Chapter 6

Modulation of pentose phosphate pathway augments the efficacy of 2-deoxy-D-glucose in COVID-19 management

Simran Chopra[1], Ashrit Nair[1], Navneet Sharma[2], Viney Jain[3] and Rakesh Kumar Sharma[4]

[1]*Department of Textile and Fibre Engineering, Indian Institute of Technology Delhi, New Delhi, Delhi, India,* [2]*Amity Institute of Pharmacy (AIP), Amity University, Noida, Uttar Pradesh, India,* [3]*Jain Vishva Bharti Institute, Ladnun, Rajasthan, India,* [4]*SGT University, Gurugram, Haryana, India*

6.1 Introduction

Scientists and clinicians the world over are exploring various drug targets and potential therapeutics for COVID-19 caused by SARS-CoV-2 [1]. On May 8, 2021, Defence Research and Development Organisation (DRDO) announced that it got approval for an anti-COVID-19 therapeutic application of the drug 2-deoxy-D-glucose (2-DG) from the Drug Controller General of India (DCGI) [2].

Balakrishna et al., proposed the potential benefits of using 2-DG to mitigate COVID-19 infection based on a thorough review of the literature and *in silico* studies done by conducting ligand-receptor docking, drug likeliness, bioactivity indices, and ADMETox values [3]. Khurana et al., studied the potential use of 2-DG using a computational biology approach by constructing a drug-gene-gene-disease interaction network [4]. Bhatt et al., were in agreement that the repurposed drug will be a promising cost-effective adjuvant treatment option for tackling COVID-19 [5]. This drug can be taken orally and is available in powdered form. An updated review on 2-DG has been published recently [6].

6.2 Studies on 2-deoxy-D-glucose as an adjuvant to cancer therapy and viral-induced diseases

2-Deoxy-D-glucose has been shown to promote apoptosis in cells exposed to ionizing radiation [7]. It affects the postirradiation DNA repair and recovery processes of cancer cells having a higher rate of glycolysis. 2-DG inhibits the pentose phosphate pathway (PPP) by reducing glycosylation and further inhibiting angiogenesis, which increases the apoptosis of cancer cells [8,9].

2-DG has the potential to selectively kill tumor cells, thereby remarkably improving the efficacy of radiotherapy under euoxic as well as hypoxic conditions [10].

The 2-DG-induced modification of DNA repair and radiation damage has been studied in various cancer cell models such as solid Ehrlich ascites tumor cells of mice, baby hamster kidney cell line (HNK-21), human carcinoma cervix cells, human brain tumor in organ culture, human glioma cell line (BMG-1), etc. It was observed that there was an inhibition of DNA repair in these cell lines, which was correlated with reduced glycolysis and ATP production. In a study conducted by Jain et al., the DNA repair that was assayed by radiation-induced unscheduled DNA synthesis and the repair of potentially lethal damage (PLD) in yeast cells (determined on the basis of postirradiation increase in the reproductive capacity of cells) was observed to be inhibited by 2-DG. The inhibition of the rate of repair of PLD in irradiated yeast cells was at a greater extent than the cell proliferation rate due to the reduction in the rate of ATP production governed by the glycolytic pathway [10,11]. Reduction in glycolysis was important in killing tumor cells [11].

Stem Cells. DOI: https://doi.org/10.1016/B978-0-323-95545-4.00001-3

Some of the promising preclinical and clinical studies using 2-DG are listed in Table 6.1.

The first human trial of 2-DG was conducted on a thyroid patient in 1974 at All India Institute of Medical Sciences, New Delhi, where the patient was given Iodine-131 (I-131) along with two doses of 2-DG orally (10 g/dose in 150 mL water) after 16 and 65 hours. There were no side effects observed, and there was a marked reduction in the size of the tumor. Even though the metastasis deposits were the same, the quality of life improved for the patient as the progress of the disease stopped [19].

Another human trial of 2-DG consisted of its topical application of 2-DG for the treatment of genital herpes lesions in a double-blind placebo (1:1 to 2-DG or placebo) controlled trial in 36 women at NIH. The treatment was continued for 3 weeks, and a recovery rate of 89% was observed [18].

Administration of 2-DG (1−2 mg/g body weight) in combination with the gamma irradiation to the sarcoma-180 and solid Ehrlich ascites tumor-bearing mice showed a potential increase in the tumor cell loss and animal survival [15].

TABLE 6.1 Promising preclinical and clinical studies using 2-deoxy-D-glucose.

Study	Trial Design	Endpoint	Reference
In vitro	HeLa cell culture (derived from cervical cancer cell line)	2-DG optimizes radiotherapy by the enhancement of radiation damage to cancer cells and reduced damage to normal cells	Jain et al. [11]
In vitro	Organ cultures of human cerebral glioma	2-DG-induced radiation damage in cerebral glioma cells	Dwarakanath et al. [12]
In vitro	Cell cultures of human prostate cancer cell line	2-DG-induced radiation damage in prostate cancer cells	Rae et al. [13]
In vitro	Cell cultures of breast cancer cell line and prostate cancer cell line	2-DG inhibits cancer cell growth that is further enhanced by the use of dehydroepiandrosterone	Li et al. 2015 [14]
In-vivo	Sarcoma-180 and solid Ehrlich ascites tumor-bearing mice	Decreased volume of the tumor with improved animal survival	Latz et al. [15]
In vivo	Nude mouse xenograft models of human osteosarcoma and nonsmall-cell lung cancer	A significant reduction in tumor growth was observed as compared to using either drug alone	Maschek et al. [16]
In vivo	Hypoxic cells in retinoblastoma	Reduction in tumor size by 86% when 2-DG was used as an adjuvant along with carboplatin	Boutrid et al. [17]
Human clinical trial	Genital herpes lesions	Significant cure of infection with no major adverse effects in a double-blind placebo-controlled trial (1:1 2-DG or placebo) for 3 weeks	Blough and Giuntoli [18]
Human clinical trial	Thyroid carcinoma	Significant enhancement in quality of life since the progress of the disease was arrested and the metastasis deposits were the same.	Naqvi S et al. [19]
Phase I/II	Supratentorial glioma patients	Significant enhancement in quality of life along with no toxicity in the multicenter, nonrandomized, open-label, single treatment arm (uncontrolled) trials	Mohanti et al. [20]
Phase II	Dose escalation trial in patients with malignant cerebral gliomas	Significant tolerance to the treatment with no toxicity in the multicenter, nonrandomized, open-label, single-treatment group	Singh et al. [21]
Phase III	Patients with glioblastoma multiforme and astrocytoma grade III	An improvement in Karnofsky performance scale signifying enhancement in the quality of life	Prasanna et al. [22]
Phase I/II	Solid tumor and hormone-refractory prostate cancer	Significant tolerance to the treatment with a maximum dose toleration of 60 mg/kg.	Gounder et al. [23]
Phase I	Patients with advanced solid tumors	63 mg/kg was the maximum clinically tolerable dose	Raez et al. [24]

An in vitro study of the effect of 2-DG on the HeLa cell line showed reduced aerobic glycolysis by 62% along with inhibited DNA repair, resulting in enhanced radiation damage to the cancer cell line [11].

Glioblastoma multiforme (GBM) is a fast-growing and aggressive brain tumor with an extremely low 5-year relative survival rate and is majorly dependent on glycolysis. Due to excellent DNA repair systems, these tumor cells are resistant to treatment in both hypoxic and euoxic conditions [7]. After the surgical and/or radiotherapeutic ablation of cancerous tissue, there is always a possibility of local regrowth of human glioblastomas within the cranial cavity [12]. The in vitro studies using organ cultures of human cerebral glioma concluded that higher concentrations of 2-DG (5 mM) may be required to induce radiation damage in the cerebral gliomas under euoxic conditions; however, this requirement could be decreased in hypoxic conditions [12].

Phase I/II clinical trials were conducted in patients affected by GBM [20,21]. Four weekly fractions of 5 Gy/fraction of gamma radiation to the whole brain were administered 2−3 weeks postsurgery. 2-DG was administered orally (200 mg/kg body weight) after overnight fasting and also 20−30 minutes prior to irradiation (5 Gy). This schedule of the combination of 2-DG and radiotherapy was given once weekly spanning over days 1, 8, 15, and 22 with a total radiation dose of 20 Gy given over a period of 21 days. Two weeks after the fourth week, a CT scan was carried out and radiotherapy was given to the residual tumor (plus 3 cm margin) at a dosage of 14 Gy in seven fractions at five fractions per week. No acute toxicity or late radiation damage was observed. Moreover, a significant enhancement in the quality of life with a moderate increase in survival was observed [20]. To determine the tolerance and safety of escalating the 2-DG dosage, dose optimization studies were undertaken during the combined treatment of 2-DG and radiotherapy in GBM patients. The dosage of 2-DG was administered in incremental doses of 200−250−300 mg/kg body weight. The dosage included seven weekly fractions of combined treatment of 2-DG along with radiation at a dose of 5 Gy/fraction, including the postsurgery treatment of the residual tumor plus a 3 cm margin. Patients with dosages up to 250 mg/kg showed excellent tolerance to the treatment with no toxicity [21].

In another study conducted on GBM and astrocytomas grade III patients, after overnight fasting 100 mL at a dose of 250 mg/kg was given orally to patients for 25 minutes of irradiation followed by 5 Gy radiation to the tumor bed and 3 cm around the tumor once a week for 7 weeks. A re-exploration surgery was performed, where it was found that the tumor was necrotic, soft, and suckable signifying clinical improvement [22].

In an in vivo study conducted by Maschek et al., the efficacy of the combination of adriamycin or paclitaxel with 2-DG in nude mouse xenograft models of nonsmall-cell lung cancer and human osteosarcoma was observed. In both cases, a significant reduction in tumor growth was observed as compared to using either drug alone [16].

In another trial, 2-DG was used as an adjuvant for targeting the hypoxic cells in retinoblastoma. Carboplatin (31.25 μg/20 μL) along with 2-DG (500 mg/kg) was given to sixteen-week-old $LH_{BETA}T_{AG}$ mice. It was observed that therapy alone with carboplatin resulted in a 52% tumor size reduction, whereas monotherapy of 2-DG resulted in a 49% tumor size reduction. However, on using 2-DG as an adjuvant along with carboplatin then a reduction in tumor size was observed to be 86% [17].

A phase I/II trial was conducted on twelve patients for the treatment of advanced solid tumors and hormone-refractory prostate cancer. The drug was administered orally on a daily basis continuously for 2 weeks of every 3-week cycle. The dose was escalated from 30 to 45 mg/kg and finally up to 60 mg/kg. The treatment was well endured with no mortality and maximum tolerance of dose not exceeding 60 mg/kg [23].

In an in vitro study conducted by Li et al., the efficacy of 2-DG increased on using dehydroepiandrosterone against breast cancer and prostate cancer cell line [14].

A phase I trial was conducted on patients with advanced solid tumors in which patients were administered 2-DG orally once daily for 7 days every other week starting at a dose of 2 mg/kg, and docetaxel was administered intravenously at 30 mg/m^2 for 3 of every 4 weeks beginning on day 1 of week 2. Following dose escalation, a group of patients was then treated with 2-DG for 21 days or every day of each 4-week cycle for up to 12 cycles. It was observed that the maximum clinically tolerable dose was 63 mg/kg, and 66% of patients showed progressive disease as their best response [24].

In an in vitro study by Rae et al., 2-DG was used to sensitize prostate cancer cells to radiotherapy. There was a concentration-dependent decrease in clonogenic survival on the simultaneous administration of 1−10 mM 2-DG along with 1−4 Gy X-rays [13].

2-DG was found to interfere with the Kaposi's sarcoma-associated herpesvirus (KSHV) genome replication, resulting in decreased virion production during the lytic phase of virus infection, a requirement for KSHV tumorigenesis. 2-DG at low doses targeted the KSHV replication in lytic phase cells without inducing cytotoxicity and at high doses kills the KSHV-infected cells at the latent stage. Therefore, 2-DG targets the KSHV at the viral and cellular levels [25]. The inhibitory effect of 2-DG has been observed on human papillomavirus 18 (HPV 18) as well [26].

Around one-tenth of cancer incidences are virus related, suggesting the increased uptake of glucose and aerobic glycolysis to be a common trait of viral infection. 2-DG may be a promising drug to reduce viral-induced tumors regardless of the route it takes [9]. Thus, the study of 2-DG on noncancerous viral infections is of vital importance.

The application of the 2-DG for the therapeutic management of COVID-19 has been developed by the Institute of Nuclear Medicine and Allied Sciences (INMAS), DRDO, Delhi, and Dr. Reddy's Laboratories (DRL), Hyderabad, with the help of the Centre for Cellular and Molecular Biology (CCMB), Hyderabad. DRL-INMAS conducted phase II Trials on 2-DG in COVID patients. In phase II trials, the use of 2-DG as an adjunct to the standard of care showed benefits as compared to the standard of care alone in COVID-19 patients [27]. On November 4, 2020, permission to conduct the phase III clinical trials had been recommended by the subject expert committee, an advisory to the DCGI on applications seeking approvals for new drugs, clinical trials, and vaccines. Final approval to conduct the phase-3 clinical trials was granted on November 16, 2020. In phase III trials, it was observed that there was a symptomatic improvement in 42% of the patients and till Day 3 became free from supplemental oxygen whereas 31% were cured with just SoC. Also, a significant proportion showed RT-PCR negative conversion on treatment with 2-DG [8].

Table 6.2 summarizes the studies on 2-DG for the management of COVID-19 in India.

6.3 Pathogenicity of SARS-CoV-2 infection and probable mechanism of anti-COVID action of 2-deoxy-D-glucose

Clinically, the viral infection occurs in three phases: the viremia phase or the early infection phase, the acute or pneumonia phase, and then finally the resolution phase or the continued illness phase, which is also known as the inflammatory phase [28]. There are five steps in the life cycle of a virus with the host, that is, attachment, penetration, biosynthesis, maturation, and release [29]. The coronavirus is composed of four structural proteins termed spike (S), membrane (M), envelop (E), and nucleocapsid (N) [30]. The SARS-CoV-2 binds to the angiotensin-converting enzyme-2 (ACE-2) via the receptor binding domain (RBD) of spike protein and invades the respiratory epithelial cells [31]. The ACE-2 undergoes protease cleavage due to the transmembrane serine protease-2 followed by fusing with the human cell membrane [28,32]. The genomic RNA of the virus contains the replicase gene, which coordinates the transcription by creating two copies, one for a new genome and the other as the mRNA for the synthesis of structural and accessory proteins [28]. The new genome formed destroys the host cell, resulting in inflammatory mediators by host cells that trigger alveolar macrophages to release cytokines such as IL-1, IL-6, and tumor necrosis factor -α (TNF-α) [33]. These cytokines destroy the endothelial layer of the alveolus, resulting in alveolar collapse and impaired gaseous exchange. The increase in proinflammatory cytokine markers such as tumor necrosis factor-α (TNF-α) and interferon-γ- induced protein 10 (IP -10) may result in a cytokine storm [34]. The cytokine storm if left untreated may result in viral sepsis, multiple organ failure, and ultimately resulting in death [35].

2-DG may act at a number of points in the progression of the coronavirus disease. *In silico* studies conducted by Balakrishna et al., suggest that the structure of 2-DG fits into protease 3CL protease (3CLpro) as well as NSP15 endoribonuclease, resulting in the inhibition receptors binding SARS-CoV-2 to the host cell. The NSP15 is one of the vital NSPs for viral transcription and replication. NSP15 is an interferon antagonist that uses endoribonuclease activity-independent mechanism to inhibit interferon-β production [36]. 3CLPro plays a major part in the posttranslational

TABLE 6.2 Studies on 2-deoxy-D-glucose for the management of COVID-19.

Preclinical studies	Phase I/II clinical trials	Phase III clinical trials
In vitro cell culture studies (CCMB, Hyderabad)	Initiated by Dr. Reddy's Laboratory under CTRI number CTRI/2020/06/025664	Initiated in 27 COVID hospitals across India on 220 patients from December 20 to March 21 under CTRI number CTRI/2021/01/030231
Observation: Viral plaques in cells administered with 2-DG were significantly lower as compared to the control group.	A trial was conducted on 110 patients from May 20 to October 20. Patients showed better symptomatic cures as compared to patients who were not given 2-DG [27].	The use of supplemental oxygen was reduced drastically after the administration of 2-DG. The drug showed promising results in patients aged 65 or above.

Based on the results of these trials, DCGI accorded approval for an anti-COVID-19 therapeutic application of the drug, 2-DG.

processing of the replicase gene [37]. The viral protease is responsible for the continuation of the viral life cycle of SARS-CoV-2. Similarly, endonucleases are responsible for catalyzing the processing of viral RNAs responsible for sustaining viral replication [25]. Also, the SARS-CoV-2 enhances mitochondrial ROS production, leading to the stabilization of hypoxia-inducible-factor-1α (HIF-1α) that promotes glycolysis [38]. In another Prescience *in silico* Multi-Target Multi-Ligand Enhance Sampling Screening study (PRinMTML-ESS), the replication of SARS-CoV-2 was effectively reduced by a significant reduction in the glycolytic flux due to the competitive inhibition of glucose using hexokinase enzyme [39]. Thus, 2-DG inhibits the process of viral capsid formation by inactivating the viral proteases and halts the viral replication process by retaining the action of endoribonuclease [3]. 2-DG is having an antiinflammatory action [40] and has the potential to reduce the viral load [41]. Fig. 6.1 depicts the effect of 2-DG on viral replication.

A network-biology led computational drug repurposing strategy by Khurana et al., suggests that increased AMP levels in the cells led to the activation of AMP-activated protein kinases that further inhibits the Janus kinase (JAK1). It is also responsible for the activation of the STAT transcription factors such as signal transducers and activators of transcription-3 (STAT3). STAT 3 leads to apoptosis of cells and is responsible for the inhibition of replication of virus-infected cells [4].

The viruses are generally dependent on glycolysis to sustain their survival and proliferation. This remains the primary route that is targeted by 2-DG. In a study by Codo et al., it was noted that the SARS-CoV-2 infection is associated with an upregulation of glycolytic genes in the bronchoalveolar lavage monocytes [38]. In an experiment conducted by Bhatt et al., it was concluded that the 2-DG-induced growth inhibition of virus-infected cells is due to reduced proliferation and cytostatic effect and not due to cytotoxic effect [5]. 2-DG is a competitive inhibitor of glucose, preventing glucose from binding to the GLUT receptors, thus reducing glucose uptake. Moreover, according to the potentially relevant modes of action mentioned in (Fig. 6.1), 2-DG aggressively inhibits the survival of the virus and the infected cell. This puts an indirect brake on the proliferation of the virus and reduces the chances of increased infection, Further, viruses can alternatively follow the glutaminolysis pathway to successfully overcome the lack of energy in case of glucose shortage. One such example was seen in the replication of poliovirus in HeLa cells where supplementing

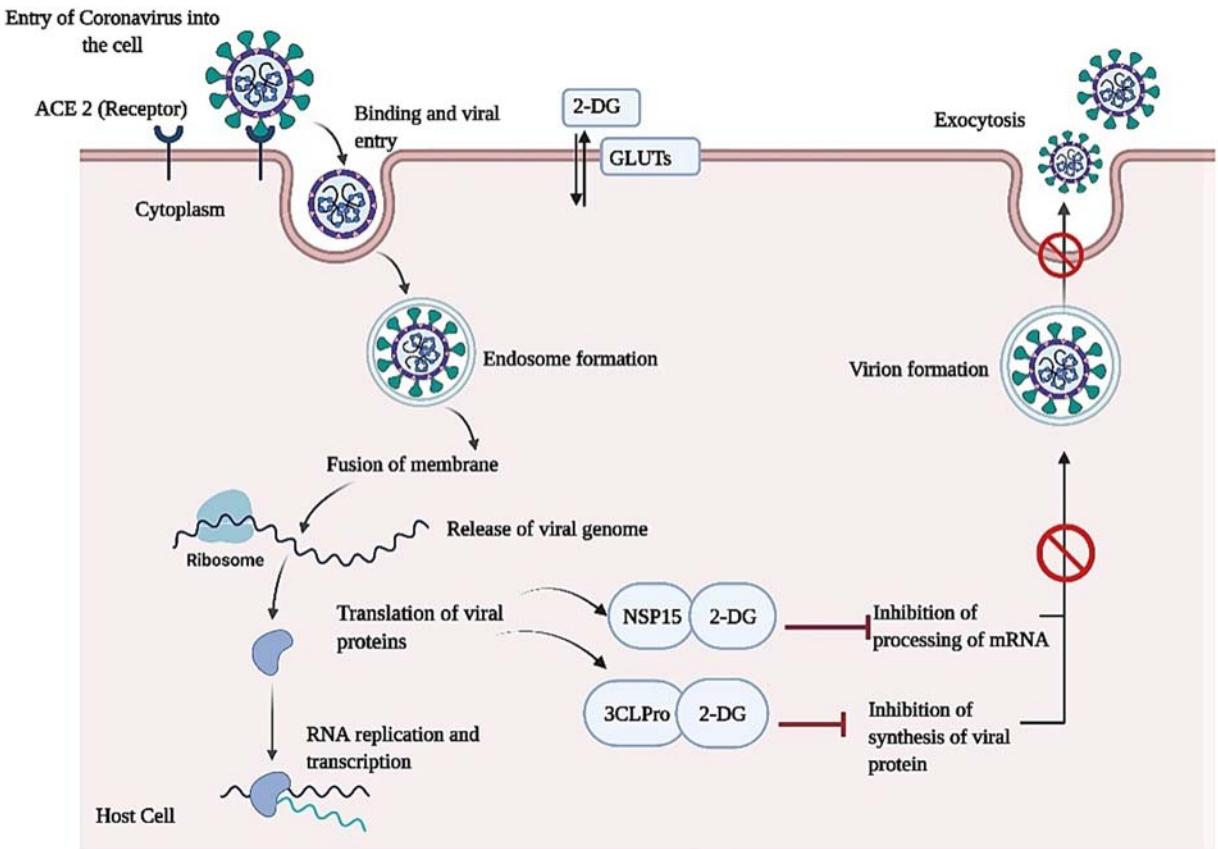

FIGURE 6.1 Effect of 2-deoxy-D-glucose on the viral replication.

glutamine restored viral replication [42,43]. Similarly, it was observed that the proliferation of the poliovirus in HeLa cells was stopped in a minimal media but with the addition of glucose, the viral titer recovered was found to be significant [44]. However, using inhibitors can minimize the already low yield of ATP during glutaminolysis and further put a stop to viral infection [45]. Dengue virus belonging to flavivirus requires glucose and glycolysis for replication [46]. Alternatively, the proliferation of the Hantaan virus depends on oxidative phosphorylation (OXPHOS) since OXPHOS provides ATP for intracellular activities. For its replication in host cells, the Hantaan virus (HTNV) exploited mitochondria OXPHOS to assist its replication in host cells [47].

An assay study using a Seahorse XF analyzer was done to study the effect of 2-DG on triple negative breast cancer cell variants such as Hs578T and its more aggressive variant, $Hs578Ts(i)_8$. In this study, it was observed that 2-DG had decreased the aggressive variant due to increased glycolysis and decreased oxidative phosphorylation, implying that 2-DG may benefit breast cancer cells [48].

Bhatt et al., observed 2-DG to be effective against B.6 and B.1.1.7 coronavirus variants as it targets the metabolic requirement of the infected host cells. 2-DG can be hypothesized as a broad-spectrum antiviral drug that can be effective against COVID-19 and its variants [5].

2-DG competes with D-glucose for entry into the cell. Inside the cells, it is phosphorylated by the hexokinase enzyme to form 2-deoxy-D-glucose-6-phosphate (2-DG-6-P) [49]. The 2-DG-6-P inhibits phosphohexose isomerase. The 2-DG-6-P does not enter further in the glycolytic pathway as it cannot be metabolized by the phosphohexose isomerase that catalyzes the conversion of glucose-6-phosphate to fructose-6-phosphate [50]. It also inhibits the hexokinase enzyme allosterically and competitively. This results in the accumulation of 2-DG-6-P in the cytosol, and glycolysis is inhibited [50,51]. Due to the inhibition of the glycolytic pathway, PPP is propagated. The increase in the PPP further increases the NADPH generation. The increase in NADPH aids in the reduction of glutathione by increasing the expression of the glutathione synthetase [52]. Due to the inhibition of glycolysis, there is a significant decrease in ATP, resulting in the activation of the AMP-activated protein kinase (AMPK). This activation triggers the phosphorylation of proteins such as tuberous sclerosis protein and the mammalian target of rapamycin (mTOR) kinase [53]. Further, the expression of p53 is induced, ultimately resulting in cell cycle arrest at the G1 phase, initiating the apoptosis of the viral cells. Another pathway for the autophagy of viral cells is stress induction in the endoplasmic reticulum due to glucose deprivation and reduction in ATP levels. This stress stimulates the production of reactive oxygen species that further catalyzes the process of cell death [54]. The 2-DG also possesses antiinflammatory properties that inhibit the M2 macrophage polarization. This polarization was responsible for airway inflammation. The AMPK activation inhibited the HIF-1α expression that further inhibits the M2 macrophage polarization [55]. The 2-DG drug inactivates the viral protease and withholds the action of viral endoribonuclease that can halt the viral replication process [56]. Thus, 2-DG is a plausible drug as it inhibits glycolysis in hypoxic conditions and glycosylation in the presence of oxygen. The inhibition of glycosylation led to ER stress, further inducing phagocytosis and apoptosis [56]. Due to the similarity in the structure of 2-DG and the structure formed when hydroxyl is eliminated from C-2 of D-mannose, 2-DG is expected to interfere with the metabolism of the D-mannose-related metabolic pathway. D-mannose is responsible for protein N-glycosylation, and 2-DG interrupts the protein glycosylation in the presence of oxygen resulting in ER stress [53,57]. The 2-DG drug also leads to the formation of defective virions that lack the ability to infect newer cells [5]. There is a depletion of intracellular ATP due to the administration of 2-DG because of which various other enzymes and ribozymes will be dysfunctional, resulting in reduced viral replication [58].

However, the proposed 2-DG drug is to be used as a supplementary drug and not to be considered a "wonder drug." Research conducted by DRL has shown a significant reduction in viral load on using 2-DG. In a study by Bhatt et al., it was observed that glycolysis is inhibited by 2-DG and consequently faster ATP production, which does not allow the virus to multiply in host cells [5]. Also, 2-DG allows the cells to recover from the stress caused by the multiplication of SARS-CoV-2 and thus reduces cell death [5]. An improvement in the symptoms has been witnessed in 42% of patients consuming 2-DG as an adjunct [8]. However, the drug doesn't directly inhibit viral replication or disrupt the viral pathways or mechanisms. Therefore, the drug is aimed toward complementing the retroviral drugs to augment the efficacy.

Recent studies have illustrated the potential of viruses to follow multiple alternative metabolic pathways to ensure survival. This is evident from studies conducted on different viruses such as the human cytomegalovirus (HCMV). The HCMV synthesizes UDP-sugar biosynthesis from pyrimidines to support virion protein glycosylation [59]. The HCMV can also follow the activation of the mechanism to switch the anaplerotic substrate to glutamine from glucose that can accommodate the energetic and biosynthetic needs of viral infection [60]. In chronic conditions, there is a possibility of utilizing glutamine as an alternate fuel. However, the potential for survival of the virus becomes aggressively lower. Due to the situation not being an ideal condition, the energy fabrication in such a scenario ensures limited products. This pathway can be targeted in the future to further enhance the efficacy of the viruses [61]. Viruses such as HCMV

also tend to manipulate host mitochondria to further propagate viral replication [62,63]. Targeting the mitochondria and designing a cocktail drug, including inhibitors such as CB-839 or Telaglenastat, can be used to inhibit viral replication. Targeting mitochondria can also be a viable strategy. The mechanism of action of HCMVs (a double-stranded DNA) [64] can be used to designate possible targets that can be pursued against SARS-CoV-2 (single-stranded RNA virus) [65]. The proposed recombinant drug can serve as a supplement to the target-specific drugs developed for antiviral actions.

The relevant modes of action of 2-DG at the entry point of the SARS-CoV-2 virus into the host cell and its effect on bioenergetics, glycosylation, ER stress, p53 expression, and cytokine storm in the virus-affected cell are summarized in Fig. 6.2.

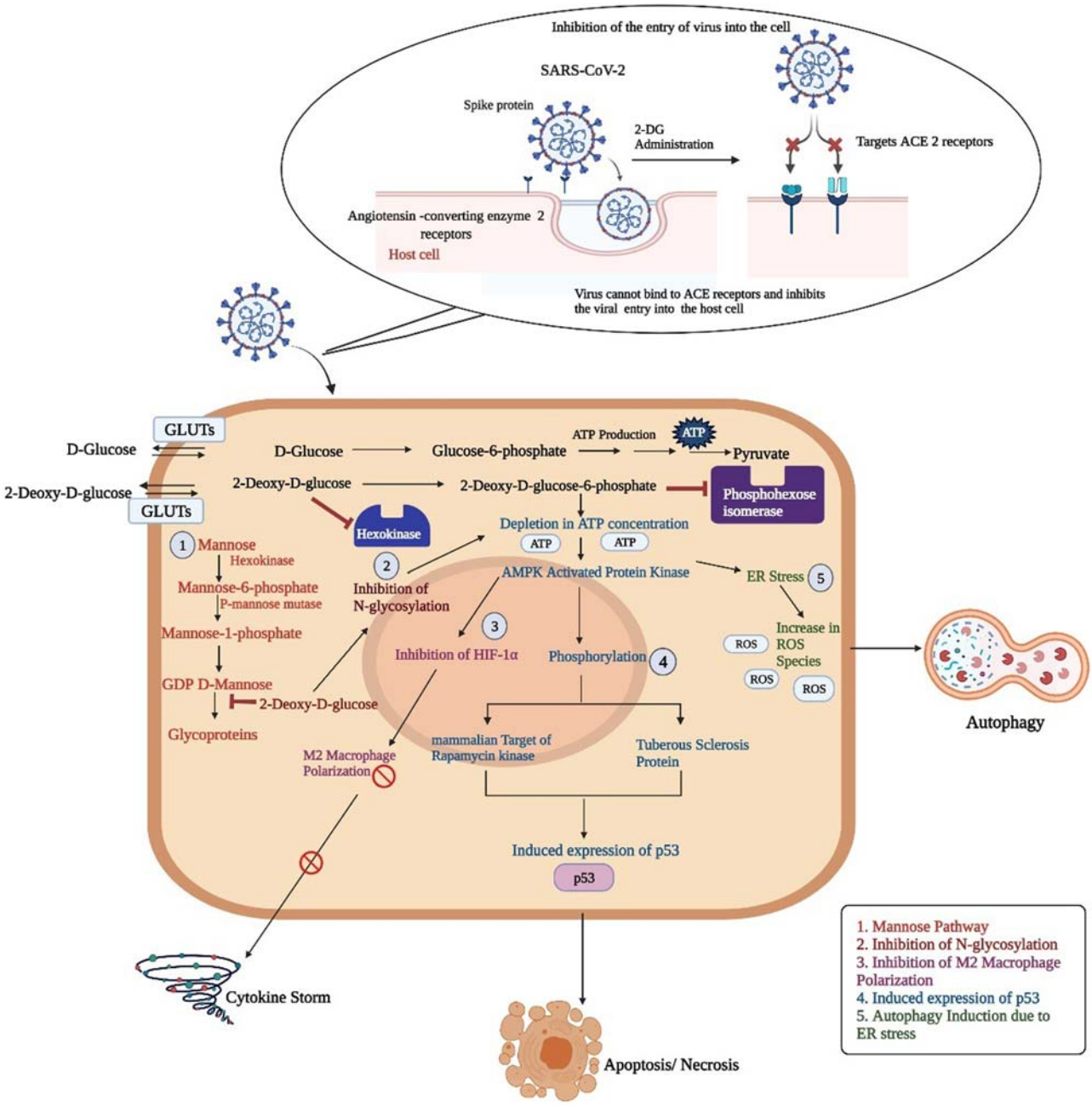

FIGURE 6.2 Possible relevant modes of action of 2-deoxy-D-glucose at the entry point of the SARS-CoV-2 virus into the host cell and its effect on the bioenergetics, glycosylation, ER stress, p53 expression, and cytokine storm in the virus-affected cell.

6.4 Safety of 2-deoxy-D-glucose drug

The symptoms that may develop after the oral administration of 2-DG are hypoglycemia, dizziness, nausea, and fatigue [20,21]. In rare cases, it may cause alterations in the regulation of the cardio-respiratory and immune systems [66]. Balakrishna et al., reported that 2-DG did not show any major signs of toxicity or side effects using the Toxicity Estimation Software Tool (T.E.S.T) [3]. Even though the half-life of 2-DG is approximately 90 minutes and it will disappear from the blood making the after-effects of 2-DG short-lived, the possibility of the development of long-term serious effects cannot be neglected [67].

6.5 The importance of 6-aminonicotinamide in the inhibition of the pentose phosphate pathway

6-Aminonicotinamide is an analog of niacin and an antagonist of nicotinamide, having cytotoxic properties and the potential to inhibit the NAD-dependent enzyme activity associated with ATP production. It was applied as an adjuvant to various cancer drugs as it can potentiate the effect of radiation and enhance the efficacy of anticancer drugs. It has been used with various other chemicals to increase the overall effect of treatment in various cancers such as breast, ovarian, and leukemia [68].

In a recent study by Bojkova et al., the levels of transketolase and transaldolase 1, the two constituents of the nonoxidative PPP branch, were found to have increased in cells infected with SARS-CoV-2. The increased levels of 6-phosphogluconate dehydrogenase (PGD) also indicated an increase in the oxidative PPP branch as well in the SARS-CoV-2 infected cells [69].

In a metabolomic study conducted by Delgado et al., it was observed that KSHV-infected samples had increased levels of PPP intermediates such as ribose-5-phosphate and ribulose-5-phosphate, suggesting an increase in nucleotide synthesis [70]. In another study by Thai et al., it was concluded that there was an increase in the flux of adenovirus infection through glycolysis and also potentially through the nonoxidative arm of the PPP that provides nucleotides for adenoviral DNA replication [71]. Thus, it can be said that viral RNA production and DNA replication is dependent on the PPP pathway along with the glycolytic pathway.

The metabolism of the host cells is altered by the viruses in a similar way to the Warburg effect, that is, by enhancing glycolysis [72]. The enhanced aerobic glycolysis was observed in the Zika virus with upregulation of glycolytic genes such as membrane glucose transporter 1 (GLUT 1) and various enzymes of glycolysis [73]. The reprogramming of viral proteins is responsible for the host cell metabolism and activation of signaling pathways such as PI3/AKT/mTOR and MAPK/ ERK; these pathways are responsible for the replication of MERS-CoV [72,74] Since both MERS-CoV and SARS-CoV-2 are betacoronaviruses, they share various similarities, and thus it can be considered that the Warburg effect promotes the signaling pathways in SARS-CoV-2 replication [72,75]. Thus, it cannot be neglected that the virus may promote alternative pathways to adapt nutrient conditions to bioenergetic and biosynthetic demands [72]. Therefore, it is essential to inhibit both the glycolytic and PPP pathways to alleviate the proliferation of the virus in infected cells.

The inhibitory effect of 6-AN on the PPP pathway has been observed in the mouse oocyte−cumulus complexes and in bovine pronuclear oocytes [76,77]. In a study by Ren et al., 6-AN was found to be effective against hepatitis B virus infection as it inhibited the expression levels of HBsAg (Hepatitis B surface antigen) in both in vivo and in vitro conditions [68].

6.6 Studies on 6-aminonicotinamide and 2-deoxy-D-glucose combination

The combination of 2-DG and 6-AN was found to be effective in P-31NMR spectroscopic studies on perfused EAT cells [78]. The ratio of β-ATP/Pi was taken as a measure of cellular energy status. On the administration of 2-DG alone, even though the ratio reduced to 50%, upon the removal of 2-DG, the decrease in ratio could be recovered. On the other hand, in the administration of a 2-DG and 6-AN combination, the peak of β-ATP decreased sharply. On perfusion, with a drug-free medium, the energy-linked DNA repair of EAT cells could not be recovered for up to 12 hours [79].

Varshney et al., have shown the effect of radiosensitization using a combination of 2-DG and 6-AN on murine Ehrlich ascites tumor-bearing mice. Administration of a combination of 2-DG (2 g/kg) and 6-AN (2 mg/kg) before irradiation led to the complete regression of tumors in 80% of animals with survival for more than 300 days [80].

Since 2-DG and 6-AN have the potential to cause CNS disturbances when administered independently, the use of a combination of 2-DG and 6-AN may have proven to be of major concern. It may have resulted in respiratory failure or

irreversible brain damage [80]. However, no animals showed any mortality, and in fact no major change in vital parameters was observed apart from temporary lethargy and marginal hyperventilation [80].

In another study conducted by Varshney et al., two human tumor cell lines, namely cerebral glioma, BMG-1, and squamous carcinoma cells, 4197, were considered. It was observed that the combination of 2-DG and 6-AN along with 2.5 Gy enhanced the cell death of both 4197 cells and BMG-1 by approximately 50% and 35%, respectively. In BMG-1 cells, administration of 2-DG (5 mM), 6-AN (5 μM) along with 2.5 Gy resulted in 70% growth inhibition [81].

In a study by Sharma et al., two malignant cell lines that were head and neck squamous carcinoma and cerebral glioma were studied. In this study, the level of nuclear-erythroid-related factor-2 (Nrf2) was analyzed as an enhanced level of Nrf2 was associated with cancer chemo- and radioresistance. A decrease in the level of Nrf2 was observed in both the irradiated malignant cells at 24 hours following treatment with a combination of 2-DG and 6-AN [82].

The administration of 6-AN alone could possibly lead to vitamin B1 deficiency syndrome along with transient neurological disturbances and teratogenic effects, whereas the dosage of the combination of 2-DG and 6-AN was observed to be lower than the individual use, resulting in overall reduced undesirable toxicity along with improved radiotherapy of cancer [81,83]. The increase in the reduced form of glutathione may act as a modulator of DNA repair and also acts as a radioprotector against DNA damage [84]. Therefore, the action of 2-DG on the inhibition of DNA repair under euoxic conditions is reversible in the presence of respiratory metabolism [85], and in order to diminish this reversibility of effects of 2-DG on bioenergetics, suitable modulators such as 6-AN may be used. 6-AN can potentially alter the energy-yielding pathways. Therefore, the combination of 2-DG and 6-AN will be promising in inhibiting the glycolytic and PPPs along with diminishing the reversibility effects of 2-DG.

6.7 Probable mechanism of action of combination of 2-deoxy-D-glucose and 6-aminonicotinamide

The administration of 6-aminonicotinamide (6-AN) as the inhibitor of the PPP has been shown to result in synergistic effects with 2-DG [80,81]. 6-AN is a nicotinamide analog and possesses antimitochondrial properties [79,82]. This antagonist of niacin competes with niacin in NAD(P^+) utilizing biological pathways to form 6-aminonicotinamide adenine dinucleotide phosphate (6-ANAD(P^+)). The 6-ANAD(P^+) inhibits reactions in which NAD and NADP played a part as coenzymes. In the PPP, the conversion of 6-phosphogluconate to ribulose-5-phosphate is inhibited due to the

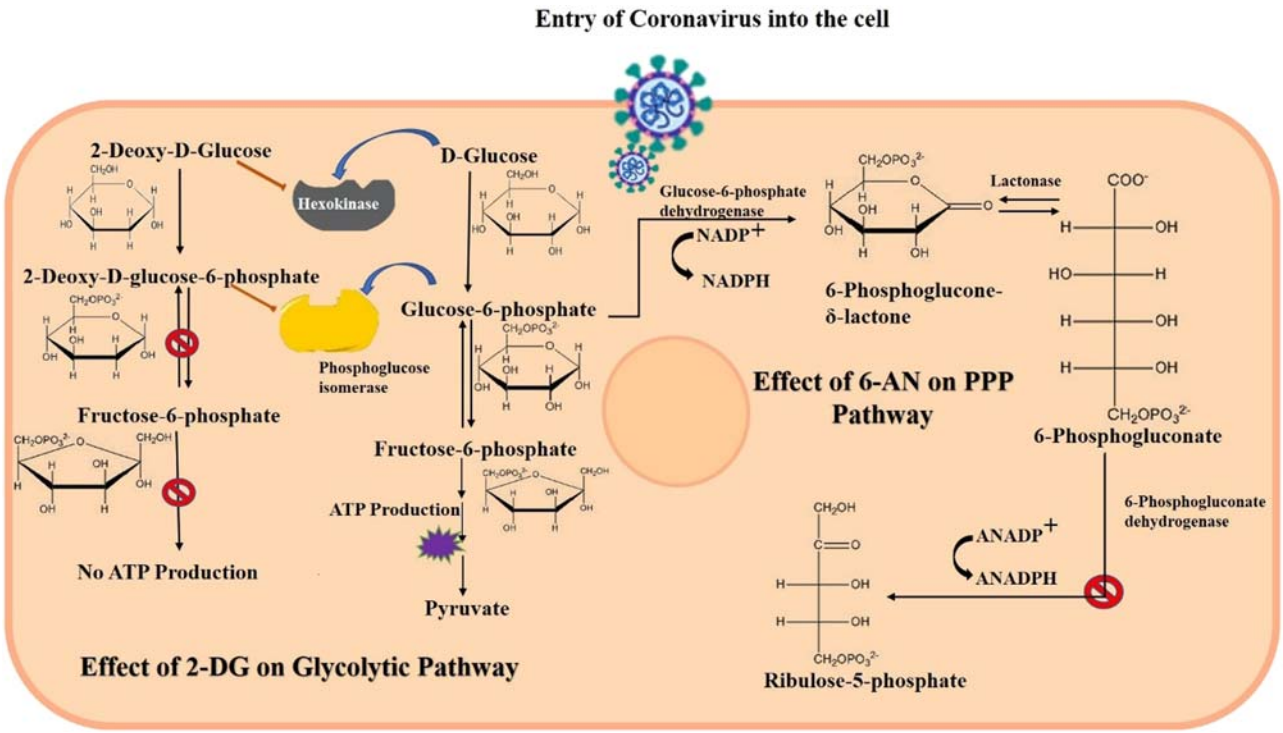

FIGURE 6.3 Effect of combination of 2-deoxy-D-glucose and 6-aminonicotinamide on glycolytic and pentose phosphate pathways.

inhibition of the enzyme 6-PGD, resulting in the accumulation of 6-phosphogluconate (6-PG); this step was necessary for the synthesis of coenzyme NAD^+, NADPH, and ribose unit essential for biosynthesis and DNA repair [79,85]. DNA repair does not allow the apoptosis of cells. Further chemical or enzymatic degradation of 6-ANAD(P^+) is not possible, resulting in the inhibition of glycolysis, the oxidative part of the PPP pathway, and oxidative phosphorylation occurring in the mitochondria.

The virus-infected cells may undergo an enhanced PPP pathway to provide an alternative pathway for glucose metabolism [72]. To stop the pathogenesis of the COVID-19 infection, 2-DG in combination with 6-AN can result in simultaneous inhibition of glycolysis and PPP proving it to be a better strategy. The combination of 2-DG and 6-AN will result in a compromised supply of NADPH, and the ratio of ATP/Pi will decrease. This decrease in the ATP/Pi ratio can result in the death of virus-infected cells. The 2-DG and 6-AN combination will inhibit the proliferation of the virus in the infected cells by provoking growth inhibition at the G1 phase. However, no inhibition occurs at the G2/M phase. This leads to cell cycle arrest at the G2/M phase accompanied by apoptosis of the cells [85]. It has been observed that 2-DG in combination with 6-AN can inhibit the bioenergetics of cells to such an extent that the recuperation of these cells was practically not possible up to 12 hours after withdrawal of the drug [78]. The combination of 2-DG and 6-AN is expected to result in selectively enhanced damage in the virus-infected cells with mitochondrial dysfunction and noncoordinated expression of antioxidant enzymes. The combination of 2-DG and 6-AN can lead to enhanced ROS-mediated cell killing along with the inhibition of repair and recovery processes. The effect of the 6-AN and 2-DG combination on the glycolytic and PPP has been shown in Fig. 6.3.

6.8 Conclusion

The 2-DG has received emergency use authorization as an adjuvant treatment along with the standard of care. However, additive effects could be observed if combined with 6-AN. Thus, a combination of 2-DG and 6-AN may act as a more efficacious treatment to curb the spread of COVID-19 as 6-AN can supplement the apoptosis of viral cells induced by 2-DG due to its effect on glycolysis and potentially through the nonoxidative arm of the PPP. However, further, in vitro and in vivo investigatory studies are required to gain a deeper understanding of their combined effects.

Acknowledgment

The authors thank their respective organizations for their scientific support.

Conflict of interest

The authors of the current review article declare no conflict of interest

References

[1] Gil C, Ginex T, Maestro I, Nozal V, Barrado-Gil L, Cuesta-Geijo MÁ, et al. COVID-19: drug targets and potential treatments. J Med Chem 2020;63(21):12359−86.
[2] DCGI approves anti-COVID drug developed by DRDO for emergency use [press release]. 2021.
[3] Balkrishna A, Thakur P, Singh S, Dev S, Jain V, Varshney A, et al. Glucose antimetabolite 2-Deoxy-D-glucose and its derivative as promising candidates for tackling COVID-19: insights derived from in silico docking and molecular simulations. Authorea 2020;. Available from: https://doi.org/10.22541/au.158567174.40895611.
[4] Khurana P, Varshney R. A network-biology led computational drug repurposing strategy to prioritize therapeutic options for COVID-19. Heliyon 2021;8(5):e09387. Available from: https://doi.org/10.1016/j.heliyon.2022.e09387.
[5] Bhatt AN, Kumar A, Rai Y, Kumari N, Vedagiri D, Harshan KH, et al. Glycolytic inhibitor 2-Deoxy-D-glucose attenuates SARS-CoV-2 multiplication in host cells and weakens the infective potential of progeny virions. Life Sci 2022;295:120411. Available from: https://doi.org/10.1016/j.lfs.2022.120411.
[6] Bere J, Jonnalagadda ND, Kappari L, Karangula J, Boggula N, Kappari V. 2-Deoxy-D-glucose: an update review. J Innov Dev Pharm Tech Sci (Jidpts) 2021;4(5).
[7] Dwarakanath B, Singh D, Banerji AK, Sarin R, Venkataramana N, Jalali R, et al. Clinical studies for improving radiotherapy with 2-deoxy-D-glucose: present status and future prospects. J Cancer Res Ther 2009;5(9):21.
[8] Sahu KK, Kumar R. Role of 2-deoxy-D-glucose (2-DG) in COVID-19 disease: a potential game-changer. J Family Med Prim Care 2021;10(10):3548.
[9] Xi H, Kurtoglu M, Lampidis TJ. The wonders of 2-deoxy-d-glucose. IUBMB life 2014;66(2):110−21.

[10] Jain VK, Höltz GW, Pohlit W, Purohit SC. Inhibition of unscheduled DNA synthesis and repair of potentially lethal X-ray damage by 2-deoxy-D-glucose in yeast. Int J Radiat Biol Rel Stud Phys. Chem Med 1977;32(2):175–80.
[11] Jain V, Kalia V, Sharma R, Maharajan V, Menon M. Effects of 2-deoxy-D-glucose on glycolysis, proliferation kinetics and radiation response of human cancer cells. Int J Radiat Oncol Biol Phys 1985;11(5):943–50. Available from: https://doi.org/10.1016/0360-3016(85)90117-8.
[12] Dwarakanath BSR, Jain VK. Modification of the radiation induced damage by 2-deoxy-D-glucose in organ cultures of human cerebral gliomas. Int J Radiat Oncol Biol Phys 1987;13(5):741–6. Available from: https://doi.org/10.1016/0360-3016(87)90293-8.
[13] Rae C, Sey CH, Mairs RJ. Radiosensitization of prostate cancer cells by 2-deoxyglucose. Madridge J Oncog 2018;2(1):30–4.
[14] Li L, Fath MA, Scarbrough PM, Watson WH, Spitz DR. Combined inhibition of glycolysis, the pentose cycle, and thioredoxin metabolism selectively increases cytotoxicity and oxidative stress in human breast and prostate cancer. Redox Biol 2015;4:127–35.
[15] Latz D, Thonke A, Jüling-Pohlit L, Pohlit W. Tumor response to ionizing radiation and combined 2-deoxy-D-glucose application in EATC tumor bearing mice: monitoring of tumor size and microscopic observations. Strahlenther Onkol 1993;169(7):405–11.
[16] Maschek G, Savaraj N, Priebe W, Braunschweiger P, Hamilton K, Tidmarsh GF, et al. 2-Deoxy-d-glucose increases the efficacy of adriamycin and paclitaxel in human osteosarcoma and non-small cell lung cancers in vivo. Cancer Res 2004;64(1):31–4.
[17] Boutrid H, Jockovich M-E, Murray TG, Pina Y, Feuer WJ, Lampidis TJ, et al. Targeting hypoxia, a novel treatment for advanced retinoblastoma. Investig Ophthalmol Vis Sci 2008;49(7):2799–805.
[18] Blough HA, Giuntoli RL. Successful treatment of human genital herpes infections with 2-deoxy-D-glucose. JAMA. 1979;241(26):2798–801.
[19] Naqvi S, Joshi K, Basu A, Jain V, editors. Optimizing cancer therapy—Studies on the combination of 131-I with 2-deoxy-d-glucose in the treatment of thyroid cancer. Bombay, India: Abstr VI Ann Conf Soc Nucl Med;; 1974.
[20] Mohanti BK, Rath GK, Anantha N, Kannan V, Das BS, Chandramouli BA, et al. Improving cancer radiotherapy with 2-deoxy-D-glucose: phase I/II clinical trials on human cerebral gliomas. Int J Radiat Oncol Biol Phys 1996;35(1):103–11. Available from: https://doi.org/10.1016/s0360-3016(96)85017-6.
[21] Singh D, Banerji AK, Dwarakanath BS, Tripathi RP, Gupta JP, Mathew TL, et al. Optimizing cancer radiotherapy with 2-deoxy-D-glucose. Strahlentherapie und Onkol 2005;181(8):507–14.
[22] Prasanna VK, Venkataramana NK, Dwarakanath B, Santhosh V. Differential responses of tumors and normal brain to the combined treatment of 2-DG and radiation in glioablastoma. J Cancer Res Ther 2009;5(9):44.
[23] Gounder MK, Lin H, Stein MN, Goodin S, Bertino JR, DiPaola RS. Phase I trial of 2-deoxyglucose for treatment of advanced solid tumors and hormone refractory prostate cancer: a pharmacokinetics (PK) assessment. AACR 2010;.
[24] Raez LE, Papadopoulos K, Ricart AD, Chiorean EG, DiPaola RS, Stein MN, et al. A phase I dose-escalation trial of 2-deoxy-D-glucose alone or combined with docetaxel in patients with advanced solid tumors. Cancer Chemother Pharmacol 2013;71(2):523–30.
[25] Leung HJ, Duran EM, Kurtoglu M, Andreansky S, Lampidis TJ, Mesri EA. Activation of the unfolded protein response by 2-deoxy-D-glucose inhibits Kaposi's sarcoma-associated herpesvirus replication and gene expression. Antimicrobial Agents Chemother 2012;56(11):5794–803.
[26] Maehama T, Patzelt A, Lengert M, Hutter KJ, Kanazawa K, Zur Hausen H, et al. Selective down-regulation of human papillomavirus transcription by 2-deoxyglucose. Int J Cancer 1998;76(5):639–46.
[27] Bhatt AN, Shenoy S, Munjal S, Chinnadurai V, Agarwal A, Kumar AV, et al. 2-Deoxy-D-glucose as an adjunct to standard of care in the medical management of COVID-19: a proof-of-concept & dose-ranging randomised clinical trial. medRxiv 2021;.
[28] Leap J, Villgran V, Cheema T. COVID-19: epidemiology, pathophysiology, transmission, symptoms. Crit Care Nurs Q 2020;43(4):338–42.
[29] Yuki K, Fujiogi M, Koutsogiannaki S. COVID-19 pathophysiology: a review. Clin Immunol 2020;215:108427.
[30] Bosch BJ, Van der Zee R, De Haan CA, Rottier PJ. The coronavirus spike protein is a class I virus fusion protein: structural and functional characterization of the fusion core complex. J Virol 2003;77(16):8801.
[31] Dhochak N, Singhal T, Kabra S, Lodha R. Pathophysiology of COVID-19: why children fare better than adults? Indian J Pediatr 2020;87:537–46.
[32] Hörnich BF, Großkopf AK, Schlagowski S, Tenbusch M, Kleine-Weber H, Neipel F, et al. SARS-CoV-2 and SARS-CoV spike-mediated cell-cell fusion differ in their requirements for receptor expression and proteolytic activation. J Virol 2021;95(9).
[33] Saha A, Saha B. Novel coronavirus SARS-CoV -2 (Covid-19) dynamics inside the human body. Rev Med Virol 2020;30(5).
[34] Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 2020;579(7798):270–3. Available from: https://doi.org/10.1038/s41586-020-2012-7.
[35] Tan M, Liu Y, Zhou R, Deng X, Li F, Liang K, et al. Immunopathological characteristics of coronavirus disease 2019 cases in Guangzhou, China. Immunology. 2020;160(3):261–8.
[36] Liu X, Fang P, Fang L, Hong Y, Zhu X, Wang D, et al. Porcine deltacoronavirus nsp15 antagonizes interferon-β production independently of its endoribonuclease activity. Mol Immunol 2019;114:100–7.
[37] Sharma A, Goyal S, Yadav AK, Kumar P, Gupta L. In-silico screening of plant-derived antivirals against main protease, 3CLpro and endoribonuclease, NSP15 proteins of SARS-CoV-2. J Biomol Struct Dyn 2020;1–15.
[38] Codo AC, Davanzo GG, de Brito Monteiro L, de Souza GF, Muraro SP, Virgilio-da-Silva JV, et al. Elevated glucose levels favor SARS-CoV-2 infection and monocyte response through a HIF-1α/glycolysis-dependent axis. Cell Metab 2020;32(3):437–46 e5.
[39] Ghosh B, Roy S, Singh JK. The mode of therapeutic action of 2-deoxy D-glucose: anti- viral or glycolysis blocker? ChemRxiv 2021;. Available from: https://doi.org/10.26434/chemrxiv-2021-2z6ln.
[40] Choi SY, Heo M-J, Lee C, Choi YM, An I-s, Bae S, et al. 2-deoxy-d-glucose ameliorates animal models of dermatitis. Biomedicines. 2020;8(2):20.

[41] Bojkova D, Klann K, Koch B, Widera M, Krause D, Ciesek S, et al. Proteomics of SARS-CoV-2-infected host cells reveals therapy targets. Nature. 2020;583(7816):469–72.
[42] Darnell Jr JE, Eagle H. Glucose and glutamine in poliovirus production by HeLa cells. Virology. 1958;6(2):556–66.
[43] Sanchez EL, Lagunoff M. Viral activation of cellular metabolism. Virology. 2015;479:609–18.
[44] Eagle H, Habel K. The nutritional requirements for the propagation of poliomyelitis virus by the HeLa cell. J Exp Med 1956;104(2):271–87.
[45] Hirabara SM, Gorjao R, Levada-Pires AC, Masi LN, Hatanaka E, Cury-Boaventura MF, et al. Host cell glutamine metabolism as a potential antiviral target. Clin Sci 2021;135(2):305–25.
[46] Fontaine KA, Sanchez EL, Camarda R, Lagunoff M. Dengue virus induces and requires glycolysis for optimal replication. J Virol 2015;89 (4):2358–66.
[47] Dong Y, Zhang X, Li M, Ying Q, Feng Y, Li Z, et al. Hantaan virus replication is promoted via AKT activated mitochondria OXPHOS. bioRxiv 2022;.
[48] O'Neill S, Porter RK, McNamee N, Martinez VG, O'Driscoll L. 2-Deoxy-D-Glucose inhibits aggressive triple-negative breast cancer cells by targeting glycolysis and the cancer stem cell phenotype. Sci Rep 2019;9(1):1–11.
[49] El Mjiyad N, Caro-Maldonado A, Ramírez-Peinado S, Muñoz-Pinedo C. Sugar-free approaches to cancer cell killing. Oncogene. 2011;30 (3):253–64.
[50] Pelicano H, Martin D, Xu R, Huang P. Glycolysis inhibition for anticancer treatment. Oncogene. 2006;25(34):4633–46.
[51] Samal KC, Panda B, Behera L. Anti-Covid drug: 2-deoxy-D-glucose and its mechanism of action. Biotica Res Today 2021;3(5):345–7.
[52] Miwa H, Shikami M, Goto M, Mizuno S, Takahashi M, Tsunekawa-Imai N, et al. Leukemia cells demonstrate a different metabolic perturbation provoked by 2-deoxyglucose. Oncol Rep 2013;29(5):2053–7.
[53] Pajak B, Siwiak E, Sołtyka M, Priebe A, Zieliński R, Fokt I, et al. 2-Deoxy-d-glucose and its analogs: from diagnostic to therapeutic agents. Int J Mol Sci 2020;21(1):234.
[54] Shutt D, O'Dorisio MS, Aykin-Burns N, Spitz D. 2-deoxy-D-glucose induces oxidative stress and cell killing in human neuroblastoma cells. Cancer Biol Ther 2010;9(11):853–61.
[55] Zhao Q, Chu Z, Zhu L, Yang T, Wang P, Liu F, et al. 2-Deoxy-d-glucose treatment decreases anti-inflammatory M2 macrophage polarization in mice with tumor and allergic airway inflammation. Front Immunol 2017;8.
[56] Thirumalaisamy R, Riaz Khan VA, Sivasankar C, Deepa M. Hyaluronic acid - 2-deoxy-D-glucose conjugate act as a promising targeted drug delivery option for the treatment of COVID-19. Int J Adv Sci Eng 2021;. Available from: https://doi.org/10.29294/IJASE.7.4.2021.1995-2005.
[57] Khaitan D, Chandna S, Arya MB, Dwarakanath B. Differential mechanisms of radiosensitization by 2-deoxy-D-glucose in the monolayers and multicellular spheroids of a human glioma cell line. Cancer Biol Ther 2006;5(9):1142–51.
[58] Yokananth R, Prashanth TL, Sekar Y. Implications of 2-deoxy-D-glucose as a therapeutic molecule in treating COVID-19 patients. Phar Innov. J 2022;11(1):01–9.
[59] DeVito SR, Ortiz-Riaño E, Martínez-Sobrido L, Munger J. Cytomegalovirus-mediated activation of pyrimidine biosynthesis drives UDP–sugar synthesis to support viral protein glycosylation. Proc Natl Acad Sci 2014;111(50):18019–24.
[60] Chambers JW, Maguire TG, Alwine JC. Glutamine metabolism is essential for human cytomegalovirus infection. J Virol 2010;84(4):1867–73.
[61] Bharadwaj S, Singh M, Kirtipal N, Kang SG. SARS-CoV-2 and glutamine: SARS-CoV-2 triggered pathogenesis via metabolic reprograming of glutamine in host cells. Front Mol Biosci 2021;7:627842.
[62] Combs JA, Monk CH, Harrison MA, Norton EB, Morris CA, Sullivan DE, et al. Inhibiting cytomegalovirus replication through targeting the host electron transport chain. Antivir Res 2021;194:105159.
[63] Combs JA, Norton EB, Saifudeen ZR, Bentrup KHZ, Katakam PV, Morris CA, et al. Human cytomegalovirus alters host cell mitochondrial function during acute infection. J Virol 2019;94(2):e01183–19.
[64] Liu Y-T, Strugatsky D, Liu W, Zhou ZH. Structure of human cytomegalovirus virion reveals host tRNA binding to capsid-associated tegument protein. Nat Commun 2021;12(1):1–9.
[65] Hadi J, Dunowska M, Wu S, Brightwell G. Control measures for sars-cov-2: a review on light-based inactivation of single-stranded rna viruses. Pathogens. 2020;9(9):737.
[66] Vijayaraghavan R, Kumar D, Dube S, Singh R, Pandey K, Bag B, et al. Acute toxicity and cardio-respiratory effects of 2-deoxy-D-glucose: a promising radio sensitiser. Biomed Env Sci BES 2006;19(2):96–103.
[67] Dwarakanath B, Jain V. Targeting glucose metabolism with 2-deoxy-D-glucose for improving cancer therapy. Fut Oncol 2009;5(5):581–5.
[68] Ren F, Yang X, Hu Z-W, Wong VKW, Xu H-Y, Ren J-H, et al. Niacin analogue, 6-aminonicotinamide, a novel inhibitor of hepatitis B virus replication and HBsAg production. EBioMedicine. 2019;49:232–46.
[69] Bojkova D, Costa R, Reus P, Bechtel M, Jaboreck M-C, Olmer R, et al. Targeting the pentose phosphate pathway for SARS-CoV-2 therapy. Metabolites. 2021;11(10):699.
[70] Delgado T, Sanchez EL, Camarda R, Lagunoff M. Global metabolic profiling of infection by an oncogenic virus: KSHV induces and requires lipogenesis for survival of latent infection. PLoS Pathog 2012;8(8):e1002866.
[71] Thai M, Graham NA, Braas D, Nehil M, Komisopoulou E, Kurdistani SK, et al. Adenovirus E4ORF1-induced MYC activation promotes host cell anabolic glucose metabolism and virus replication. Cell Metab 2014;19(4):694–701.
[72] Icard P, Lincet H, Wu Z, Coquerel A, Forgez P, Alifano M, et al. The key role of Warburg effect in SARS-CoV-2 replication and associated inflammatory response. Biochimie. 2021;180:169–77.

[73] Singh S, Singh PK, Suhail H, Arumugaswami V, Pellett PE, Giri S, et al. AMP-activated protein kinase restricts Zika virus replication in endothelial cells by potentiating innate antiviral responses and inhibiting glycolysis. J Immunol 2020;204(7):1810–24.
[74] Kindrachuk J, Ork B, Mazur S, Holbrook MR, Frieman MB, Traynor D, et al. Antiviral potential of ERK/MAPK and PI3K/AKT/mTOR signaling modulation for Middle East respiratory syndrome coronavirus infection as identified by temporal kinome analysis. Antimicrobial Agents Chemother 2015;59(2):1088–99.
[75] Rabaan AA, Al-Ahmed SH, Haque S, Sah R, Tiwari R, Malik YS, et al. SARS-CoV-2, SARS-CoV, and MERS-COV: a comparative overview. Infez Med 2020;28(2):174–84.
[76] Comizzoli P, Urner F, Sakkas D, Renard JP. Up-regulation of glucose metabolism during male pronucleus formation determines the early onset of the s phase in bovine zygotes. Biol Reprod 2003;68(5):1934–40.
[77] Downs SM, Humpherson PG, Leese HJ. Meiotic induction in cumulus cell-enclosed mouse oocytes: involvement of the pentose phosphate pathway. Biol Reprod 1998;58(4):1084–94.
[78] Sharma R, Hanssum H, Jain V. In vivo NMR spectroscopic studies on the bioenergetic changes induced by metabolic modulators in Ehrlich ascites tumour cells. Indian J Biochem Biophys 1996;33(2):122–30.
[79] Sharma R, Maitra A, Jain V. Effects of 6-aminonicotinamide and 2-deoxy-D-glucose combination on the bioenergetics of perfused Ehrlich ascites tumour cells as monitored by 31 P-MR spectroscopy. Indian J Biochem Biophys 2000;37(5):307–12.
[80] Varshney R, Gupta S, Dwarakanath BS. Radiosensitization of murine ehrlich ascites tumor by a combination of 2-deoxy-D-glucose and 6-aminonicotinamide. Technol Cancer Res Treat 2004;3(6):659–63.
[81] Varshney R, Dwarakanath B, Jain V. Radiosensitization by 6-aminonicotinamide and 2-deoxy-D-glucose in human cancer cells. Int J Radiat Biol 2005;81(5):397–408.
[82] Sharma PK, Varshney R. 2-Deoxy-D-glucose and 6-aminonicotinamide-mediated Nrf2 down regulation leads to radiosensitization of malignant cells via abrogation of GSH-mediated defense. Free Radic Res 2012;46(12):1446–57.
[83] Herter FP, Weissman SG, Thompson HG, Hyman G, Martin DS. Clinical experience with 6-aminonicotinamide. Cancer Res 1961;21(1):31–7.
[84] Chatterjee A. Reduced glutathione: a radioprotector or a modulator of DNA-repair activity? Nutrients. 2013;5(2):525–42.
[85] Bhardwaj R, Sharma PK, Jadon S, Varshney R. A combination of 2-deoxy-D-glucose and 6-aminonicotinamide induces cell cycle arrest and apoptosis selectively in irradiated human malignant cells. Tumor Biol 2012;33(4):1021–30.