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Use of 2-deoxy-D-glucose in virology research: A mini review

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Abstract
2-deoxy-D-glucose, widely known as 2-DG, is a synthetic analogue of glucose that has biological effect on metabolic pathways – glycolysis and pentose phosphate pathway; on signalling pathways - 5′-AMP-activated kinase – AMPK, mammalian target of rapamycin complex 1 – mTORC1; and on biosynthesis of lipids and protein-N-glycosylation, thereby demonstrating its role as an anti-cancer, anti-inflammatory, calorie restriction mimetic, and anti-viral agent. Although the property of 2-DG as an anti-viral agent was widely known during the COVID-19 pandemic, its potential to inhibit influenza virus was evaluated for the first time in the year 1959. Subsequently, experimental studies pertaining to inhibition of viral infection by 2-DG was explored in viruses belonging to the family of Paramyxoviridae, Rhabdoviridae, Togaviridae, Herpesviridae, Flaviviridae, Adenoviridae and Retroviridae. The anti-viral potential of 2-DG in viruses experimented since the mid 1900s has been reviewed here in brief.

Keywords: 2-DG, glucose analogue, anti-viral, glycolysis, viral replication, metabolic pathway

Introduction
2-deoxy-D-glucose, widely known as 2-DG, is a synthetic analogue of glucose. Structurally, glucose and 2-DG varies at the second carbon position, *i.e.*, a hydroxyl group is present in glucose, whereas a hydrogen is present in 2-DG. This structural difference of the presence of a hydrogen in 2-DG inhibits the function of hexokinase and phosphohexose isomerase (Sols and Crane, 1954) [19], which are essential enzymes for the conversion of glucose to pyruvate – the glycolysis pathway. Thus, inhibition of glycolysis by 2-DG, followed by depletion of adenosine triphosphate (ATP), establishes 2-DG as an anti-metabolite of glucose (Xi *et al*., 2011) [25]. In addition to inhibition of glycolysis, 2-DG has also been reported to modulate adenosine-activated protein kinase (AMPK), tumor suppressor gene p53 and protein N-glycosylation in endoplasmic reticulum (ER), thereby demonstrating its role as an anti-cancer, anti-inflammatory, calorie restriction mimetic, and anti-viral agent (Pajak *et al*., 2019) [16]. Although, the various physiological effects of 2-DG have been reviewed well in earlier papers, the use, and effects of the same in viruses of veterinary importance have not been coalesced. Hence, this short review provides information on the experimental use of 2-DG on viruses since the mid 1900s.

History
Upon perusal of literatures, it was observed that the earliest research report on the use of 2-DG was documented in the year 1959, where 2-DG was evaluated for its potential to inhibit the synthesis of influenza virus in chicken chorio-allantoic membrane (Kilbourne *et al.*, 1959) [12]. This study reported that 2-DG intervenes in the synthesis and elongation of oligosaccharides in the viral envelope glycoproteins. Based on this pioneering study, many experimental studies pertaining to inhibition of viral infection by 2-DG was explored subsequently, which included viruses belonging to the family of Paramyxoviridae, Rhabdoviridae, Togaviridae (Kaluza *et al*., 1972) [10], Herpesviridae (Courtney *et al*., 1973) [4], Flaviviridae (Woodman *et al*., 1977) [23], Adenoviridae (Xi *et al.* 2014) [26] and Retroviridae (Blough *et al*., 1986) [2].

Biological Effects of 2-DG

The biochemical structure of 2-DG differs from the glucose at the second carbon position, where the hydroxyl group is substituted by a hydrogen. 2-DG has been reported to have biological effect on metabolic pathways – glycolysis (Woodward *et al*., 1952) [24], pentose phosphate pathway (Young and Mayor, 1979) [27]; on signalling pathways - 5′-AMP-activated kinase – AMPK (Hardie and Lin, 2017) [8], mammalian target of rapamycin complex 1 – mTORC1 (Jung *et al.*, 2010) [9]; and on biosynthesis of lipids and protein-N-glycosylation (Datema *et al*., 1978) [5].

The mechanism of action of 2-DG in glycolysis is well studied. First and foremost, inside a cell, 2-DG is converted to phosphorylated 2-DG (2-DG-6-P) by hexokinase, the rate limiting enzyme in the glycolysis pathway. Secondly, the 2-DG-6-P should be metabolized into the subsequent component with the help of phospho-glucose isomerase (PGI). However, this does not occur due to inhibition of PGI by 2-DG-6-P, and thus there occurs increased intracellular accumulation of 2-DG-P. The accumulated 2-DG-P component now inhibits the hexokinase enzyme, which leads to cessation of glycolysis pathway ensuing ATP depletion. Thus, 2-DG causes depletion of ATP and deprivation of glucose levels (Chen *et al*., 1992; Demetrakopoulos *et al*., 1982; Goel, 2021; Wick *et al*., 1957) [3, 6, 7, 22].

These depleted levels of ATP lead to alteration in the ATP/AMP ratio, thus activating the AMP-activated protein kinase (AMPK) and the deprivation of glucose levels lead to production of reactive oxygen species (ROS), which in turn activates AMPK pathway (Jung *et al*., 2010) [9]. Activation of AMPK pathway leads to endoplasmic reticulum (ER) stress and autophagy by direct phosphorylation of TSC1 proteins in the mammalian target of rapamycin kinase (mTORC) complex. The prolonged accumulation of self-degraded products of autophagy leads to induction of apoptosis. Further, 2-DG has been reported to have effects on structural and storage carbohydrates, and glycosphingolipids (Jung *et al*., 2010) [9].

The inhibition of hexokinase and PGI enzymes by 2-DG has effects on pentose phosphate pathway also. The inhibition of these enzymes leads to cessation of synthesis of fructose-6-phosphate, an essential glycolytic mediator in the synthesis of ribulose-6-phosphate in the non-oxidative branch of pentose phosphate pathway. Whereas in the oxidative branch, the levels of glucose-6-phosphate, the precursor of ribose-5-phosphate is decreases by 2-DG. Thus, 2-DG has been reported to have effects on both oxidative and non-oxidative branches of pentose phosphate pathway leading to impairment of nucleotide synthesis (Xi *et al*., 2014) [26].

2-DG in viral replication

Experimentation of 2-DG in viral replication has been reported since the late 1950s, with the first report on the significant reduction of influenza virus concentration in the chorio-allantoic membrane of embryonated chicken egg (Kilbourne, 1959) [12]. Ever since then,

2-DG has been explored as an anti-viral agent in Herpesviridae (Courtney *et al*., 1973; Radsak *et al*., 1981) [4, 17], Flaviviridae (Woodman *et al*., 1977) [23], Orthomyxoviridae (Kaluza *et al*., 1972; Kilbourne, 1959) [10, 12], Rhabdoviridae (Kaluza *et al*., 1972) [10], Retroviridae, Paramyxoviridae (Tripathy and Mohanty, 1979) [21], Togaviridae (Kaluza *et al*., 1972) [10] and Picornaviridae. The details of previous studies detailing the effect of 2-DG on various viruses have been tabulated (Table 1).

The mechanism of anti-viral activity of 2-DG include: (i) false incorporation of 2-DG into the structure of the viral capsid thereby compromising the infectious nature of the virus; (ii) interference in the glycoprotein folding process in the endoplasmic reticulum which leads to stress in endoplasmic reticulum thereby activating unfolded protein response leading to shut down of protein synthesis and blocking the viral replication; (iii) inhibition of glycolysis thereby leading to deprivation of building blocks required for viral replication (Mesri and Lampidis, 2021) [14]. Deprivation of building blocks is the mechanism of inhibition of severe acute respiratory syndrome related coronavirus – 2 (SARS-CoV-2), wherein glycolysis that provides energy, and glycosylation that provides glycoprotein for production of spike protein are inhibited (Mesri and Lampidis, 2021) [14].

Table 1: Previous studies of effects of 2-DG on virus replication of various viruses

S. No.	Virus family	Virus studied	Species affected	Model under study	Effect of 2DDG on viral replication	Highlights of the study involved	Reference
1.	Adeno-associated virus	Adeno-associated virus along with Herpes Simplex virus coinfection	Humans	Hep-2 cells	+/-	▪ Inhibited early viral protein synthesis ▪ Inhibited assembly of virus into capsid configuration	(Xi *et al*., 2014) [26]
2.	Flaviviridae	Japanese B encephalitis virus	Humans	BHK-21 cells	+	▪ Inhibited assembly of virus and further maturation into infectious form	(Woodman *et al*., 1977) [23]
3.	Herpesviridae	Cytomegalovirus	Humans	Diploid human embryonic lung cells	+	▪ Interfered with mRNA synthesis essential for viral replication ▪ Depleted UTP pool	(Radsak *et al*., 1981) [17]
4.	Herpesviridae	Herpes Simplex virus	Humans	Vero cells	+	▪ Attachment and penetration of virus to host cell is decreased resulting in reduced infectivity	(Spivack *et al*., 1982) [20]
5.	Herpesviridae	Herpes Simplex virus	Humans	BSC1 cells	+	▪ Yield of infectious viral particles reduced	(Courtney *et* al., 1973) [4]
6.	Herpesviridae	Herpes Simplex virus	Humans	Rabbits	+	▪ Reduction in the ocular herpetic keratitis lesions was observed	(Ray *et* al., 1974) [18]
7.	Herpesviridae	Herpes Simplex virus – 1	Humans	Mice and Guinea pigs	-	▪ No effect was observed on skin lesions ▪ Did not clear the infection	(Kern *et al*., 1982) [11]
8.	Herpesviridae	Herpes Simplex virus – 1	Humans	Women	+	▪ Infection was cleared in more than 85% of the women infected with Herpes virus	(Blough and Giuntoli, 1979) [1]
9.	Herpesviridae	Infectious bovine rhino-tracheitis viral infection	Bovine	Calves	+/-	• Injection of 2DDG on a daily basis – No protection • Ocular instillation – Reduced viral-	(Mohanty *et al*., 1980) [15]

						induced conjunctivitis and keratoconjunctivitis	
10.	Herpesviridae	Pseudorabies	Porcine	Primary rabbit kidney cells	+	▪ Production of non-infectious viral particles ▪ Fusion of virus into host cell is inhibited	(Ludwig and Rott, 1975) [13]
11.	Orthomyxoviridae	Fowl plague virus	Avian	Primary chicken fibroblast cells	+	▪ Significantly inhibited the production of viral particles	(Kaluza *et al*., 1972) [10]
12.	Orthomyxoviridae	Influenza virus	Humans	Chorio-allantoic membrane of chicken embryo	+	▪ Viral concentration was 8 to 32-fold lesser upon comparison with control at 8 hours post inoculation	(Kilbourne *et al.*, 1959) [12]
13.	Paramyxoviridae	Bovine respiratory syncytial virus	Bovine	Bovine turbinate cell cultures	+	▪ Replication and cytopathic effects – reversibly inhibited ▪ Mature virions lacked characteristic surface projections	(Tripathy and Mohanty, 1979) [21]
14.	Paramyxoviridae	Newcastle disease virus	Avian	Primary chicken fibroblast cells	+	▪ Significantly inhibited the production of viral particles	(Kaluza *et al*., 1972) [10]
15.	Retroviridae	Human Immunodeficiency Virus	Humans	H9 / CEM / HUT-78 / MT-4	+	▪ Blocked the formation of syncytia ▪ Inhibits viral fusion, attachment and penetration	
16.	Rhabdoviridae	Vesicular stomatitis virus	Bovine and porcine	BHK or HeLa cells	+	▪ Significantly inhibited the production of viral particles	
17.	Togaviridae	Semliki Forest virus	Humans	BHK or HeLa cells	+	▪ Significantly inhibited the production of viral particles	
18.	Togaviridae	Sindbis virus	Humans	BHK or HeLa cells	+	▪ Significantly inhibited the production of viral particles	

Conclusion

In conclusion, 2-DG has been explored for its anti-viral property for the past five decades. Effects of 2-DG on viral replication have been studied on viruses belonging to the family of Paramyxoviridae, Rhabdoviridae, Togaviridae, Herpesviridae, Flaviviridae, Adenoviridae and Retroviridae. Anti-viral approaches using 2-DG gained significance in the inhibition of SARS-CoV-2. 2-DG is considered as a potential anti-viral therapeutic agent that is reported to selectively accumulate in infected cell, however, their effects on normal cells need to be studied further for effective use of the same.

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