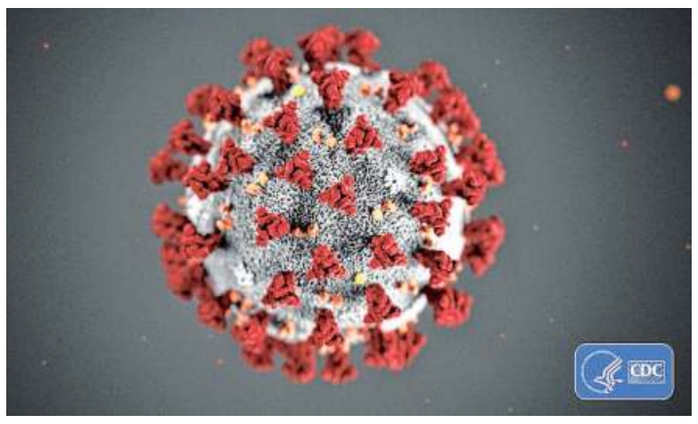
A step closer to developing a potent drug against novel coronavirus

The presence of the inhibitor in the lungs even after 24 hours is significant because the virus affects the lungs

R. PRASAD

Designing better antivirals that would prevent the novel coronavirus (SARS-CoV-2) from infecting human cells may now become possible thanks to a team of researchers producing the crystal structure of the main protease of the virus. Main virus protease is an enzyme that processes proteins critical to virus development. An antiviral that blocks this enzyme, as in the case of drugs used against HIV virus, effectively prevents the virus from replicating. Hence, such an inhibitor will be effective against the novel coronavirus. The results of the study were published in the journal *Science*.



Home strike: A team of researchers has deciphered the crystal structure of the main protease of the virus. ▪ AP

Deciphering key enzyme

A team led by Rolf Hilgenfeld from the University of Lubeck, Germany developed the crystal structure of main protease of the virus at 1.75 angstrom resolution. And by redesigning an existing inhibitor developed for other coronaviruses, the researchers have been able to develop a potent inhibitor that can effectively block the enzyme and neutralise the novel coronavirus. "Based on the structure, we developed the lead compound into a potent inhibitor of the SARS-CoV-2," they write.

Main virus protease is one of the best characterised drug targets among coronaviruses. The inhibitor against the main protease targets a specific region of the enzyme. And any antiviral that targets this region of the enzyme will be specific to the virus and will not be toxic to human cells.

The researchers had earlier designed broad-spectrum inhibitors of the main proteases of other coronaviruses. One of the inhibitors showed good antiviral activity against other coronaviruses. Now, the team chose that inhibitor and modified it to increase the amount of time the drug is present in the body and to improve its solubility in plasma.

After the modification, the half-life of the inhibitor (compound 13a) increased three-fold, and the solubility improved by a factor of about 19. And to enhance the antiviral activity, the researchers further modified the inhibitor (compound 13b).

The researchers found that the IC50 (concentration of the compound to produce 50% inhibition) to inhibit the novel coronavirus is 0.90 microMolar. The inhibitor showed good potency to block the replication of the virus at half maximal effective concentration of 1.75 micromolar. In human cells infected with the novel coronavirus, a higher half maximal effective concentration of the inhibitor was required.

Effective inhibitor

The metabolic stability of the 13a inhibitor originally modified was found to be "good" in both mouse and human microsomes (a fragment of endoplasmic reticulum and attached ribosomes). Even at the end of 30 minutes, around 80% of the residual compound in mouse and 60% in human cells remained metabolically stable. When the inhibitor was administered subcutaneously into mice, the inhibitor was present in the plasma for as long as four hours but was excreted via urine for up to a day.

The half-life of the compound 13b was found to be 1.8 hours. But most importantly, even after 24 hours there was some amount (33 nanogram per gram) of the compound 13b in the lung tissue. The presence of the inhibitor in the lungs even at the end of a day is particularly signifiicant as the virus affects the lungs.

No adverse effects

The team tested for any adverse effects when mice inhaled the inhibitor 13b. "Inhalation was tolerated well and mice did not show any adverse effects, suggesting that this way, direct administration of the compound to the lungs would be possible," they write.

Given the "favourable results" the study provides a "useful framework for development" of drugs to combat the novel coronavirus, the authors claim in the paper.

'The vaccine was rapidly synthesised as novel coronavirus sequence was available'

R. PRASAD

Just over three months after the genome sequence of the novel coronavirus (SARS-CoV-2) was shared by Chinese researchers, an messenger-RNA (mRNA) vaccine is being tested at Seattle in a Phase-1 clinical trial on 45 healthy volunteers between the ages of 18 to 55 years over a period of approximately six weeks.

The trial began on March 16 at the Kaiser Permanente Washington Health Research Institute (KPWHRI) in Seattle. The study is evaluating different doses of the experimental vaccine for safety and its ability to induce an immune response in participants.

The vaccine (mRNA-1273) was developed by National Institute of Allergy and Infectious Diseases (NIAID), U.S., scientists and their collaborators at the biotechnology company Moderna, Inc., based in Cambridge, Massachusetts. The Coalition for Epidemic Preparedness Innovations (CEPI) supported the manufacturing of the vaccine candidate for the Phase 1 clinical trial.

Virologist **Gagandeep Kang,** who is the executive director of the Translational Health Science and Technology Institute (THSTI), Faridabad, in an email to *The Hindu* explains how the mRNA vaccine is developed and how it has become possible to get the vaccine ready for testing so quickly.

What makes the use of messenger-RNA (mRNA) for the vaccine different from the conventional vaccines and how does it work?

Most of the vaccines we know are based on a whole organism (bacteria or virus, living or dead) or a part of an organism. Usually these organisms cause disease, but to make vaccines, the organism are manipulated by heat, chemical or other biological approaches to ensure that their pathogenicity has been removed. These vaccines, when given to a person, act like an infection, but without producing disease. The parts of the organism which are recognised by the immune response are called antigens, and when they are recognised, an immune response is made, as either antibodies or activated immune cells that protect from disease when the same infection is seen again.

Unlike a usual vaccine, RNA vaccines work by the introduction of an mRNA sequence into the host's cells. This mRNA codes for a disease-specific antigen. Once inside a cell, the mRNA instructs the cell to produce the antigen, which is recognised by the immune system which makes an antibody or cellular response.

Currently, two forms of mRNA vaccines are been widely developed against multiple pathogens: conventional mRNA vaccines and self-amplifying mRNA vaccines, which are derived from positive strand RNA viruses.

Gagandeep Kang ▪ SPECIAL ARRANGEMENT

How has it become possible to develop an mRNA vaccine in just about three months? Is it necessary to have the virus to develop the vaccine?

In part, reseachers were able to quickly develop mRNA-1273 because of prior studies of related coronaviruses that cause SARS and MERS.

We have incredible genome sequencing capacity now, and got the SARS-CoV-2 sequence in early January 2020. Since all you need for the mRNA vaccine is the sequence of the pathogen, a vaccine could be rapidly synthesised in the laboratory.

The mRNA can be made synthetically by *in vitro* transcription or reading of a plasmid DNA template, with a recombinant RNA polymerase. A cap and tail are attached to from a mature mRNA sequence.

No, the virus is not required, but the genome sequence [of the virus] is needed. The messenger RNAs are produced synthetically and this is what makes the technology rapid and reproducible.

Since a particular protein (spike protein) found on the virus is what binds to receptors found on human cells and then infect them, will the vaccine use mRNAs produced for this protein?

The first vaccine to enter human trials is Moderna's mRNA-1273. This is a novel lipid nanoparticle-encapsulated mRNA vaccine that codes for the full-length prefusion stabilised spike (S) protein.

How do vaccine developers know which mRNAs of the spike protein are produced? How do they select the correct ones to be included in the vaccine?

The gene sequences of the proteins of coronaviruses are known. Even with a novel virus, it is possible to figure out which sequence codes for which protein. Only one mRNA is included and it is selected based on sequence matching.

Are the mRNAs used in the vaccine packed in some protective material or used bare?

In the case of Moderna, the mRNA is stabilised so that is protected from enzymes that might break it down.

Has this vaccine already been tested in animals for safety or is it tested on human volunteers bypassing animal trials? How ethical is this?

The US FDA has approved studies to proceed in parallel so human studies are also being done with the same product. The mRNA platform technology by which the vaccine was made has already been used safely in 1,700 volunteers for other mRNA vaccines. So the FDA will have considered that in its decision. There are always worries about ethics when testing in emergencies, because safety should be paramount for vaccines, but there is, so far, no safety signal [adverse effects] from the Moderna mRNA platform.

With regard to the parallel studies in animals also, one unusual aspect of the mRNA-1273 vaccine is that although other formulations of similar vaccines have been tested on animals, this particular vaccine construct has not been evaluated in the appropriate animal model which is a transgenic mouse expressing the ACE2 receptor. Tal Zaks, Chief Medical Officer of Moderna was quoted in *STAT,* a news outlet, as saying: "I don't think proving this in an animal model is on the critical path to getting this to a clinical trial," He also pointed out that National Institute of Health scientists are "working on nonclinical research in parallel."

Has there been any instance before when an mRNA vaccine has been tested on humans?

Many candidate vaccines have been produced using this technology and are in various phases of testing. Currently, there are over 20 candidates for infectious diseases and cancers from multiple groups. This is a new technology and, as yet, there are no licensed products.

Will mRNA vaccine produce better protection against the virus than vaccines developed through the conventional route?

We do not know yet what will work and how well, which is why multiple approaches are being taken for vaccine development.

How long will it take before we have a vaccine for commercial use?

It will take at least a year, if not longer.

Long-tailed macaques show rich tool-use behaviour

Tool use and object manipulation were observed in six behavioural contexts involving eight different types of objects

SHUBASHREE DESIKAN

In recent times, there has been a lot of interest among primatologists in studying object handling and tool-use in non-human primates such as apes and chimpanzees. A study from IISER Mohali has looked into how long-tailed macaques *(Macaca fascicularis umbrosus)* in Greater Nicobar Island handle objects and use tools to simplify their efforts.

The researchers observed interesting behaviour related to object manipulation and tool use in six behavioural contexts involving eight different types of objects. They also saw that males were more frequently involved in tool use than females. The results of the study are published in the *International Journal of Primatology.*

There is a crucial difference between tool use and object use. A tool helps the user get better outcomes. Jayashree Mazumder, first author of the paper explains in an email to *The Hindu:* "When we change either the function or structure... of an object, we make it a tool. But when we use an item in the manner it is supposed to be used, we are not making it a tool... it is an object use."

Identifying individuals

Observing the long-tailed macaques from a distance of about 10 metres for close to four months, Ms Mazumder, who is working for her PhD at IISER Mohali, has developed a catalogue of the individuals studied. Each individual was identified based on marks on the face or body. "Identifying adults is easy. They are like humans with distinct features, for example, presence of black or white spots in different locations of the face, scar marks, body size, sex and behaviour. The juveniles and sub-adults were slightly difficult but they too can be identified in a similar fashion," says Ms Mazumder.

Stefano S.K. Kaburu, professor at the University of Wolverhampton, U.K., a co-author of the paper introduced her to the behavioural data collecting software and guided her in designing the study methods.

Prevalent in males

As per their observations, 14 individuals used tools, and tool-use was more common among males. "The biased nature of tool-use could be due to many reasons. It has been hypothesised that the weight of the individual has something to do with the tool-culture. Again, the tool activity itself also defines who uses them more often, says Ms Mazumder.

Dextrous primate: A long-tailed macaque in Great Nicobar island uses a scraping tool to process the food before consuming it. ▪ JAYASHREE MAZUMDER

She gives the example of how among chimpanzees, females excel in fishing, which they learn from their mothers. Males, on the other hand, become adept in hunting, which they pick up from their peers. "Thus there could be social, ecological as well as demographic factors that could decide how tool-culture is divided among the animals. But we need more studies to come to any conclusion," she says.

According to her, the most exciting part was how the macaques decide what tool and technology to use. "Some of the macaques had few trials and errors, but it did not take them long to understand that the technique or tool was not providing the best outcome, and therefore, they were very quick in switching," she says.

Though the long-tailed macaques are further from humans in relatedness than chimpanzees or apes, this study could offer a perspective on evolutionary origins of tool use behaviour.

Handed down: Research suggests prehistoric humans played the 'drum' from whatever material they found suitable, borrowing from our ancestral primate cousins. ▪ K. V. SRINIVASAN

Musical scales are a prehistoric gift to us

SPEAKING OF SCIENCE

D. BALASUBRAMANIAN

During the last a few months, several groups have come up with interesting publications on how music affects the mind. The first is a report on March 1 from a group from Indiana University in the U.S., stating that music may overcome delirium in critically ill patients (https://doi.org/10.4037/ajcc2020175). Such patients experience acute mental disturbance, with speech disorder and hallucinations. The researchers attempted to try music as a drug-free intervention in 117 such patients, and gave half of them music - either their own personally chosen music (PM), or relaxing slow tempo music (STM), and compared them with a control group which was not offered music. The music was offered to the experimental group for 1 hour, twice daily for a week, and their progress noted. Results revealed that such music delivery (PM or STM, either was OK) reduced the incidence of delirium. When audio-books were offered instead of music, it did not help! The STM chosen had relaxing (60-80 beats per minute) classical music, native American flute sounds, or relaxing piano music – all preselected by a board-certified music therapist. They concluded that music is a useful non-pharmacological intervention for critically ill patients.

A little earlier was published a report in *Current Science* (118 (4), 612-620; 2020) from Dr. B. Geethanjali of SSN College, Chennai, and her colleagues, titled "Evaluating the effect of music intervention on hypertension". They did a randomised controlled assessment of 200 high-blood-pressure patients, measuring their heart rate, respiratory rate (RR) and mean arterial pressure (MAP), and found that these parameters declined after music intervention for one month. The researchers chose to offer music intervention, along with the regular treatment, and chose the raga Hindolam (or Malkauns) – a pentatonic, 'low arousal', and pleasant one. (As we all know and experience, fast music and rhythms are 'high arousal', and excite us).

About this time also, the well known music therapist, Rajam Shankar of Hyderabad came out with a scholarly and well-researched monograph: "the healing power of music", with details on the kind of ragas that can be used in therapy, and a detailed description of as many as 35 known Carnatic music ragas (many common to Hindustani music too), and some case studies.

A 'universality' to music appreciation

Note that while America's Indiana University researchers used music that was familiar to the patients of the 'Western' cultural background, and the Chennai authors used the music familiar in the South, the question is can music penetrate cultural differences with its ability to evoke emotions? This is the question that was studied by the brain researcher Nandini Chatterji Singh of the National Brain Research Centre at Manesar, Haryana, and the results of her studies have appeared six months ago in the journal *PLos One* (https://doi.org/10.1371/journal.pone.0222380). Here, she and her group played excerpts from twelve ragas from Hindustani music, online to 144 people from many parts of India, and 112 participants from non-Indian cultural backgrounds (from the U.S., the U.K., parts of Europe, Japan, Korea). They played the *aalap* part (a slow paced introduction of the swaras of the raga, which define the sequence of the notes of the octave, with no rhythm) followed by the *gat* (the same melodic sequence of the swaras but in a faster pace, and with the accompaniment of a percussion instrument (usually a tabla) with an explicit rhythmic cycle). These were played on a sarod. When ragas such as Hansadhwani were played, both the 'encultured' listeners from India and the 'non-encultured' group from abroad felt 'happy' or 'romantic', and when the raga Marwa was played, they described a feeling of 'sadness'. The non-encultured group responded to the rhythmic part, the *gat,* more readily. This, the researchers point out, is in agreement with other reports wherein American members in the audience reacted more readily when they witnessed traditional Indian classical dance. There thus appears to be an 'universality' in emotions in the auditory domain. They further note that a similar kind of reaction when foreigners were invited to listen to music of the Javanese people.

Ancestral gift!

This raises the question of how this universality has come about, and how music across the world uses the basic tonal alphabets and rhythms. Is this an evolutionary gift to us, much as DNA sequences are? What are the origins of music in us humans? A whole field termed 'biomusicality' has come about since the 1990s, which studies the origins of music, what areas of the brain are involved in music processing and the functions, uses and costs of music making, and what universal features can be detected across various cultures. Some researchers have suggested that prehistoric humans played the 'drum' from whatever material they found suitable, and that it is an evolutionary borrowal from our ancestral cousins, the primates. And some archaeologists have looked at the kind of music from prehistoric, Paleolithic ages of humans (Neanderthals) about 4000-5000 years ago. The first such prehistoric musical instrument was a 'flute', made of the bone of a young bear, in Slovenia; this find has at least three holes in the hollowed out bone, perhaps there were more, which were broken away when found.

Another set of flutes, found in the Jiahu region of China, was dated even earlier (7000 -8000 years ago, using isotope dating methods), and when the researchers played them (vertically like a shehnai), they found the music reminded them of the traditional do, re, fa, so la, ti (or sa, re, ga, ma pa..) scale! (More on this in my earlier column in *The Hindu,* of October 14, 1999). Recall what Saint Thyagaraja wrote: Sobhillu Saptaswara (worship the goddess presiding over the seven swaras- from the navel to the heart to the throat, tongue and the nostrils)!

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