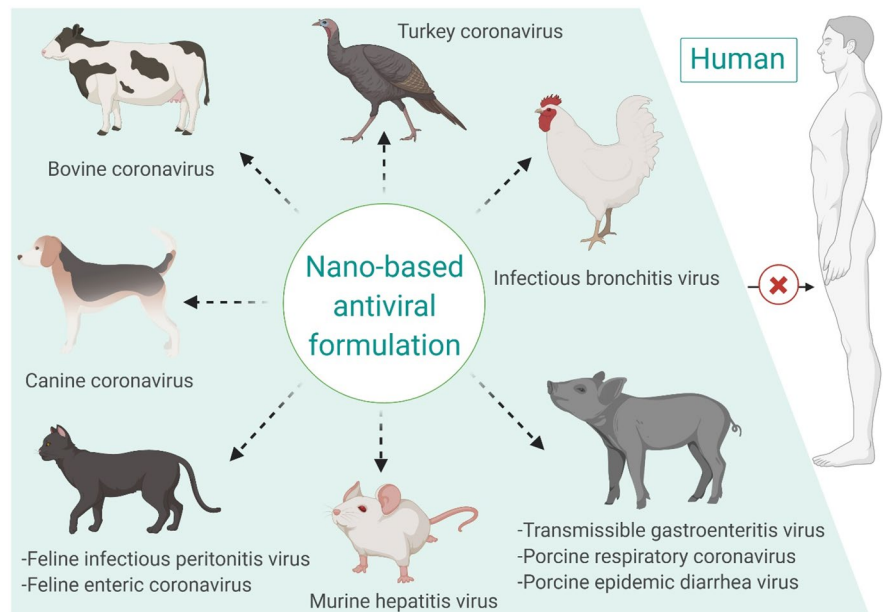
important to unleash the power of nanotechnology to contain the spread of viral infections before transmission to other species. To this end, this review presents the comprehensive outline of various nanoformulations to combat the virus infections caused by animal coronavirus, which includes nanoparticles-based antiviral agents and adjuvant-based nanovaccines. Figure 1 shows the schematic of the animals susceptible to coronavirus infections and nanoformulations developed to combat these viral infections.

Nanoparticles-based antiviral agents against non-human coronaviruses

Different types of nanostructured materials are explored as an antiviral agent against animal coronavirus. This can be classified depending on the composition of the nanomaterial, which is grouped as follows, carbon-based, metallic, and polymer-based nanoparticles. Table 2 outlines the various nanoformulations developed to target the animal coronavirus and their efficacy information.

Carbon-based nanomaterials

The use of nanomaterials such as carbon dots as an antiviral agent against coronavirus is well studied. Very recently, cationic carbon dots (CDs) of size 1.5 nm synthesized using curcumin were reported for the antiviral property against PEDV (Ting et al. 2018). For the concentrations (15.6 to 125 µg/mL) of CDs tested against Vero cells, cytotoxicity studies showed that cell viability of > 90% was maintained even after 48 h incubation.

Table 2 Nanoparticles-based antiviral agents against animal coronaviruses

Nanomaterial (size)	Genus (host)	Species	Efficacy	References
Carbon-based nanomaterials				
Curcumin cationic carbon dots (1.5 nm)	Alpha CoVs (Pigs)	Porcine epidemic Diarrhea Virus	125 µg/mL, 50% viral inhibition	63
Glycyrrhizic acid-based carbon dots (11.4 nm)	Alpha CoVs (Pigs)	Porcine epidemic Diarrhea Virus	0.90 mg/mL, 70% viral inhibition	64
Metallic nanoparticles				
Spherical silver nanoparticles (20 nm), silver nanowires (60 & 400 nm), silver colloids (10 nm)	Alpha CoVs (Pigs)	Transmissible gastroenteritis virus	12.5 µg/mL, 67.35% viral inhibition	65
Graphene-silver nanocomposites (5 & 25 nm)	Alpha CoVs (Cats)	Feline coronavirus	0.1 mg/mL, 25% viral inhibition	66
Glutathione capped-silver sulfide nanoclusters (2.5 nm & 4.1 nm)	Alpha CoVs (Pigs)	Porcine epidemic diarrhea virus	46 µg/mL, 3.0 virus log reduction	67
Gold/silver nanorods (aspect ratio is 3:1)	Alpha CoVs (Pigs)	Porcine epidemic diarrhea virus	0.04 µM, inhibited viral entry, decreased caspase-3 activity, Generation of reactive oxygen species	68
Tellurium nanoparticles coated with bovine serum albumin, nanostars (57 nm)	Alpha CoVs (Pigs)	Porcine epidemic diarrhea virus	30 µg/mL, dose-dependent viral inhibition	69
Glutathione capped-ZnS nanoparticles	Alpha CoVs (Pigs)	Porcine epidemic diarrhea virus	0.90 mg/mL, dose-dependent viral inhibition	70
Polymeric nanoparticles				
Curcumin encapsulated chitosan nanoparticles	Alpha CoVs (Cats)	Feline infectious peritonitis virus	20 µM, 73.97% viral inhibition	71
Diphyllin using polyethylene glycol-poly lactic acid-co-glycolic acid nanoparticles (40 nm)	Alpha CoVs (Cats)	Feline infectious peritonitis virus	Therapeutic index up to 800-folds	72

Fig. 2 Schematic shows the multi-site inhibition of Porcine epidemic diarrhea virus (PEDV) by curcumin-based carbon dots (CDs)
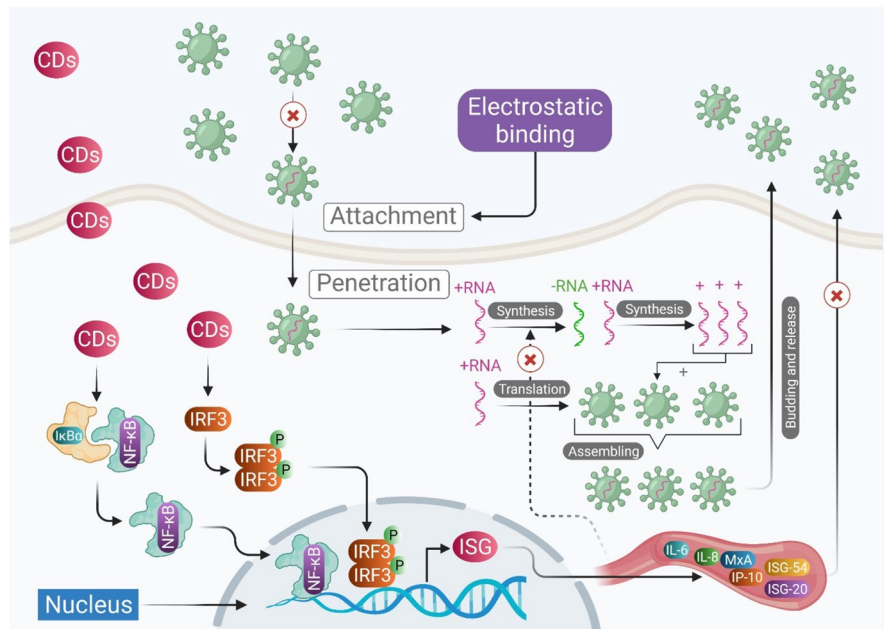
Decreased levels of expression of PEDV N proteins in Vero cells confirmed the antiviral property role of cationic-CDs (125 μg/mL). Owing to the positive surface charge (+15.6 mV), CDs interact with the virus or the cell membrane. Mechanistic studies revealed that CDs work against PEDV through multiple routes, (a) due to structural changes induced in the surface protein of PEDV, 50% viral entry is inhibited, (b) downregulated negative-strand viral RNA synthesis, (c) inhibits virus budding, and (d) inhibits reactive oxygen species (ROS) formation in PEDV infected cells (Fig. 2). Upon examination of the effect of these carbon dots on innate immune responses showed that the expression of interferon-inducible protein 10, interferon-stimulated genes (ISG) such as ISG-54, MxA, ISG-20, and pro-inflammatory cytokines-interleukins (IL) such as IL-6 and IL-8 were induced (Fig. 2). Moreover, CD-treated cells showed high levels of phosphorylated interferon regulatory factor 3 (IRF3) and p65 proteins which indicate protein phosphorylation trigger innate immune responses.

In a separate study, the antiviral activity of Glycyrrhizic acid-CDs against PEDV coronavirus was reported (Tong et al. 2020). In vitro cytotoxicity studies using Vero and PK-15 cells showed that > 70% cell viability was maintained at 0.90 mg/mL of CDs after 24 h. Compared to the control group, CD-treated group demonstrated a noticeable decrease in PEDV virus-infected PK-15 cells, which is attributed to the antiviral property of CDs against PEDV.

Metallic nanomaterials

Silver nanostructures

Silver-based nanostructures (Ag NS) like spherical nanoparticles (20 nm), silver nanowires (60 and 400 nm), and silver colloids (10 nm) were investigated for antiviral activity against TGEV (Lv et al. 2014). Cytotoxicity studies revealed that anti-TGEV activity of Ag NS was found to be dose-dependent with ~67.35% reduction in viral titer at 12.5 μg/mL. Ag NS inhibited TGEV virus through interaction with surface proteins and also diminished the infectivity of virus.

The antiviral efficacy of graphene-silver (G-Ag) nanocomposites against enveloped feline coronavirus (FCoV) was studied (Chen et al. 2016). Cytotoxicity studies revealed that 50% cytotoxic concentration (CC_{50}) of G-Ag nanocomposites against *Felis catus* whole fetus-4 cells was found to be 19.7 mg/mL. G-Ag nanocomposites inhibited 25% of FCoV infections at 0.1 mg/mL.

Spherical-shaped glutathione capped-Ag_2S nanoclusters (NCs) of size 3.7 nm have been shown to inhibit PEDV (Du et al. 2018). Cytotoxicity effect of Ag_2S NCs was evaluated at concentration ranging from 23 to 184 μg/mL against Vero cells. Results indicated that 90% of cell viability was maintained at

46 μg/mL of Ag_2S NCs. Further studies revealed that these NCs inhibit the replication of PEDV by downregulating the expression of PEDV N protein.

Apart from spherical-shaped nanoparticles, anisotropic nanostructures are also explored to determine the antiviral efficacy. In a recent report, the virucidal activity of Au@Ag core/shell bimetallic nanorods (Au@AgNRs) against PEDV was described (Du et al. 2020). About 90% of cell viability was seen at 0.04 μM of Au@AgNRs and the morphology of the Vero cells was not affected at this concentration. Through plague assay, the antiviral property of Au@AgNRs was confirmed at 0.04 μM. These silver nanostructures were shown to inhibit PEDV virus by downregulating the expression levels of PEDV N protein, blocking the viral entry, and suppressing caspase-3-mediated apoptosis and mitochondrial membrane potential.

Overall, the mechanism of antiviral action of silver nanoparticles occurs (a) through interfering with the virus binding and attachment to host cell receptor and subsequently cellular entry is prevented, (b) bind to the viral genome (DNA or RNA), and inhibit the replication of virus inside the host cells (Salleh et al. 2020). Coronavirus is a single-stranded RNA virus which utilizes the host cellular machinery to produce viral proteins and gets packaged into mature virions

and released out of cell. Collectively, silver-based nanostructures have also been studied against the animal coronavirus such as TGEV (Lv et al. 2014), FCoV (Chen et al. 2016), IBDV (Chen et al. 2016), and PEDV (Du et al. 2018). Interaction of silver nanoparticles with the surface proteins of the virus leads to the reduced viral infectivity of TGEV (Lv et al. 2014). Dose-dependent antiviral inhibitory effects were observed for G-Ag nanocomposites against IBV and FCoV (Chen et al. 2016). On the other hand, these silver-based nanostructures have downregulated the expression of PEDV N protein and thus inhibit the viral replication (Du et al. 2018) (Fig 3).

Tellurium nanoparticles

Tellurium is a metalloid element reportedly used for the synthesis of star-shaped tellurium nanoparticles (57 nm) coated with bovine serum albumin (Te/BSA) and mercaptoethane sulfonate was used as a surface modifier (Zhou et al. 2020a). In vitro cytotoxicity studies showed that 90% of Vero cells were viable in the presence of 30 μg/mL of Te/BSA nano-star. These nanostructures demonstrated significant antiviral efficacy against PEDV infected cells at concentration of 30 μg/mL.

Fig. 3 Graphical representation of the mechanism of antiviral action of gold/silver nanorods (Au@AgNRs) against porcine epidemic diarrhea virus (PEDV)
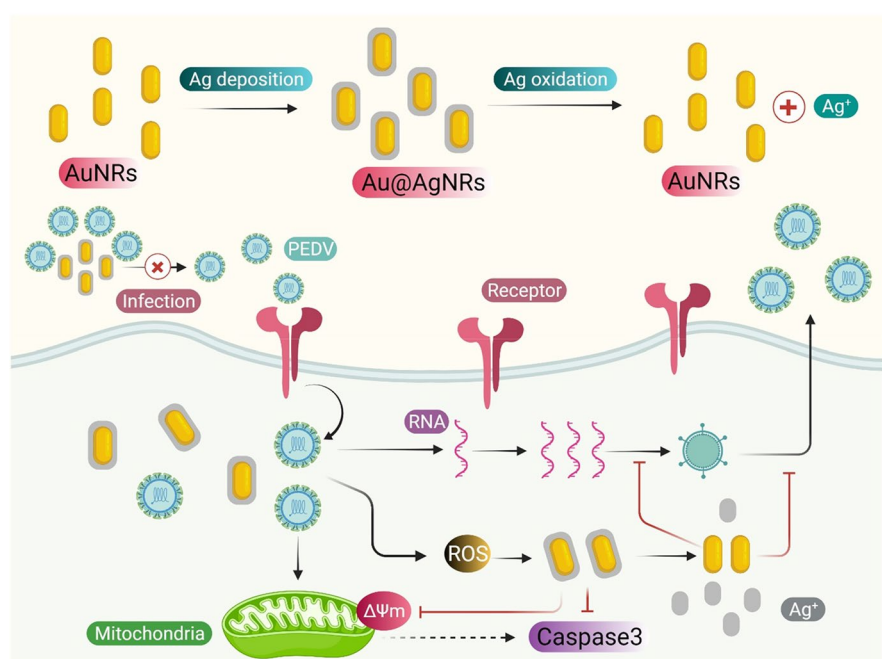
Zinc sulfide nanoparticles

Synthesis of zinc-sulfide (ZnS) nanoparticles of average dia. 3.8 ± 0.5 nm, as an antiviral agent using glutathione (GSH) act as a reducing and capping agent was reported (Zhou et al. 2020b). Investigation of the cytotoxicity effects of GSH-ZnS NPs against A549 cells, PK-15 cells, Vero Cells indicated that 90% viability was observed with these cells at the concentrations of 0.90 mg/mL. Importantly, GSH-ZnS NPs showed dose-dependent antiviral activity against PEDV and PRV and inhibited the virus propagation through inducing interferon (IFN)-β signaling pathway. Luciferase reporter assay showed that GSH-ZnS NPs failed to activate the promoters of key transcription factors, IRF3, and nuclear factor-kappa B (NF-κB) which is known to regulate the IFN-β promoter.

Polymeric nanoparticles

Using ionic gelation method, spherical- and cuboidal-shaped curcumin encapsulated chitosan (Cur-CS) nanoparticles (330 nm) were prepared (Ng et al. 2020). Cur-CS nanoparticles showed no cytotoxic effect against Crandell-Rees feline kidney (CrFK) cells at 250 μg/mL. These nanoparticles possess antiviral activity by inhibiting 73.97% of FIPV at 20 μM concentration.

To target the deadly viral disease, feline infectious peritonitis in cats, nanoparticulate of vacuolar ATPase blocker, diphyllin was developed as an antiviral agent (Hu et al. 2017). As part of the FIPV inhibitory mechanism, acidification of endosomes is essential for virus uncoating and cellular entry step. Further, encapsulation of the drug diphyllin using polyethylene glycol-poly lactic acid-co-glycolic acid nanoparticles (PEG-PLGA) nanoparticles revealed that the drug toxicity was significantly reduced and antiviral activity was increased. Overall, an improvement in therapeutic index of up to ~800-folds was monitored. In vivo studies have shown that diphyllin nanoparticles were well tolerated in mice, after intravenous administration.

Adjuvant-based nanoparticle vaccine against animal coronavirus

In order to protect the animals from coronavirus and related complications, nanovaccines can be readily formulated with endogenous antigens/adjuvants to induce the mucosal immunity. Some of the adjuvant-based nanovaccines known for preventing the animals from coronavirus are outlined in Table 3.

Adjuvant nanovaccines prepared from citrate-capped colloidal gold nanoparticles (15 nm) conjugated with swine transmissible gastroenteritis virus

Table 3 Adjuvant-based nanovaccines for coronavirus disease prevention in animals

Nanovaccine (nanomaterial size)	Genus (host)	Species	Efficacy	References
Colloidal gold nanoparticles conjugated with swine transmissible gastroenteritis virus (15 nm)	Alpha CoVs (Pigs)	Swine transmissible gastroenteritis virus	Increased plasma IFN-γ levels demonstrated immunomodulatory activity	74
PLGA-entrapped inactivated PEDV antigen-based vaccine (100–600 nm)	Alpha CoVs (Pigs)	Porcine epidemic diarrhea virus	Increase in the PEDV-specific IgG and IgA antibodies, lymphocyte proliferation, and IFN-υ levels	75
IBV-Flagellin-Self-Assembling Protein Nanoparticle vaccine (22.8 nm)	Gamma CoVs (Birds)	Infectious bronchitis virus	Improved antibody response, higher stimulation index, reduced viral shedding and lesions in the trachea	76
Quil-A and chitosan adjuvant based nanocarriers encapsulated with Plasmid DNA vaccine (< 100 nm)	Gamma CoVs (Birds)	Infectious bronchitis virus	Increased immunogenicity, reduced viral shedding, low clinical severity, increased humoral and adaptive cellular responses	77

(STG) were investigated for their immunomodulatory properties (Staroverov et al. 2011). In this study, three different groups of rabbits were immunized, viz., (a) STG virus, (b) colloidal nanogold conjugated with virus, and (c) colloidal gold nanoparticles, and the levels of inflammation markers, IFN-γ was examined. Compared to control group (475 ± 35 pg/mL), the concentration of plasma IFN-γ found in colloidal nanogold conjugated with virus immunized animals and non-conjugated virus, and colloidal gold was found to be 1067 ± 10 pg/mL, 910 ± 14 pg/mL, and 515 ± 7 pg/mL respectively. The increased plasma IFN-γ observed in animals immunized with colloidal nanogolds-virus conjugate demonstrated prominent immunomodulatory activity and the gold nanoparticles act as both adjuvant and carrier.

The use of poly (D, L-lactide-co-glycolide) (PLGA) nanoparticle and PEDV killed vaccine antigens in the preparation of vaccines against PEDV virus for piglets was reported (Li et al. 2017). Compared to the antigens alone, nanoformulated antigen demonstrated a significant increase in the PEDV-specific IgG and IgA antibodies in pregnant sows. Moreover, an enhanced response in lymphocyte proliferation and IFN-υ levels were noticed in pregnant sows. These results also corroborate to the less mortality rate observed with nanoformulated antigen-treated piglets. Intranasal administration of these nanovaccines has been shown to improve the systemic and mucosal immune responses of neonatal piglets. Thus, PLGA-entrapped inactivated PEDV antigen acts as a promising vaccine for protecting the suckling piglets from PEDV infections.

The preparation of IBV-Flagellin-Self-Assembling Protein Nanoparticle (SAPN) vaccine for Infectious bronchitis virus (IBV) was developed (Li et al. 2018). Importantly, IBV spike proteins were incorporated as the adjuvant component along with flagellin. When the immunized chickens (nanovaccine) were challenged with IBV viral load, an improved antibody response was observed as that of the negative control, Flagellin-SAPN. Moreover, higher stimulation index was evidenced in ex vivo proliferation tests on peripheral mononuclear cells derived from IBV-Flagellin-SAPN immunized chickens. Reduction in the viral shedding and tracheal lesions were the promising positive effects of the nano-adjuvant vaccine to protect the chickens from IBV-related viral infections.

In a separate study, Quil-A and chitosan (QAC) adjuvant-based nanocarriers of size < 100 nm were formulated (Chandrasekar et al. 2020). This adjuvant type vaccine system was further encapsulated with plasmid DNA vaccine (pQAC-N) encoding for IBV nucleocapsid protein N. Intranasal vaccine administration showed an improvement in immunogenicity in birds and also protects them from IBV infections. Markedly, both the adaptive and humoral cellular responses were increased in immunized chickens post treatment. Reduced viral shedding and low clinical severity were also confirmed with pQAC-N immunized birds. Apparently, chickens and chicken embryos did tolerate the pQAC-N (100 μg) nanocarriers administered through in ovo and intranasal routes.

Repurposing existing nanoformulations to combat animal coronavirus

Chang et al. (Chang et al. 2021) reported the antiviral effectiveness of the metal nanoparticle composite, TPNT1 against SARS-CoV-2. This nanocomposite contains gold nanoparticles (1 ppm), silver nanoparticles (5 ppm), zinc oxide nanoparticles (60 ppm), and chlorine dioxide (42.5 ppm), which acts by blocking the viral entry into the cell. Silver nanoparticles (1–10 ppm) of average size 10 nm reportedly destroy the extracellular SARS-CoV-2 through blocking viral entry into host cells while nanoparticle concentrations of 20 ppm and above exerted cytotoxic behavior (Jeremiah et al. 2020).

Based on the information of genetic similarity between the coronavirus and other well-studied viruses, some of the existing antiviral drugs could be a potential candidate against coronaviruses. United States-Food and Drug Administration (USFDA)-approved anemia drug, iron-oxide nanoparticles (IONPs), could be repurposed as a potential antiviral drug against SARS-CoV-2. Preliminary docking studies suggested that these IONPs interact with the protein of coronavirus and inactivates it (Abo-Zeid et al. 2020). The FDA-approved drug ivermectin formulated in orally administrable nanoparticles form performs as a potent antiviral agent against coronavirus by lowering the expression of angiotensin-converting enzyme 2 (ACE2) receptor and spike protein (Surnar et al. 2020). One of the most promising therapeutic approaches against COVID-19 is the antiviral small

interfering RNA (siRNA) delivery through inhalation which could be designed in conjunction with FDA-approved lipid or polymer or the lipid-polymer hybrid nanoparticulate systems for improved efficacy (Ullah et al. 2020).

Synthesis of plant-based nanoformulations is continuously growing area of research (Ishak et al. 2019) and nanoparticles produced by this approach also exhibited antiviral activity against RNA viruses that infect humans. Under this approach, different plant extracts and their constituents have been employed to prepare nanoparticles from metals such as silver, gold, and ceria. Besides, carbon dots and chitosan-based nanoformulations were also developed. Among different nanomaterials, silver nanoparticles are the most widely studied nanomaterial prepared from various plant sources such as *Cinnamomum cassia*, *Andrographis paniculata*, *Phyllanthus niruri*, *Tinospora cordifolia*, *Lampranthus coccineus*, *Malephora lutea*, *Ricinus communis*, *Curcuma longa*, and *Panax gingseng* which exerted antiviral properties against H7N3 influenza A virus (Fatima et al. 2016), Chikungunya virus (Sharma et al. 2019), Hepatitis virus-10 (Haggag et al. 2019), Coxsackievirus B4 (Haggag et al. 2019), Coxsackievirus B3 (Ben Salem et al. 2012), Respiratory syncytial virus (Yang et al. 2016), and Influenza A virus (strain A/PR/8) (Sreekanth et al. 2018). Biogenic gold nanoparticles (11 nm) prepared using the garlic extract showed antiviral activity against Measles virus (Meléndez-Villanueva et al. 2019). Cerium oxide nanoparticles (14 nm) generated from the fruit extract of plant *Hyphaene thebaica* displayed virucidal activity against Sabin-like poliovirus (Mohamed et al. 2020). Chitosan nanoparticles (29–39.5 nm) synthesized using the Rhizome extract of *Curcuma longa* exhibited antiviral efficacy against Hepatitis virus C (Loutfy et al. 2020).

In order to combat the COVID-19 pandemic caused due to the newly emerged coronavirus, SARS-CoV-2, researchers from worldwide have put extensive efforts to develop vaccines to protect humans. In developed countries like the USA, after a series of clinical testing to probe the vaccine effectiveness and safety, USFDA and Centre for disease control (USA) have authorized the use of vaccines namely Pfizer-BioNTech (BNT162b2 vaccine), Moderna (mRNA-1273 vaccine), and Johnson & Johnson's Janssen. Besides, vaccines such as AstraZeneca and Novavax COVID-19 vaccine are undergoing 3rd phase of clinical trials (Centers for Disease Control and Prevention xxxx). Among them, Pfizer-BioNTech and Moderna are the mRNA-based vaccines, where the mRNA which encodes for the SARS-CoV-2 spike protein is encapsulated within lipid nanoparticles (Malik et al. 2021). Janssen vaccine is based on stable DNA molecule extracted from adenovirus (non-replicating viral vector) which was substantially modified to express a vital portion of SARS-CoV-2 virus particle against which the body generates immunogenic response. AstraZeneca/Oxford COVID-19 vaccine, reputedly known as Covishield, is a replicating viral vector-based vaccine. With the help of recombinant DNA technology, the gene which encodes for coronavirus spike protein is inserted into the Chimpanzee adenovirus, which allows them to enter the cells but does not replicate inside them. Novavax COVID-19 vaccine is a protein subunit type vaccine which contains SARS-CoV-2 glycoprotein nanoparticle vaccine with Matrix M protein as adjuvant (Malik et al. 2021).

In addition, there are vaccines developed based on inactivated viruses, namely, Sinopharm, Coronavac (Sinovac), and Covaxin (Bharat Biotech) (Kyriakidis et al. 2021). Very recently, Sinopharm vaccine received the approval from World Health Organisation (WHO) for emergency use after this vaccine has been tested for its safety, efficacy, and quality. Although these inactivated viruses do not replicate inside the host cells, it boosts the immune system of humans to confront the viral infections. Sputnik, an adenoviral vector-based vaccine developed by Russia, contains few parts of SARS-CoV-2 gene which generates the immune response (Kyriakidis et al. 2021). During the preclinical studies, in vitro cell cultures and in vivo (animal model) studies have been employed to investigate the safety, preliminary efficacy, toxicity, and pharmacokinetic parameters of these human vaccines. Considering this, vaccines for animal coronaviruses could be easier to develop, as their regulations are relatively less as compared to human vaccination. As a logical extension, the technologies and strategies used in the preparation of these human vaccines can be readily applied in the development of animal vaccines to protect them from coronavirus. Alternatively, repurposing existing potent vaccines against the coronavirus infections in animals is the most realistic approach.

Future perspectives

In order to overcome the pandemic situation prevailing due to the emergence of viral infections in animals, nanoparticles-based antiviral agents and vaccines were developed and critically evaluated. It is known that traditional bhasma preparations contain certain percentage of nano-sized particles. With the wisdom of ancient ayurveda, the bhasma prepared from certain metals like gold, silver, and copper showed superior immunomodulatory and antiviral properties, although it is not thoroughly explored like conventional antiviral drugs studied against RNA viruses including SARS-CoV-2 (Sarkar and Mukhopadhyay 2021). Similarly, the nanoparticles are also generated by various approaches — top-down and bottom-up methods. However, systematic evaluation and clinical studies are still lacking for most of the nanoparticle preparations. While developing nanoformulations, it is crucial to understand the benefit/risk ratio and safety profile of the formulated drug/vaccine (Alphandéry 2020). Detailed in vitro toxicity, in vivo toxicity, animal pharmacology, and clinical studies are necessary to translate the products from bench to bed side, after passing the statutory and regulatory requirements. Some of the limitations faced by several researchers worldwide are requirement of high-level sophisticated biosafety cabinets and biocontainment facilities, rigorous ethical considerations, and regulatory procedures, choosing an appropriate animal model, educational training, standard operating procedures and skills for handling, and few others.

Conclusions

In this review, the impact of nanotechnology-enabled antiviral formulations to combat the deadly coronavirus infections in animals was presented. Lack of outbreak control and disease eradication plan could be detrimental to human society and global economy. To meet the societal requirements towards managing diseases in economically important animal population, this review summarizes the developments and recent advances in the nanoparticles-based antiviral agents and nanovaccines developed against animal coronaviruses. Different classes of nanomaterials, namely, carbon-based, polymeric, and metal-based, have been established for their virucidal activity against animal coronavirus. Among the nanostructured materials investigated, silver nanoparticles and its composites are found to be the best nano-drug candidate with broad-spectrum antiviral activity against various enveloped animal coronaviruses like FCoV, TGEV, and PEDV. Besides, carbon dots also possess strong inhibitory properties against animal coronavirus which acts by multimodal mechanism of action. As a preventive measure, nanovaccine formulations containing nanoparticles from gold, PLGA, chitosan, and viral protein are established. Here, the nanomaterials form the part of or as a complete adjuvant employed in the preparation of nanovaccines against animal coronaviruses. These nano-adjuvant vaccines induce the defense mechanism in animals by increasing their immunomodulatory properties and antibody response. On the other hand, repurposing the currently available nano-based antiviral drugs and vaccines as a viable treatment strategy can be possibly explored to eradicate the transmission and spread of the coronavirus infections from animals to other vulnerable species.

Declarations

Conflict interest The authors declare no conflicts of interest.

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