COMMENTARY

2-Deoxy glucose for COVID-19 treatment needs cautious handling

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2-Deoxy glucose (2-DG), the metabolic analogue of D-glucose is a glycolytic inhibitor. It acts primarily by accumulating as 2-deoxyglucose-6-phosphate in the cells which cannot be metabolized further. This analogue of glucose in combination with other drugs, has been used in anti-cancer therapy, owing to the dependency of tumour cells on the glycolytic pathway for energy, also termed as the 'Warburg effect'. We have examined the effect of 2-DG on tumour angiogenesis and found it to be an effective anti-angiogenic compound at very low concentration of 10 μM (ref. 1). Though 2-DG appears to be a promising compound for the management of pathological conditions that involve high rate of glycolysis, including COVID-19, we would like to raise a few points of concern based on research from our laboratory and across the globe.

Induction of endoplasmic reticulum stress and autophagy

2-DG at relatively higher concentrations induces endoplasmic reticulum (ER) stress. At a concentration above 100 μM, it inhibits N-linked glycosylation resulting in the accumulation of mis-folded proteins and thereby causing ER stress[2]. 2-DG also induces autophagy in a variety of cells *in vitro* as well as *in vivo*[3,4]. 2-DG treatment increases the expression of autophagic markers like LC3-II and Beclin–I (refs 3, 5). Autophagy is a ubiquitous, self-degradative process which maintains homeostasis and regulates many physiological and pathological processes such as tumorigenesis, neurodegeneration, ageing, infection, etc.[6]. Autophagy plays a housekeeping role in the cells by clearing aggregated/misfolded proteins, damaged organelles like mitochondria, endoplasmic reticulum, peroxisomes and also removes intracellular pathogens[6]. SARS-CoV-2 has been reported to inhibit ER stress and evade autophagy, rendering it replicative advantage within the host cell[7]. Therefore, a drug like 2-DG which induces both these cellular processes logically appears to be beneficial for the treatment of the infection.

2-DG as an anti-viral agent

It has been reported that 2-DG inhibits the multiplication of enveloped viruses such as Herpes simplex virus[8], measles virus and respiratory syncytial virus[9], Semiliki Forest virus and Sindbis virus[10]. It also inhibits the expression of viral genes and viral replication[11]. In a study conducted to assess the effect of 2-DG on rhinovirus infection *in vivo*, it was found that it inhibited viral load and inflammation compared with placebo-treated mice[12]. These reports are in agreement with the present findings of 2-DG being effective against SARS CoV-2 infection. In addition, *in silico*-based molecular docking approach has revealed that the efficient binding of 2-DG with viral main protease 3CLpro and NSP15 endoribonuclease, could incapacitate SARS-Cov2 by inactivating the viral receptors[13].

Caution during 2-DG treatment

Cytotoxicity and brain function

The main concern of using 2-DG for therapy is that it may affect the functioning of highly glycolytically active organs like the brain, especially the hypothalamus which uses glucose as the primary fuel for energy. Administration of 2-DG at higher concentrations (0.2 g/kg) on mice shows reduced uptake of food, toxicity to the cardiac system and increased mortality[14]. Previous reports have also suggested adverse outcomes for treatment with 2-DG (250 mg/kg) in cases of psychiatric diseases and stress[15–17], prostate cancer[18] and glioma[19–21], which include dizziness, fatigue, restlessness and prolonged QTs. A drug with negative outcomes in clinical trials at higher concentrations must be handled with caution and care.

Complications anticipated on use in co-morbid patients

2-DG is the substrate analogue of glucose and competes with the latter to occupy the active site of enzymes. Therefore, the efficacy of 2-DG mediated treatment would largely depend on the duration of treatment and the metabolic status of the subject[22,23]. Also, its effectiveness in patients with diabetic complications raises concern.

2-DG is capable of getting accumulated in almost all the glycolytically active cells, especially cancer cells. Cancer cells being reprogrammed to carry out aerobic glycolysis would rather accumulate more of 2-DG during treatment, and an increase in dosage to compensate for such effects may lead to detrimental effects on brain functioning[24]. Therefore, the use of 2-DG in patients with conditions related to hypoxia and malignancies should be considered carefully. Pharmacological doses of 2-DG (500 mg/kg) were found to increase local cerebral blood flow, significantly in the cerebral cortex, basal ganglia and thalamic nuclei in the brain of conscious, unrestrained adult rat[25]. There are normal cells in the body like the endothelial cells which prefer aerobic glycolysis and would accumulate 2-DG. This, in turn may contribute to endothelial and vascular dysfunction[26]. 2-DG also causes a fall in blood pressure and decrease in respiratory frequency at doses of 500 and 100 mg/kg (ref. 27). It induces metabolic stress that has a profound effect on innate immune mechanism, predominantly downregulating the production of T-helper 1 cell population[28].

Last, but not the least, concern is the availability of medication without prescription. If 2-DG is made available across the counter, its indiscriminate use will lead to further health complications. Being a drug that has been used and failed as a standalone anti-tumour agent due to its cytotoxic effects, the usage of 2-DG for the treatment of COVID-19 has to be carefully weighed for its advantage over the complications it may contribute to.

This note does not intend to deter the use of 2-DG for the treatment of COVID-19. Rather, it raises concerns based on the available literature and scientific data on 2-DG.

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Criteria for the identification of suspect SARS-CoV-2 reinfection cases

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Owing to limited knowledge about the SARS-CoV-2 virus, in the initial few months of the pandemic, COVID-19 patients were not expected to be reinfected[1]. As the pandemic progressed and more information about the disease has now become available, consensus among health experts has started emerging that those who have recovered from COVID-19 can be reinfected. This has been acknowledged by various organizations[2]. According to a report published by Centers for Disease Control and Prevention (CDC), 'cases of reinfection with COVID-19 have been reported but remain rare'[3]. Multiple criteria are being used across different geographies to identify a reinfection[2]. However, there is no well-defined and consistent definition for deciding what constitutes true SARS-CoV-2 reinfection. This note aims at establishing a criterion for the identification of cases of reinfection. After studying different approaches that are being used globally, the goal is to determine a criterion that is accurate and practical in its application on a large scale in India.

Identification of reinfection

Identification of SARS-CoV-2 reinfections is critically necessary for public health control and related risk assessments. Reinfection refers to a case where a person who has been infected previously (confirmed tested positive) and thereafter recovered (tested negative at least once) is found to be infected again (tested positive again after at least one negative test). However, it is important to make sure that these cases are reinfections because SARS-CoV-2 residue can remain in the body for several weeks[4]. Therefore, ensuring whether these are actual cases of reinfection and not residual infection from the initial infection is necessary.

There are various methodologies under consideration for the identification of reinfection episodes based on the following approaches.

Using genetic sequencing data. CDC has set up a gold-standard investigation protocol for the confirmation of SARS-CoV-2 reinfection[2]. This includes a comparison between viral genome sequences from the first infection and reinfection. The two sequences should not differ significantly for reinfection[2], as the virus is expected to mutate at the rate of about two single nucleotide variants (SNVs) per month[2]. The protocol requires a positive confirmatory test of the first infection and virus detection across two distinct time periods (without any specification on the time frame of the tests). Along with the positive confirmatory test, genetic sequencing data are also needed to support the conclusion with a high probability that reinfection has occurred. However, it mentions that reinfection cannot be confirmed if clinical specimens from the initial SARS-CoV-2 illness are not available.

Analysing survey data available on potential reinfection cases. Many countries have identified potential cases of reinfection and there are a few organizations that are tracking the confirmed[5,6] and suspected[7] number of SARS-CoV-2 reinfections. The confirmed cases indicate that after being infected for the first time, reinfection can occur as early as 10 days. However, the average time period noted in one of the trackers[5] (organizations that are tracking reinfection instances) is 78 days[5], whereas another study found the median time for reinfection to be 64.5 days with the time range for reinfection being 45 to 129 days[6]. According to a report published by Pan American Health Organization/ World Health Organization (PAHO/WHO), the time range for a suspected case is ≥90 days from the first SARS-CoV-2 infection, or it can follow a period ≥45 days from the first infection with SARS-CoV-2 (ref. 2).

Based on these studies and the data on reinfection available, a possible way of identifying a set of potential reinfection cases statistically is to use the average days it takes for a person to test positive again after recovering from the infection. This method, although not definitive, will significantly decrease the number of cases that need to be clinically investigated further to arrive at the true instances of reinfection.