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MISIÓN

La Revista de Farmacología de Chile es considerada el órgano oficial de difusión científica y de opinión de la Sociedad de Farmacología de Chile. En un principio esta revista nació como un remozado libro de resúmenes del XVIII Congreso Latinoamericano de la Asociación de Farmacología realizado en Chile el año 2008. Desde 2009 y hasta ahora la Revista de Farmacología de Chile ha recibido varios trabajos originales de investigación y diversas revisiones de temas farmacológicos relevantes. La Revista de Farmacología de Chile aborda temas relacionados con la farmacología básica y experimental, así como investigaciones clínicas. Las áreas temáticas principales son: farmacocinética, farmacodinamia, farmacología cardiovascular, farmacología pulmonar, farmacología endocrina, neurofarmacología, farmacología clínica, estudios preclínicos, estrés oxidativo, fitofarmacología, ciencias farmacéuticas, química-médica y toxicología. También la revista actualmente permite divulgar opiniones sobre los principales temas de salud relacionados con medicamentos en Chile, la presentación de líneas de investigación de laboratorios nacionales en donde se realizan investigaciones farmacológicas, información de curso y programas de postgrados nacionales en farmacología y la publicación de resúmenes científicos del Congreso Anual SOFARCHI.

Audiencia:

La Revista de Farmacología de Chile esta dirigida a farmacólogos nacionales e internacionales interesados en la divulgación de la farmacología. También está dirigida a estudiantes de pregrado de carreras universitarias del área de la salud y ciencias biomédicas, y a estudiantes de postgrado que cursen maestrías y doctorados en farmacología.

Periodicidad:

Se editarán hasta 3 números anuales (Abril, Agosto y Diciembre) en formato digital. El número de Diciembre incluirá trabajos originales y los resúmenes del Congreso Anual de la Sociedad de Farmacología de Chile.

Temas a Publicar:

- Artículos originales en Farmacología Básica, Farmacología Clínica, Farmacoterapia y Toxicología.
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- Artículos de revisión de temas farmacológicos importantes sobre las diversas temáticas de la disciplina.
- Artículos de Información de nuevos fármacos incorporados al arsenal terapéutico nacional.
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- Promoción de actividades académicas, congresos y cursos nacionales e internacionales en farmacología.
- Publicitar las ofertas de trabajo de inserción académica en Universidades Chilenas y extranjeras, así como las oportunidades de inserción laboral en la industria privada ligada al desarrollo de fármacos.

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WCP2023
2 - 7 JULY 2023
GLASGOW | SCOTLAND

Overview

The first World Congress of Pharmacology was held in Stockholm, Sweden in 1961. Two years later the second congress took place in Prague, Czechoslovakia. Until 1990, the congresses were then held every three years, alternating with the world congresses of the closely related sister biomedical unions, the International Union of Biochemistry and Molecular Biology (IUBMB) and the International Union of Physiology (IUPS).

After 1990, the congresses moved to a four-year interval, with the World Conferences of Clinical Pharmacology and Therapeutics (CPT), which were organised by the IUPHAR Division on Clinical Pharmacology, taking place on the even years between the basic science congresses.

As the relationship between the pre-clinical and therapeutic axes of the subject became increasingly important, IUPHAR opted to combine the scientific programs into a single venue. In 2010, the first combined congress took place in Copenhagen during the 16th World Congress of Basic and Clinical Pharmacology (WorldPharma2010).

Congress Selection Process

Upcoming IUPHAR Congresses

WCP2023
2 - 7 JULY 2023
GLASGOW | SCOTLAND

The IXXth World Congress of Basic and Clinical Pharmacology 2023 (WCP2023) will be held July 02 - 07, 2023 (subject to confirmation) in Glasgow, Scotland, United Kingdom. It will be hosted by the British Pharmacological Society. Please visit **wcp2023.org** for additional information.

The XXth World Congress of Basic and Clinical Pharmacology 2026 (WCP2026) will be held in July 2026 in Melbourne, Australia. It will be hosted by the Australasian Society of Clinical and Experimental Pharmacologists and Toxicologists. More information will be posted as it becomes available.

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INTERNATIONAL UNION OF BASIC AND CLINICAL PHARMACOLOGY

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SOCIEDAD DE FARMACOLOGÍA DE CHILE

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REVIEW ARTICLES

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REVIEW ARTICLE

The science behind the development of anti-SARS-CoV-2 vaccines.

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ABSTRACT

The science behind vaccines started almost three centuries ago with the development of the smallpox vaccine and currently they are considered the most potent strategy for saving lives worldwide. The first strategy used to vaccinate was the utilization of attenuated microorganisms-used until nowadays-followed by killed versions of the pathogens. Next, the era of rational design of vaccines started with the use of polysaccharides linked to proteins in order to induce immune memory, followed with the genetic engineering revolution that allowed the production of novel vaccines based on antigenic protein subunits. Without doubt the SARS-CoV-2 pandemic accelerated pre-existing scientific developments made for previous virus that were seen as a threat. The capabilities built to synthesize segments of DNA or RNA were especially relevant, as well as the experience of testing their performance by means of in vivo models, to develop vaccines to deal with the SARS-CoV-2 pandemic at a pace never seen before. All the vaccines currently approved for emergency use are either based on killed SARS-CoV-2 or codify for the Spike protein of the viral surface, either as DNA or RNA. The vaccines were studied in pre-clinical experimental animals from rodents to non-human primates to determine their tolerability, safety, immunogenicity and efficacy. Subsequently, their safety and efficacy were assessed in clinical trials. The vaccines currently approved for emergency use showed to be safe, exhibiting mild to moderate adverse effects, mostly within the expected for a vaccine. Their range of efficacy varied from 62 to 95 %. The interim analysis of their phase III clinical trials, as well as, all the preceding scientific research, allowed them to obtain the emergency use authorization by several regulatory agencies, as well the recognition by the WHO. Time will show, which ones of these vaccines will achieve a regular authorization by the main regulatory agencies of the world.

Keywords: *vaccines, SARS-CoV-2, COVID-19, killed vaccine, mRNA vaccine, adenoviral vector vaccine.*

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1) Introduction

Vaccines have been around for almost three centuries saving more than 7000 deaths a day. Vaccines were and will continue to be the most powerful intervention for world health. In the XX century, smallpox was eradicated, and other infectious diseases were controlled, especially those affecting children and infants (De Gregorio E. & Rappuoli R., 2014).

The first report of immunity to natural infection came from the Plague of Athens (431 bce to 404 bce)(Thucydides, 1989). Then, the first approximation to the current concept of vaccination started in China with the process known as "variolization", that used pustules of people infected, with less aggressive forms of smallpox to inoculate healthy individuals either nasally or into small cutaneous scratches (Needham J.,1980). The breakthrough came with the experiments of Edward Jenner in 1796, who used the pustules of cowpox to inoculate healthy individuals. This represents the first platform used to produce vaccines, which is used until the current days: alive attenuated microorganisms (De Gregorio E. & Rappuoli R., 2014).

A century after the birth of the first vaccine, microbiologists worked over other infectious diseases and developed attenuated versions of the microorganisms under study. Thus, the current version of the BCG vaccines was produced using this strategy (Calmette A. & Guérin C.,1914). Also, passive immunization was discovered on those days using the sera from infected individuals. Next, chemically inactivated toxins were successfully used for vaccination (De Gregorio E. & Rappuoli R., 2014).

For vaccines against viral infections, the development of methods for viral growth within cell cultures was needed. This allowed the emergence of viral attenuated (Sabin A.B. et al., 1954) and inactivated (Salk J.E. et al, 1954) polio vaccines in the 1950´s. Followed by measles, mumps and rubella in the next decade. The latter and others of more recent appearance like varicella zoster, rotavirus and influenza are used nowadays (De Gregorio E. & Rappuoli R., 2014).

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Next in development, came polysaccharide vaccines that marked the switch from an empirical approach to a rational design by conjugating protein to the polysaccharides in Porder to trigger immunological memory (De Gregorio E. & Rappuoli R., 2014). Then, the revolution of DNA recombinant technology flourished, and a next-generation vaccines emerged, which were built from an antigenic protein of the microorganisms assembled in a virus like particle (VLP). This strategy is utilized for the currently used Hepatitis B virus and Human Papilloma Virus vaccines (Valenzuela P. et al., 1982; De Gregorio E. and Rappuoli R., 2014).

In 2013, the first genome-based vaccine was developed and licensed in Europe, Australia and Canada for meningococcus serotype B strain. This paved the way to a new era for vaccines in which the genomic information gave a powerful microbial-antigen discovery tool (De Gregorio E. & Rappuoli, R. 2014). The latter was further strengthened by the advances in techniques supporting three-dimensional study of antigenic proteins, that allowed to design vaccines based on proteins or segment of them, by modifying their structure in order to make them more immunogenic (Dormitzer P. R., 2008). Next, the materialization of capabilities for the *in vitro* synthesis of large segments of DNA and RNA marked and other advancement towards the rational vaccine design. The first experience with this synthetic biology approach was for the vaccine designed for the avian influenza virus H7N9, that allowed the production of a subunit vaccine which was tested in Phase I trials and showed immunogenicity (Bart S.A., 2014). In addition, for the same virus, an mRNA nano lipid-encapsulated vaccine was ready to inoculate experimental animals eight days after the virus was first reported, and neutralizing antibody production was reported three weeks after the second immunization (Geall A.J. et al 2012; Hekele A., 2013). These technologies accelerated the development of vaccines in preparedness for a potential pandemic.

2) Anti-SARS-CoV-2 vaccines

The pandemic caused by SARS-CoV-2 was officially declared by WHO on March 11 2020 (Cucinotta D. & Vanelli M., 2020). Immediately, the race for a vaccine started against the clock, with the shared participation of very well-known and trusted strategies, like killed virus and subunits vaccines, with novel and never licensed platforms like mRNA and DNA based vaccines. In the next sections, we discuss the main platforms that have been authorized for emergency use to date, that had to demonstrate quality, safety, efficacy, immunogenicity and efficacy. Before, it is important to recall the fundamental function of the Spike protein (S) from SARS-CoV-2 that allows the attachment and entry of the virus to the target cells through the recognition of the angiotensin-converting enzyme 2 (ACE-2) receptor. The later has made the S protein an attractive target to develop vaccines anti SARS-CoV-2, being known that its S1 subunit contains the receptor binding domain (RBD) that recognizes ACE-2, and the S2 subunit contains the cleavage site required for the virus to fuse with the target cells (Chung J. Y.& Thone M. N., 2021). The conception of the S protein as a valuable antigenic target for vaccine design and neutralizing antibodies induction was possible because of the previous experiments conducted with the predecessors coronaviruses; SARS-CoV-2 and MERS (Mercado N.B. et al., 2020a).

2.1. Killed virus:

This platform is one of the most currently used, being easy to produce by growing the virus within mammalian cell cultures, like Vero cells and then, using heat, formaldehyde or ß propiolactone to inactivate them. They are safer than attenuated virus, since they cannot reproduce and nonetheless, they have all the antigens of the original virus to induce the immune response. However, their incapability to replicate reduces the immunogenicity and adds the requirement of adjuvants and boosts (Chung J. Y. & Thone M.N., 2021). The two approved SARS-Cov-2 inactivated vaccines, were adsorbed with $Al(OH)_3$ and produced in Vero cells (Wang H. et al., 2020).

The Sinopharm COVID-19 (BBIBP-CorV) vaccine was granted WHO approval on May 7th, 2021. The analysis included quality, safety and efficacy, as well as risk managements plans and programmatic suitability, such as cold chain requirements. In addition, the evaluation included the on-site inspection of the production facilities (WHO, 2021a). In addition, it is approved for emergency use in 53 countries (Moodie, n.d.). The first immunogenicity studies were performed in Balbc mice at 2, 4 or 8 µg/dose, finding 100% seroconversion at day 7. Also, a 0/7, 0/14 and 0/21 schedule of immunization was tested finding that the middle and high dose at 0/21 showed the highest level of neutralizing antibodies at 7 days after the second injection. Next, the immunogenicity was demonstrated in rats, Guinea pigs, rabbits and cynomolgus monkeys, obtaining similar results. Then, rhesus macaques immunized with the low or high dose at a 0/14 scheme were challenged intratracheally with SARS-CoV-2, ten days after the last dose. A reduction in viral detection by PCR was shown by throat and anal swab analysis. In addition, acute toxicity experiments were performed with Sprague-Dawley rats, injecting BBIBP-CorV at 24 µg/rat. No macroscopic or histopathological changes in comparison to control rats were observed. Guinea pigs were used for anaphylaxis evaluation, finding no signs of allergic reactions. Finally, long term toxicology studies were performed with Cynomolgus monkeys, subjected to four weekly injections of the three doses. The gross

anatomy and histopathology showed no differences with control animals injected with physiological solution, but granulomatous inflammation was observed for all doses with a trend to diminish at day 25 after injection (Wang H., et al., 2020). The BBIBP-CorV vaccine was then studied in a phase I trial enrolling healthy volunteers of 18-80 years old, receiving 0, 2, 4 or 8 µg protein on a 0/28 days immunization schedule. Fever was the most frequent systemic adverse reaction, being all the effects mild or moderate. In patients of the 18-59 years group, seroconversion was observed starting at day 7 for the three doses tested, however for the participants aged 60 years and older, seroconversion was detected at day 7 for the 4 and 8 µg doses and at day 14 for the 2µg dose. Next, a phase II trial was conducted with 448 participants, randomly assigned to four immunization schedules: the 4µg dose was tested at three immunizations schedules (0/14; 0/21 or 0/28) while the 8µg dose was used in a single dose scheme. Each schedule had a placebo counterpart. The most common side effects were pain at the site of injection and fever. Neutralizing antibodies were detected after 28 days of the last inoculation in all the immunization schedules tested (Xia S. et al., 2021). To date, phase III clinical trials data for this vaccine are not published.

The other vaccine based in killed virus is Coronavac (Sinovac) that has been approved for emergency use in 29 countries (Moodie, n.d.); it was recently approved by WHO (WHO, 2021b) and since May 4, 2021 is being evaluated by EMA (EMA, 2021). At the preclinical in vivo assays, Balb/c mice and Wistar rats were used to assess immunogenicity for 0, 1.5, 3, or 6 mg per dose and a 0/7 schedule. Anti-S, anti-RBD and anti N-specific antibodies were determined at week one and six after immunization. The highest titers were found at week six for the concentration of the Anti-S and Anti-RBD specific antibodies. On the other hand, neutralizing antibodies for both tested species and several strains, circulating by the time these assays were performed, were found at week 7. Next, immunogenicity and efficacy were assessed in rhesus macaques which were immunized in a 0/7/14 either with 3µg or 6 µg dose. In both immunization schemes antibodies anti-S and anti-RBD were observed reaching a maximum at week three. Then, the animals were challenged with the virus and evaluated by histopathological analysis of lung tissue and PCR from throat and anal swabs to detect viral mRNA. Lungs of infected macaques showed slight and focal histopathological changes in a few lobes of the lungs as opposed to control group that exhibited severe interstitial pneumonia. The highest evaluated dose for inoculation resulted in no detectable viral copies in the pharynx, crissum, or lung. Another study performed in macaques using a low and medium dose (1,5 and 6 µg) determined that the vaccine did not produce fever, weight loss, lack of appetite or changes in mental health, hematological and biochemical parameters (Gao, Q. et al, 2020).

The Coronavac vaccines was analyzed first in a phase I/II trial recruiting healthy adults aged 18-50 years. Two doses were studied, 3 or 6 µg/dose against placebo and two inoculation schemes 0/14 or 0/28, both in phase I and II. In the phase I study, 144 participants were enrolled, and their most common adverse effect was pain in the site of injection. Only one case of acute hypersensitivity was reported in the 6 µg/dose group and all the adverse events were mild in severity and disappeared within 48 h. At 28 days post second dose, the seroconversion of neutralizing antibodies in the 0/14 schedules were 25%, 83% and 0% for the 3, 6 and 0 µg/dose groups, respectively. On the other hand, for the 0/28 schedule, the seroconversion of neutralizing antibodies at 28 days after the second dose was 83%, 79%, 4 % for the 3, 6 and 0 µg/dose groups, respectively. The phase II, enrolled 448 participants, finding pain in the site of injection as the most common effect, with no serious vaccine-related side effect reported within 28 days of the second injection. With regard to neutralizing antibodies to live SARS-CoV-2, they were higher, above 90%, in the participants receiving the vaccines compared with the placebo group for all the schedules and doses (Zhang, Y. et al., 2021). On table 1, a summary of the phase 3 design for Coronavac (Sinovac) is shown. The phase 3 trial performed in Brazil was presented to WHO and showed an efficacy of 51% in prevention of symptomatic disease, after a 0/14 vaccination schedule, 14 days after receiving the second dose (WHO, 2021b). The results of the study performed in Turkey were published and showed, in consistency with previous Phase I/II, that the most frequent local adverse event was pain in the site of injection and the most common systemic effect was fatigue. The calculated efficacy to prevent PCR-confirmed symptomatic infection was 83,5% (Tanriover M.D. et al., 2021). Interestingly, an effectiveness prospective study, performed in Chilean population, showed a 65,9% effectiveness to prevent symptomatic infection, 87,5% for the prevention of hospitalization, 90,3% for the prevention of Intensive Care Unit admission and 86,3% for prevention of COVID-19 associated death (Jara A. et al., 2021).

2.2. Nucleic acid

2.2.1 mRNA: The use of mRNA is well suited for rapid manufacture and modification of the immunogen, which may explain their early appearance during the pandemic (Pardi N. et al., 2018). The Moderna vaccine mRNA-1273, codifies for the prefusion S stabilized protein and it is encapsulated within a lipid nanoparticle. It is currently approved for emergency use in 53 countries and listed by WHO for emergency use (Moodie, n.d.). Studies performed in multiple mice strains showed that this

vaccine elicited robust binding and pseudovirus neutralizing antibodies with two injections three weeks apart. The immunized animals and controls were challenged with SARS-CoV-2 adapted to infect mice and a dose-dependent protection was found (Corbett K.S. et al., 2020). Next, studies in non-human primates were performed using Indian-origin rhesus macaques that were immunized with 10, 100 µg mRNA-1273 or phosphate buffer saline on a 0/4 week schedule. At 4 weeks after the last dose, they were virally challenged by intratracheal and intranasal route. Antibodies to the conformationally defined S prefusion protein were found in macaques immunized with low and high doses, as well as neutralizing antibodies against a pseudovirus. There was an increase in antibodies titers four weeks after the second vaccination and this was dose dependent. The antibody titers were higher than the ones found in convalescent-phase serum specimens. In addition, four weeks after the second immunization the macaques exhibited Th1 immune response, which were higher in the 100µg group. Then, in the viral challenge experiments, it was shown that overall levels of sub-genomic viral RNA in BAL fluid and nasal swab specimens were statistically lower in the immunized groups than in the non-immunized group. This was demonstrated for the 10 and 100µg group. For the group of animals that received 100µg of mRNA-1273 no signs of lung inflammation, viral mRNA or antigen were detected in any of them (Corbett K.S. et al., 2020). The clinical phase II for this mRNA vaccine was performed enrolling 300 participants of 18-55 years, who were randomly assigned to groups that received doses of 25, 50, 100 or 250 µg and 300 participants of 56-70 years receiving the 25, 50 or 100 µg doses. In both age cohort the most frequent adverse effects were pain at the site of injection, headache and fatigue. Binding and neutralizing antibodies against SARS-Cov-2 at day 14 exceeded the levels observed in convalescent sera of people recovered from COVID-19 (Chu L. et al., 2020). For the phase III trial 30.420 volunteers were randomly assigned to receive the vaccine at 100 µg or placebo in a 0/28 schedule (on Table 1 the details of the design can be observed). The efficacy to prevent illness, including severe disease was determined as 94,1%, showing no important differences through key secondary analysis such as analysis at 14 days after the first dose, previous SARS-CoV-2 infections or age group above 65 years and older. The efficacy of the mRNA-1273 vaccines to prevent serious COVID-19 was informed as 100%. Local adverse effects were more frequent within the patients that received the vaccines in comparison to placebo, being pain at the site of injection reported in more than 75% of the participants. Headache, fatigue and myalgia were the most frequent systemic adverse effects observed in more than 50% of the volunteers, being more frequent after the second dose (Baden L.R. et al., 2021).

The Pfizer/Biontech BNT162b mRNA vaccine encodes the full-length transmembrane S protein on its prefusion state encapsulated in lipid nanoparticles. It has been approved for emergency use in 88 countries, the Caribbean Regulatory System Emergency Use Recommendations and it is listed by WHO for emergency use (Moodie, n.d.). The mice preclinical studies showed that a single dose of BNT162b mRNA at 0,1, 1 or 5 µg stimulated IgG antibody production against S1 and the RBD domain in a dose-dependent manner. In addition, splenic CD4+ and CD8+ IFN-γ producing lymphocytes were detected, exhibiting a Th1 phenotype after stimulation with S protein. Next, a study was performed with non-human primates injected with saline, 30 or 100 µg of BNT162b mRNA in a 0/21 schedule. There were detectable RBD-binding antibodies by day 14 after the first dose and they further increased at day 7 after the second dose. There was a strong boosting effect on the antibody's titers after the second dose. A CD4+ cell induction was observed accompanied of IFN-γ, IL-2 or TNF production. Also, CD8+ lymphocytes that produced IFN-γ were detected. Next, the macaques were virally challenged intratracheally and intranasally, after 41 to 55 days of immunization. There was no viral mRNA detection of the virus from the BAL or nasal swabs of immunized animals at any time. The phase I clinical trials for these vaccines were performed with two groups of healthy volunteers, 18-55 and 65-85 years old. At this stage the volunteers were assigned to placebo or one of two vaccines: BNT162b1 or BNT162b2, the first encoded for a secreted trimerized RBD and the second for a membrane-anchored full length spike protein stabilized on its prefusion state. Three doses were tested, 10, 20, 30 and 100 µg and the schedule of administration was 0/21 and one group received a single dose. This trial´s results, supported the advancement to the next steps with the BNT162b2 mRNA, because it exhibited lower frequency and severity of systemic adverse effects, especially in older population (Walsh E.E. et al., 2020). In addition, a phase I/II trial performed in Germany arrived to the same conclusion, as the study cited before plus a superior cellular immune response based on CD4+ and CD8+ responses specific to SARS-CoV-2 epitopes, including RBD (Khehra N. et al., 2021). Next the phase II/III trial was performed, and the detail of its design is shown in Table 1. The calculated efficacy was 95% with no relevant differences among several groups such as age, sex, ethnicity, obesity and elderly. The most frequent local side effect of this vaccine was pain on the site of injection and none of the effects achieved grade 4. The systemic side effects more frequently seen included headache and fatigue. Fever also appeared after the second dose and lasted no more than two days (Polack F.P. et al., 2020). A phase III trial was later conducted to test the safety and efficacy of this vaccine in 2260 adolescents aged 12 to 15 years. This study showed an efficacy of 100% with a robust

immune response. No major differences in adverse effects were reported in comparison with the 16-25 years group (Khehra N. et al., 2021). After the real-world use of this vaccine, it has been informed that in the UK, the first dose prevented 61% of symptomatic disease and 80% of deaths by COVID-19 in people aged 70 and over. After two doses the protection for the same age group raised to 85-90% (Iacobucci, G., 2021).

2.2.2 DNA: All the emergency-approved vaccines that deliver DNA coding the gene for the S protein are delivered using adenoviral vectors (Chung J.Y. & Thone, M.N., 2021). The first one achieving approval was developed by the University of Oxford and the pharmaceutical company Astrazeneca. The ChAdOx1 nCOVID-19 vaccine (AZD1222) contains the S codifying gene within the ChAdOx1 simian replicative-incompetent simian adenovirus. This vaccine has been approved for emergency use in 110 countries, it is endorsed by the Africa Regulatory Taskforce, is recommended by the Caribbean Regulatory System and listed by the WHO for emergency use (Moodie, n.d.). The first *in vivo* preclinical assay was performed using two mice strains, Balb/c and CD1. As a control, a Green Fluorescent Protein containing ChAdOx1 virus was used (ChadOx1GFP). This was a one-shot schedule and both IgG antibodies against S protein and virus antibodies were detected on days 9-14 after immunization. In addition, a cellular Th1 response was found. Next, immunogenicity and efficacy were studied on rhesus macaques. A prime-only or a prime-boost regimen were examined, using the ChadOx1GFP as a control group. No adverse effects were observed, and IgG anti-S antibodies were detected by day 14, however a significant increase was observed after the boost. Neutralizing anti SARS-CoV-2 antibodies were also higher after the second administration. Then, the animals were challenged with 2,6 x10^6 units of SARS-CoV-2 at the upper and lower respiratory tracts of rhesus macaques. The clinical outcome was worse in control animals compared with the immunized ones. Infectious virus was detected in prime vaccinated animals at day 1 and 3 post infection in controls and prime vaccinated groups but only on day 1 post infection in prime-boost animals. Histopathological data from the lungs of euthanized macaques showed that the vaccinated animals exhibited neither signs of viral pneumonia or immune-enhanced inflammatory disease, nor viral antigens. These signs were present in the lungs of control animals. In addition, high level of genomic RNA within the lungs of the placebo group was found, and in the prime group it was found to be significantly lower compared to the control group. However, within the prime-boost group it was found that two out of six animals showed the viral genome (van Doremalen N. et al., 2020). The phase I/II study performed in the UK enrolled 1077 healthy volunteers aged 18-55 years. They were immunized with either 5x 10^{10} viral particles or a placebo consisting of a meningococcal conjugated vaccine, in a single dose schedule, with the exception of 10 unblinded participants who received a 1/28 prime-boost schedule. There were no serious adverse reactions reported and the most common local or systemic observed effects included fever, pain, chills, muscle ache, headache and malaise. T cell response was detected at day 14 and antibodies anti S protein raised by day 28 and increased with the boost. Neutralizing antibodies correlated with anti S protein IgG measured by ELISA (Folegatti P.M. et al., 2020). These results allowed to advance to safety and efficacy trials, whose arrangement details are shown in Table I. The interim analysis, including participants in UK and Brazil mainly aged 18-55 years, showed an efficacy of 62,1% for participants that received two standard doses. In safety aspects, control and vaccinated patients presented adverse effects (89 vs 79, respectively), including a case of transverse myelitis found 14 days after a booster and found possibly related to the ChAdOx1 nCOVID-19 vaccine and the independent neurological committee classified it as an idiopathic, short segment, spinal cord demyelination (Voysey M. et al., 2021). Other cases of transverse myelitis were discarded as related to the vaccine. It is interesting to specify that at this point no findings of vaccine-induced thrombotic thrombocytopenia were reported. Afterwards, when the vaccine was in massive use in Europe, a safety signal emerged, notifying that, otherwise healthy individuals, developed thrombocytopenia and thrombosis in unusual locations such as cerebral and/or splanchnic veins. The frequency of this events is low, approximately 7-10 per million people that received the vaccine. Currently, it is understood as an autoimmune reaction, associated with antibodies against platelet factor 4, resembling a variant of autoimmune heparin-induced thrombocytopenia (Arepally G.M. and Ortel T.L., 2021).

The Gamaleya Institute developed the Gam-COVID-Vac, also known as Sputnik V, provisionally approved in Russia after the end of phase II clinical assays (Logunov D.Y. et al., 2020). It is composed of two adenoviral vectors, rAd type 26 and rAd type5, each containing the full-length S protein. This heterologous approach minimizes the immune response against vector components, that are often seen with this platform, and that might attenuate the efficacy of viral vector-based vaccines, however, no publications were found describing the preclinical assays made before clinical trials. Two phase I/II non-randomized assays were performed in hospitals in Russia, in both volunteers were 18-60 years old. For the Phase I, the participants were inoculated on day cero either with one dose of rAd26-S or one dose of rAd5-S and safety was followed 28 days. Next, phase II started with a prime-boost scheme of 0/21 days, in which first rAd type 26 was administered intramuscularly and then, at day 21 rAd type5 was inoculated by the same

route. Most of the adverse effects were mild like pain on the site of injection, fever, headache, asthenia, muscle and joint pain; and no serious adverse effects were reported. Antibodies to the S protein and neutralizing antibodies increased significantly at day 14 and during the follow period (day 42) they continued increasing. The cellular antigen-specific immune response to the virus was detected as CD4+ and CD8+ proliferating cells and IFN-γ production. This cellular response peaked at day 28 post vaccination (Logunov D.Y. et al., 2020). Subsequently, a phase III clinical assay interim analysis was published, and details of its design are presented in Table 1. The results showed an efficacy in preventing PCR-confirmed infection of 91,6%. No serious adverse effects were associated to the vaccine and the most common adverse effects were flu-like symptoms, injection site reactions, headache and asthenia. The level of seroconversion for neutralizing antibodies was 95-83% for the vaccinated group. The levels of neutralizing antibodies were comparable among ages and gender. Secretion of IFN-γ, as a measure of cellular immune response upon *in vitro* exposure to S protein, was shown to be significatively elevated at day 28 post inoculation in vaccinated participants compared to their levels at day 0 (Logunov D.Y. et al, 2021).

Cansino Biologicals, on the other hand, developed Convidencia, a non-replicating adenovirus type-5 (Ad5)-vectored COVID-19 vaccine, that codifies for the spike protein (Jain S. et al., 2020). It is approved in Chile, China, Hungary, Mexico and Pakistan (Moodie, n.d.). The non-clinical assays were not published. A first-in-human trial was performed recruiting healthy volunteers aged 18-60 years, which were administered the vaccine intramuscularly at $5x10^{10}$, $1x10^{11}$ or $1,5x10^{11}$ viral particles per inoculation. The most common local adverse reaction was pain on the site of injection and the most common systemic reaction was fever, fatigue, headache and muscle pain. Humoral response, detected by ELISA or neutralizing antibodies peaked at day 28 after vaccination and specific T cellular immune response peaked at day 14 (Zhu F.C. et al, 2020a). These results encouraged the advancement of the research to a phase II trial performed in a single center in Wuhan, China. It recruited participants aged 18 and older and they were assigned to receive a single dose of $1x10^{11}$ viral particles/mL, $5x10^{10}$ viral particles/mL or placebo. In the groups that received the vaccine, the seroconversion rates were 95% and 96%, for the highest and lowest dose groups, respectively. Neutralizing antibodies were detected in both dose groups. In addition, IFN-γ was found in 90% and 88 % of the volunteers that received the $1x10^{11}$ viral particles/mL and $5x10^{10}$ viral particles/mL, respectively. No serious adverse events were reported (Zhu F.C. et al., 2020b). The phase III interim analysis that allowed the approval of this vaccine has still not been published.

Finally, Johnson & Johnson provided Jansen (Ad26.COV2.S), an adenoviral vector-based DNA vaccine, using the Adenovirus serotype 26 in an replicative incompetent version. It is approved in 49 countries, WHO and the Africa Regulatory Taskforce (ART) (Moodie, n.d.). This vaccine codifies the full length, stabilized S protein and it is administered as a single dose. The efficacy preclinical assays performed in Syrian golden hamsters treated with $1x10^{9}$ or $1x10^{10}$ viral particles, or sham immunized showed the development of an immune response after a single dose was administered by intramuscular route. The response included RBD specific antibodies and neutralizing antibodies at week 2 and 4, with both doses having comparable results. Then, the animals were challenged with SARS-CoV-2 intranasally. Vaccinated animals had less weight loss and were protected against mortality compared to sham immunized counterparts. In addition, tissue viral load was significantly lower or not detected in animals receiving the viral vector-ADN vaccine. Finally, the pathological abnormalities in the lung tissue examined were reduced in the immunized animal, showing prevention of severe clinical condition (Tostanoski L.H. et al., 2020). Studies in non- human primates were performed with rhesus macaques immunized intramuscularly with a single dose of $1x10^{11}$ viral particles. At week 4 all the macaques exhibited anti RBD domain neutralizing antibodies, as well as specific anti-S IgG and IgA in bronchoalveolar lavage fluid. Also, a Th1 and T cytotoxic cell response associated with IFN-γ production was found, showing the activation of cellular immune response. Then, the animals were SARS-CoV-2 challenged, showing robust protection evidenced as an indetectable or very low viral load (Mercado N.B. et al., 2020a; Mercado, N.B. et al., 2020b). Subsequently, a phase I/IIa multicentric trial was performed, in which the Ad26.COV2.S vaccine was assayed in a cohort 1, consisting of 18-55 years volunteers which was split in cohort 1a (larger enrollment) and 1b (shorter enrollment for in depth analysis of immunogenicity). A cohort 2 was included for long term comparison of single dose versus a two-dose regimen (data not published to date). On the other hand, cohort 3 enrolled participants over 65 years old. Cohort 1 and 3 received the vaccine at low dose ($5x10^{10}$ viral particles/mL), high dose ($1x10^{11}$ viral particles/mL) in either single dose or a 0/56 regimen. There were, in each cohort five subgroups equally distributed (1:1:1:1:1), receiving low dose/low dose, low dose/placebo, high dose/high dose, high dose/placebo and placebo/placebo.

The most frequent local adverse event was pain at the site of injection and the most frequent systemic adverse effects were fatigue, headache and myalgia. No severe adverse effects were reported, and no participants discontinued the trial because of an adverse event. In all the groups of cohort 1, the seroconversion for antibodies against the full-

length S protein was 99% by day 29 after vaccination. On the other hand, cohort 3 participants showed at day 29 after vaccination, a 96% seroconversion. With regard to neutralizing antibodies, measured in a random group from cohorts 1a and 3 at day 29, it was observed a seroconversion of 88-96% in both groups. At day 15, Th1 specific CD4+ cells for S peptides were found in 76% of the low dose and 83% of high dose recipients from cohort 1a, the respective values for cohort 3 were, 60% and 67%. The S-specific CD8+ response at day 15, was detected in 51% of the low dose and 64% of the participants in cohort 1a, in comparison with 36% and 24 % in the low and high dose group, respectively, of cohort 3 volunteers. These results supported the advancement towards the development of two phase III trials to determine the efficacy of the lower dose of the vaccine in either a single dose or a two dose schedule (Sadoff J. et al., 2021). The phase III trial using a single dose of Ad26.COV2.S (5×10^{10} viral particles) was published and the details of its design are shown it Table 1. Reactogenicity was mild to moderate and transient. Numeric imbalances were reported for certain adverse effects in the vaccinated group, for instance venous thrombotic events (patients with predisposing factors), seizure and tinnitus, however a causal relationship with the vaccine could not be established. The efficacy of the vaccine to prevent symptomatic cases of COVID-19, 14 days after the inoculation was 66,9%, and after 28 days, it was 66,1%. This vaccine has also shown rare vaccine-induced immune thrombotic thrombocytopenic events when applied in emergency use to the general population (Arepally G.M. & Ortel T.L., 2021).

Table 1. Clinical trials performed for vaccines with emergency approval.

Platform	**Killed virus**	**Nucleic Acids**					
		RNA		**DNA**			
Vaccine	**CoronaVac (Sinovac)**	**Moderna** mRNA-1273	**Pfizer/ Biontech** BNT162b	**AstraZeneca** (AZD1222)	**Sputnik V** (Gamaleya Institute)	**Convidecia** (CanSino Biologicals)	**Ad26.COV2.S** Johnson & Johnson/Jansen
Participants randomization	Vaccine or placebo at a 1: 1 ratio. Double-blind 11800 adults (18–59 years old), and 1,260 elderlies (≥60), 14 or 28 days apart. (Brazil Phase 3 Clinical Trial). Placebo: aluminum hydroxide in a 0.5 mL solution.	30420 (1:1) Included: 14134 vaccine 14073 placebo. (Baden et al, 2021)	21,720 (18,556) BNT162b2 21,728 (18,530) placebo	5089 ChAdOx1 nCoV-19 5129 MenACWY as control.	Phase 3: General safety analysis: 9528 vaccine 3038 placebo Immunogenicity analysis: (vaccine vs placebo) 342 vs 114 receptor binding domain IgG 72 vs 28 neutralizing antibodies 44 vs 14 IFN γ Five age strata (18-30; 31-40; 41-50; 51-60 and > 60 years (Logunov et al, 2021)	Phase 3 not published. Phase 2: Adults between 18 and 60 years. Phase 2 vaccines of 1 × 10^{11} and 5 × 10^{10} viral particles and placebo; randomized at a 2:1:1 ratio.	Phase3: Participants 18 to 59 years of age than by those 60 years of age or older. 19,630 received Ad26.COV2.S and 19,691 received placebo (Sadoff et al. 2021b).
Authorized Schedule	Two doses of 3 µg/0.5 mL (equivalent to 600 SU per dose) of inactivated SARS-CoV-2 virus, and aluminum hydroxide as adjuvant, 28 days apart.	Two doses of mRNA-1273, 100 µg, 28 days apart	Two doses of BNT162b 30 µg, 21 days apart	Two doses of ChAdOx1 nCoV-19, 5 × 10^{10} viral particles, 28 days apart.	First dose of rAd26-S and second dose of rAd5, both of 10^{11} viral particles, 21 days apart. (Logunov et al, 2021)	single injection of 1×10^{11} viral particles	Single dose of Ad26.COV2.S at 5×10^{10} viral particles.
Safety Monitoring Parameters/ Frequency	Solicited and unsolicited local and systemic adverse reactions in the first 7 days after vaccination. Unsolicited local and systemic adverse reactions up to 28 days after the second dose.	Solicited local and systemic adverse events for 7 days after each injection Unsolicited adverse reactions for 28 days after each injection (Baden et al, 2021).	Registration in an electronic diary: Protocol-defined solicited local and systemic reactions or use of pain relief medication 7 days after each dose. Unsolicited adverse events through 1 month after the second dose. 3)Serious adverse events through 6 months after the second dose.	Registration in a diary card: Protocol-defined solicited local and systemic adverse reactions for 7 days after each dose. Serious adverse events following-up period of 1 year after the last dose.	Observation visits at days 28, 42 and 180. Participants reports of sign or symptoms of possible adverse effects to staff members or electronic diaries. Incidence and severity of adverse events. (Logunov et al, 2021)	Adverse events 14 and 28, and month 6 post vaccination	Daily reported solicited adverse events by subpopulation included 3356 participants, during the 7-day period after vaccine or placebo.
Efficacy Monitoring Parameters/ Frequency	Cellular immune response was assessed using a specific T-cell assays (IFN-γ, IL-6, IL2 and TNF- α) at 14 days after vaccination. Seroconversion rate 14 days after each dose. SARS-CoV-2 specific T cells after vaccination, (Cytometry and ELISPOT)/weekly since first up to 4 weeks after second dose. SARS-CoV2 neutralizing antibodies by neutralization test on the first dose day and at 42 (completed) and 56, 70, 168 and 224 days after the first dose (in course). Protection rate of a two-dose of SARS-CoV-2 vaccine against rates of hospitalization, disease severity/and death two weeks after the second dose of vaccination.	In Phase 2 Seroconversion and level of SARS-CoV-2 spike glycoprotein-specific binding antibody (bAb), on days 1, 29, 43, 57, 209, and 394 (Chu et al, 2021). In Phase 3, PCR-SARS-CoV2 after 2 symptoms.	Phase 2 (Walsh et al, 2020) Immunogenicity assessments (SARS-CoV-2 serum neutralization assay and receptor-binding domain [RBD]–binding or S1-binding IgG, at 7 days and 21 days after the first dose, and at 7 days and 14 days after the second dose.	Search of asymptomatic infections: (Phase2/3) In the COV002-UK group (N=4807), by weekly self-administered nose and throat swab for NAAT testing from 1 week after first vaccination. Immunogenicity (Phase 2): Spike protein T-cell responses and Anti-spike IgG Anti-RBD-IgG at 0,7,14 y 28 days after each dose.	Proportion of participants with PCR confirmed COVID-19, 21 days after the first dose (primary outcome). Severity of COVID-19, antibodies anti-S, anti-N, neutralizing antibodies, specific cellular immunity (secondary outcomes) (Logunov et al, 2021)	Phase 2: RBD-specific ELISA antibody Responses, at day 14 and 28 (Zhu et al.,2020).	Neutralizing-antibody, at baseline and at day 29 after vaccination in all the Phase 2 participants and on days 57 and 71 (Sadoff et al. 2021a). First occurrence of centrally confirmed moderate to severe–critical Covid-19 with an onset at least 14 days after administration and at least 28 days vaccine or placebo.

MenACWY : meningococcal group A, C, W, and Y conjugate vaccine. **NAAT:** Nucleic Acid Amplification Test. **RBD:** Receptor Binding Domain.

3) Conclusion

This pandemic catalyzed, in a speed that nobody would have envisioned, the pendant scientific developments required to put novel vaccines platforms available. Currently, coexists, the more conventional full-killed virus with the very novel nucleic acids vaccines. Time will tell which ones will advance to become licensed as pharmaceutical products and prevail on time.

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REVIEW ARTICLE

Physiopathology and clinical implications of acute respiratory failure by SARS-CoV-2 infection in obese patients: endothelial dysfunction and preliminary pharmacological therapies.

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ABSTRACT

Coronavirus disease 2019 (COVID-19) due to CoV-2 (coronavirus type 2) virus possess a particular risk of developing acute respiratory distress syndrome (ARDS) or SARS (severe acute respiratory syndrome coronavirus 2)-CoV2 in people with preexisting conditions related with endothelial dysfunction and increased pro-inflammatory state. In between these conditions, chronic systemic inflammation related to obese patients, is associated with the development of atherosclerosis, type 2 diabetes, and hypertension, comorbidities that adversely affect the clinical outcome in critical patients with COVID-19. Obesity affects up to a 40% of the general population in USA and more than 30% of the adult population in Chile. Until Julio (2021), 1,600,000 people have been infected, with 34,000 deaths. Given the coexistence of this worldwide obesity epidemic, COVID-19 negative outcomes are seriously enhanced in the current scenario. On the other hand, obesity is characterized by endothelial dysfunction observed in different vascular beds, an alteration which can be associated by impaired vasodilation, oxidative stress, and inflammatory events. Emerging evidence shows that obesity-related conditions such as endothelial dysfunction is associated with detrimental outcomes for COVID-19 evolution, especially if the patient derives to intensive care units (ICU). This implies the need to understand the pathophysiology of the infection in obese population, in order to propose therapeutic alternatives and public health policies, especially if the virus remains in the population. In this review we summarize evidence about the pathogeny of Cov-2 infection in obese individual and discuss how obesity associated inflammatory. Finally, comment the current pharmacological treatments, that are based on the reduction of the pulmonary inflammatory response such as some corticosteroids, antimalarial drugs or antibiotics. In addition to refer the use of agents such as antivirals and the agents that blocking of the effect of cytokines such as anti-IL6.

Keywords: *obesity, COVID-19 severity, endothelial dysfunction, IL-6, obesity, corticosteroids.*

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1) Introduction

In Chile, the number of infected patients with Coronavirus disease 2019 (COVID-19) reached 1.600.000 in July (2021) with more than 34.000 deaths, threatening the health system collapse due to the increasing demand for ICU. Emerging evidence suggests that obesity related conditions seem to worsen the effect of the virus respiratory infection, especially at ICU admission. Studies from Chinese cohorts of patients with COVID-19 have identified several risk factors for severe COVID-19 including age, hypertension, type 2 diabetes, and cardiomyopathy and ischemic heart disease [Zhengtong and Shubin, 2021]. However, obesity as a risk factor for detrimental outcome in patients with COVID-19, has not been fully evaluated.

The experimental and clinical evidence indicates that obesity-induced endothelial dysfunction, with a simultaneous up-regulation of pro-inflammatory cytokines.

Also, oxidative stress is a deleterious factor that systemically increases during obesity and promotes the development of diabetes, atherosclerosis, and endothelial dysfunction. In this view, some studies show higher levels of oxidative markers in obese patients with these risk factors [Duică et al., 2021, Hamed et al., 2011]. Based on this evidence, we propose that the endothelial dysfunction degree in obese patients with COVID-19 will determine at admission of ICU, a detrimental clinical evolution and poor prognosis. Therefore, the study of the pro-inflammatory profile and the level of oxidant stress in plasma of these patients during respiratory infection by SARS Cov-2 are highly relevant for an assertive prognosis, therapy and eventual post-discharge management [Moin et al., 2021].

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The systemic and molecular pathophysiological mechanisms that explain the interaction between obesity and severity due to SARS-CoV2 infection are varied; this review attempts to provide a overview regarding pathophysiology of SARS-CoV-2 pulmonary infection and relationship with endothelial dysfunction. Finally, will described some pharmacological therapies that are in using at clinical practice.

2) Pathophysiology of lung injury induced by SARS CoV-2 infection

Infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in humans is associated with a broad spectrum of clinical respiratory syndromes, ranging from mild upper airway symptoms to progressive life-threatening viral pneumonia [Puri et al., 2020]. Clinically, patients with severe COVID-19 have labored breathing and progressive hypoxemia and often need mechanical ventilatory support. Radiographically, peripheral lung
ground-glass opacities on computed tomographic (CT) imaging of the chest fulfill the Berlin criteria for ARDS [Thompson, 2017]. Histologically, the hallmark of the early phase of ARDS is a diffuse alveolar damage with edema, hemorrhage, and intra-alveolar fibrin deposition [Peñuelas et al. 2006]. Diffuse alveolar damage is a nonspecific finding, since it may have non-infectious or infectious causes, including Middle East respiratory syndrome coronavirus (MERS-CoV), SARS-CoV, SARS-CoV-2 and influenza viruses [Voltersvik, 2016]. Among the distinctive features of COVID-19 are the vascular changes associated with the severity disease. Clinically, the endothelial activation as expressed by elevated D-dimer levels, as well as cutaneous changes in the extremities suggests thrombotic microangiopathy [Toshiaki et al., 2020]. Disseminated intravascular coagulation (DIC) and large-vessel thrombosis have been linked to multisystem organ failure [Asakura et al., 2020]. Peripheral pulmonary vascular changes are less well characterized; however, vasculopathy in the gas-exchange networks, depending on its effect on the ventilation/perfussion mismatching, could potentially contribute to hypoxemia. Interestingly, the pathophysiology for COVID-19-related systemic micro-thrombosis, complicated by multiorgan failure, may be specific and, in particular, different from DIC. Indeed, in contrast to sepsis-induced coagulopathy, consumption of platelets, coagulation factors and fibrinogen as well as bleeding complications are rare in severe COVID-19 patients, suggesting that DIC is not a common complication of the disease [Fogarty et al., 2020].

In summary, to establish explanatory bonds between the puzzled concepts of COVID-19, these disorders may be due to hypoxia combined with an immuno-triggered thrombo-inflammation supported by both an endotheliopathy and a hypercoagulability state. Taking into account that the activation of the endothelium would be a relevant step, markers such as oxidative stress could show early changes at the level of the pulmonary and the other organ microvasculature (Figure 1).

Figure 1.

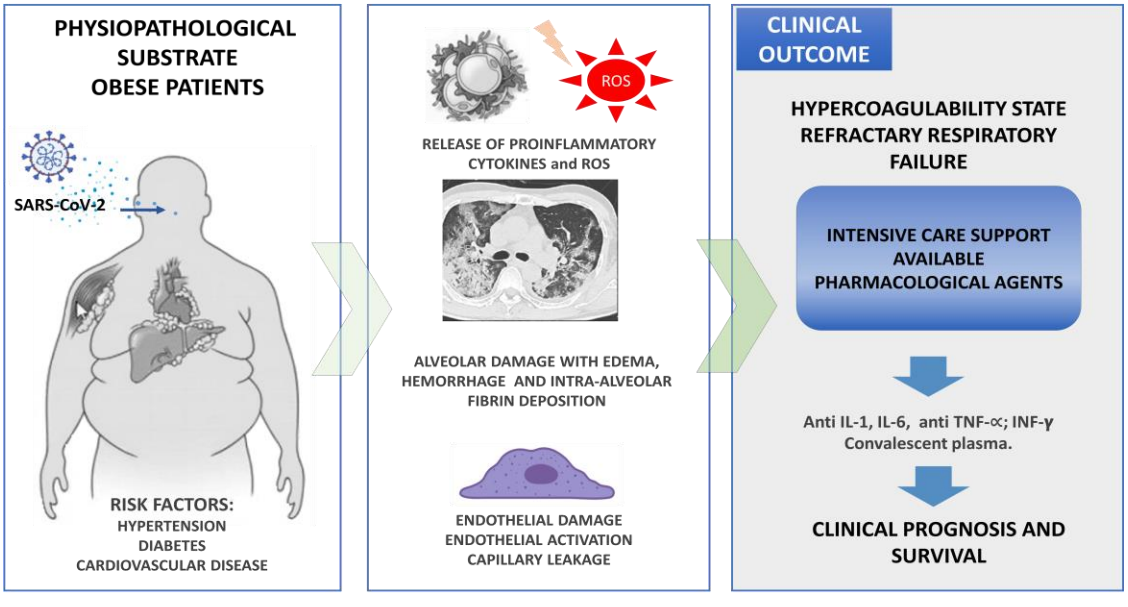

Figure 1. Simplified mechanisms that support the physiopathology, pharmacological therapies target and clinical outcomes in obese patients with pulmonary infection by SARS-CoV-2. ROS, reactive oxygen species; IL (interleukin); TNF-α, tumor necrosis factor; INF, interferon.

3) Ventilatory patterns in obese patients with SARS-CoV-2 pneumonia

In SARS-CoV-2 pneumonia that required mechanical ventilation, two different phenotypes have been described [Gattinoni et al., 2020]. The L phenotype (Low) presents with low elastance and high compliance, low ventilation/perfusion ratio, and decreased recruitability; in this case, the factor limiting pulmonary function will be perfusion. Moreover, the H phenotype (High) presents with high elastance and low compliance, pulmonary shunts, and high recruitability; the factor limiting pulmonary function will be ventilation [Costa et al., 2020].

The pathophysiological transition from Type L to Type H may be due to the evolution of the COVID-19 pneumonia on one hand and the injury attributable to high-stress ventilation, in obese patients [Wolff et al., 2021]. The possible key feature which determines the evolution of the disease, other than the severity of the disease itself, is the depth of the negative intrathoracic pressure associated with the increased tidal volume in spontaneous breathing.

In the Hospital Salvador Intensive Care Unit, we have observed an unexplained increased prevalence of patients with obesity and SARS-CoV-2 pneumonia with ventilatory phenotype H (acute respiratory distress syndrome–like). From the ventilatory point of view, the patients need high positive end-expiratory pressure and respond well to recruitment maneuvers. This ventilatory pattern has been associated with higher obesity percent of our clinical cohort and high cardiopulmonary comorbidities (hipertensive disease and chronic obstructive pulmonary disease). [Saldías et al., 2020].

The incidence of ARDS is increased in patients with obesity [Gong et al., 2010]. To date, there are no studies that mention the association between the ventilatory phenotypes of COVID-19 and obesity. We suggest that understanding these relationships is crucial and clinically important in the treatment of patients with obesity and COVID-19 pulmonary complications.

4) Endothelial dysfunction as a link between obesity and severity of SARS-CoV-2 infection

Obesity is a chronic inflammatory state associated with dysregulated endocrine and paracrine actions of adipocyte-derived factor, which in turn disrupt vascular homeostasis and cause endothelial dysfunction [Pavlov, 2020]. While the mechanisms by which obesity exacerbates COVID-19 infection are not fully understood, endothelial dysfunction may be the common link [Engin et al., 2020]. The endothelium is the cell layer lining the luminal surface of the blood vessels and, in healthy individuals, is a major regulator of vascular homeostasis [Souihol et al., 2018]. The main function of the endothelium is the barrier controlling molecules and cells transport from the bloodstream to the vessel wall and viceversa. The endothelium responds to a series of chemical and biomechanical cues by secreting factors regulating vascular tone, smooth muscle cells (SMC) proliferation and migration, immune cell adhesion, thromboresistance, and vessel inflammation [Deanfield et al., 2007]. Cardiovascular risk factors such as hypertension and diabetes mellitus, can activate the endothelium, resulting in the expression of chemokines, cytokines, such as interleukin (IL)-1, IL-6, IL-8, monocyte chemoattractant protein-1 (MCP-1), and adhesion molecules (e.g. vascular adhesion molecule, VCAM-1; intracellular adhesion molecule, ICAM-1, E-selectin) that attract and facilitate immune cell extravasation [Souihol et al., 2018]. Critical to the activation of endothelial cells (ECs) is the switch from nitric oxide (NO) signaling to reactive oxygen species (ROS) signaling. Endothelial-derived NO promotes homeostasis and maintains the vascular wall in a quiescent state by the inhibition of proinflammatory cytokine secretion, immune cell extravasation, SMC proliferation and thrombosis, preventing vascular leakage. However, excessive ROS induces NF-κB signaling, the main regulator of inflammation [Ramos-Tovar et al., 2020]. This state of oxidative stress is a common underlying mechanism for endothelial cell dysfunction in response to biochemical and biomechanical pathophysiological stimuli [Xiao et al., 2020]. Thus, endothelial dysfunction is considered one of the pathogenetic mechanisms of a whole range of inflammatory diseases [Gimbrone et al., 2016; Yuyun et al., 2018].

The pathogenetic role of the endothelial-derived factors evidences the impairment of their vital physiological properties. Thus, the detection of specific biochemical markers in the blood is an effective way for endothelial dysfunction diagnosis and characterize the vascular endothelium state. For example, in influenza virus infection, acute lung injury is associated with epithelial damage and impairment of permeability in animal models and human serum markers of cardiac dysfunction [Filgueiras-Rama et al., 2020]. However, the main clinical devastating effects are caused by endothelial dysfunction, thought to be the main mechanism leading cause for pulmonary oedema, respiratory failure and cardiovascular collapse [Vrintis et al, 2020]. In the case of SARS-CoV2 infected patients, the endothelial dysfunction would be caused by both direct virus cytopathic effect and inflammatory reaction, leading to a pro-thrombotic setting [Vrintis et al., 2020; Varga et al., 2020]. Furthermore, the immune response-induced cytokine storm, the local and systemic inflammatory response responsible for an endotheliopathy and a hypercoagulability state, leading to both systemic and macro- and micro-thrombosis [Evans et al., 2020]. However, the exact pathophysiological mechanisms leading to severe pulmonary vascular dysfunction in obese patients and ARDS have not been elucidated.

5) Pharmacological therapies against severe SARS-CoV-2 infection

The basic clinical support of a patient who arrives at the emergency department with respiratory failure due to SARS-CoV-2 does not differ from a common patient due to another severe pulmonary or systemic infection (shock). What changes

are the shorter response times in these patients. Ventilatory and gas exchange deterioration has been seen in hours, from a patient in the ward without advanced support, to the intensive and the critical care unit (ICU). Regarding specific pharmacological management, support with oxygen therapy in cases of moderate to severe hypoxemia and anti-inflammatory and immunomodulatory therapy are relevant from the point of view of their impact on mortality. We will briefly describe some drugs.

Corticosteroid in its inhaled form was employed to alter the SARS-CoV-2 replication-transcription complex and inhibit the viral ribonucleic acid (RNA) replication [Matsumaya et al., 2020]. Corticosteroid also, in intravenous administration lowered the COVID-19-associated mortality in patients with acute respiratory distress syndrome and reduced the need for oxygen supplementation [Chatterjee et al., 2020]. In the case of critical ill patients with oxygen and mechanical ventilation requirements, the administration of high doses of methylprednisolone improve the mechanical ventilatory parameters and reduced UCI days. Also, it has been demonstrated a reducing mortality intrahospital and worst outcome [Edalatifard et al., 2020]. Moreover, in adults with non-severe COVID-19 or other viral infections as influenza virus, corticosteroid was even associated with worse clinical outcomes, including a prolonged hospital stay and a higher risk of disease progression [Chatterjee et al., 2020], highlighting the potential benefits of corticosteroids in moderate to severe COVID-19 patients.

Several interleukins are held responsible for COVID-19-mediated cytokine storm (e.g. IL-1β, IL-6 and IL-18), therefore interleukin inhibitors could be advantageous. A previous study showed that anakinra, an IL-1 inhibitor, significantly lowered mortality in patients with COVID-19-induced hyperinflammation and respiratory failure, while IL-6 inhibitors (e.g. tocilizumab and sarilumab) were effective solely in patients with high CRP or low lactate dehydrogenase [Cavalli et al., 2021]. Moreover, IL-6 inhibition also improved survivals in severe COVID-19 patients receiving intensive organ support [Gordon AC et al., 2021] and was consistently associated with a lower risk of death [Khan et al., 2021]. The contribution of numerous kinases (e.g. ABL, NAK, CDK, PI3K/AKT/mTOR, ERK/MAPK and JAK) was also observed in COVID-19, opening a path for kinase inhibitors in COVID-19 management. Kinases play important roles in viral entry, replication and life cycle, intracellular membrane trafficking and possess an immunomodulatory effect that could be useful against COVID-19-mediated hyperactive immunity. Indeed, baricitinib, a janus kinase (JAK) inhibitor, inhibited major protein phosphorylation, altering the signal transduction that initiates host immune response and inflammation [Zhang et al., 2020]. The combination of baricitinib and remdesivir promoted faster recovery and accelerated clinical improvement than remdesivir alone. Moreover, it was associated with fewer serious adverse events in patients with noninvasive ventilation or high-flow oxygen support [Kalil et al., 2021]. However, a negative result was reported with imatinib, an ABL inhibitor, which was shown not to inhibit SARS-CoV-2 entry and replication in an in-vitro study [Zhao et al., 2020], indicating the prominence of several kinases among others in COVID-19 pathogenesis. Conventionally, in the presence of viral pathogens, the host cells produce and release cytokines-derived interferons (IFNs) as a self-defense mechanism. However, SARS-CoV-2 could release molecular (anti-IFN) defenses to evade host innate immunity at the early stage of infection, diminishing the effect of intrinsic IFNs in limiting viral replication and spreading [Calabrese et al., 2020]. Indeed, nebulized IFNα-2b speeded up the clearance of SARS-CoV-2 from the respiratory tract and yielded a reduction in systemic inflammation [Zhou et al., 2020]. Additionally, the combination of IFNβ-1a with antimalarial drug and/or protease inhibitors promoted discharge at day-14 and lowered the 28-day mortality [Davoudi-Monfare et al., 2020]. On the other hand, hyperimmune globulin and convalescent plasma are obtained from previously infected people with high antibody titers against specific pathogens (e.g. SARS-CoV-2). A retrospective case-control study reported that convalescent plasma reduced the oxygen demands at day-14 post-transfusion and improved survivals in severe and critically-ill COVID-19 patients [Liu et al., 2020]. However, it was opposed by randomized control trials (RCTs) which reported no benefit in all-cause mortality or other clinical outcomes compared with placebo or standard care [Janiaud et al., 2021]. Because the RCTs also included moderate to severe COVID-19 patients, this disagreement might not be due to the disease severity.

6) Conclusion

There are a number of clinical conditions associated with the severity of SARS-COV-2 infection that are not known. Therefore, the pharmacological targets that are studied should depend on those molecular pathways that show us a potential impact in real clinical conditions of critically ill patients. Regarding the clinical associated factors, the pathophysiology of obesity is known, oxidative stress and inflammation phenomena associated with the progression and severity of SARS-Cov2 infection could allow determining a therapeutic window in those patients who develop severe pneumonia and respiratory failure due to COVID -19 [Farías et al., 2017; Yonghzi, 2021]. The obese patients express basal endothelial dysfunction. The time course of inflammation and oxidation markers with endothelial dysfunction could allow predicting obese patients susceptible to ventilatory complications and organ dysfunction. Thus, it will be relevant to evaluate the cardiopulmonary function and the association with biomarkers of oxidative stress, inflammation and endothelial dysfunction in order to optimize a prognostic score in obese patients. In the future, several novel immunologic targets such as tumor necrosis factor (TNF)-α inhibitors, complement inhibitors, RLR and mTOR inhibitors, NLRP3 inflammasome inhibitors, TLR modulators, IL-18 inhibitors and possibly mesenchymal stem cell secretome could be tested due to their reported significance in SARS-COV-2 infection.

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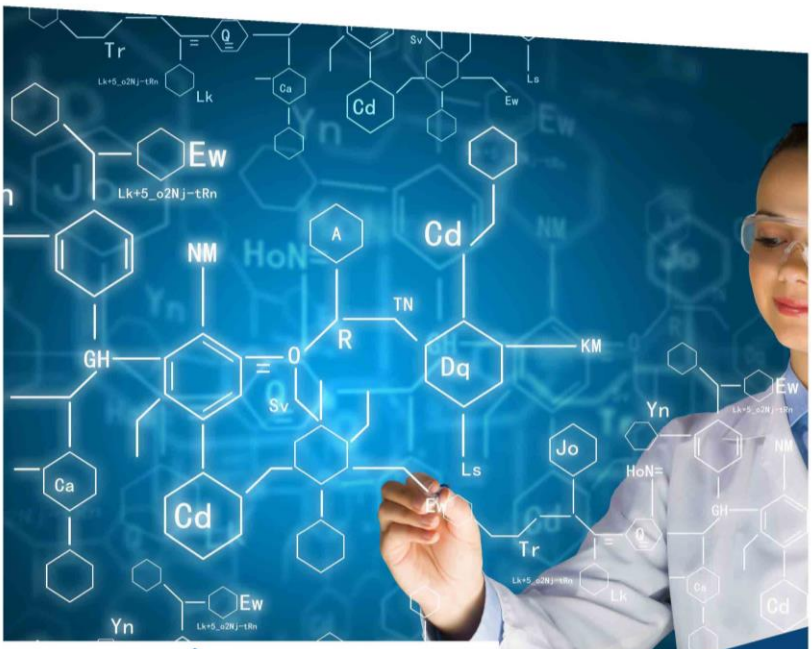

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* La Revista de Farmacología de Chile acepta trabajos en español, sin embargo previa autorización del comité editorial se podrán revisar trabajos en inglés o portugués.

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- Materiales y Métodos: Se debe detallar claramente todos los materiales y metodologías utilizadas en el desarrollo del trabajo. Además se debe explicitar en consentimiento bioético de la institución donde fue realizada la investigación. Los trabajos realizados en humanos deben incluir el apego experimental a la Declaración de Helsinki de la Asociación Medica Mundial, considerando consentimiendo informado y aprobación de comité de ética.
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Ejemplos:

Semenova M. M., Maki-Hokkonen A. M., Cao J., Komarovski V., Forsberg K. M., Koistinaho M., Coffey E. T. and Courtney M. J. (2007) Rho mediates calcium-dependent activation of p38alpha and subsequent excitotoxic cell death. Nat. Neurosci. 10, 436-443.

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ADP, CDP, GDP, IDP, UDP, 5(pyro)-diphosphates of adenosine, cytidine, guanosine, inosine, and uridine AMP, etc.-5-phosphates of adenosine, etc. ANOVA, analysis of variance ATP, etc.-5`(pyro)-triphosphates of adenosine, etc. ATPase, adenosine triphosphatase bp, base pair Ci, curie CoA and acyl-CoA-coenzyme A and its acyl derivatives (e.g., acetyl-CoA) cpm, counts per minute CNS, central nervous systemCSF, cerebrospinal fluid Cyclic AMP, 3,5-cyclic adenosine monophosphate Cyclic GMP-3,5-cyclic guanosine monophosphate DNA, deoxyribonucleic acid DNase, deoxyribonuclease DOPA, 3,4-dihydroxyphenylalanine dpm, dps-disintegrations per minute, disintegrations per second EDTA, ethylene-diaminetetraacetate EEG, electroencephalogramEGTA, ethyleneglycol bis(aminoethylether)tetraacetate ELISA, enzyme-linked immunosorbent assay FAD, FADH2, flavin-adenine dinucleotide and its reduced form FMN, flavin mononucleotide g, average gravity GABA, gamma-aminobutyric acid (not Gaba) GLC, gas-liquid chromatography GSSG, GSH, glutathione, oxidized and reduced forms h, hour HEPES, N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid HPLC, high performance liquid chromatography Ig, immunoglobulin IR, infrared kb, kilobase kDa, kilodalton ìm, micron min, minute MPP+, 1-methyl-4-phenylpyridinium MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine NAD+, NADH-oxidized and reduced forms of nicotin-amide-adenine dinucleotide NADP+, NADPH-oxidized and reduced forms of nicotin-amide-adenine dinucleotide phosphate NAD, NADP may be used when the oxidation state need not be indicated NMDA, N-methyl-D-aspartate NMN, nicotinamide mononucleotide NMR, nuclear magnetic resonance PCR, polymerase chain reaction Pi, orthophosphate (inorganic) PNS, peripheral nervous system PPi, pyrophosphate (inorganic) rpm, revolutions per minute RNA, ribonucleic acid RNase, ribonuclease RT, reverse transcription s, second SEM, standard error of mean SD, standard deviation TLC, thin-layer chromatography Tris, 2-amino-2-hydroxymethylpropane-1,3-diol UV, ultraviolet