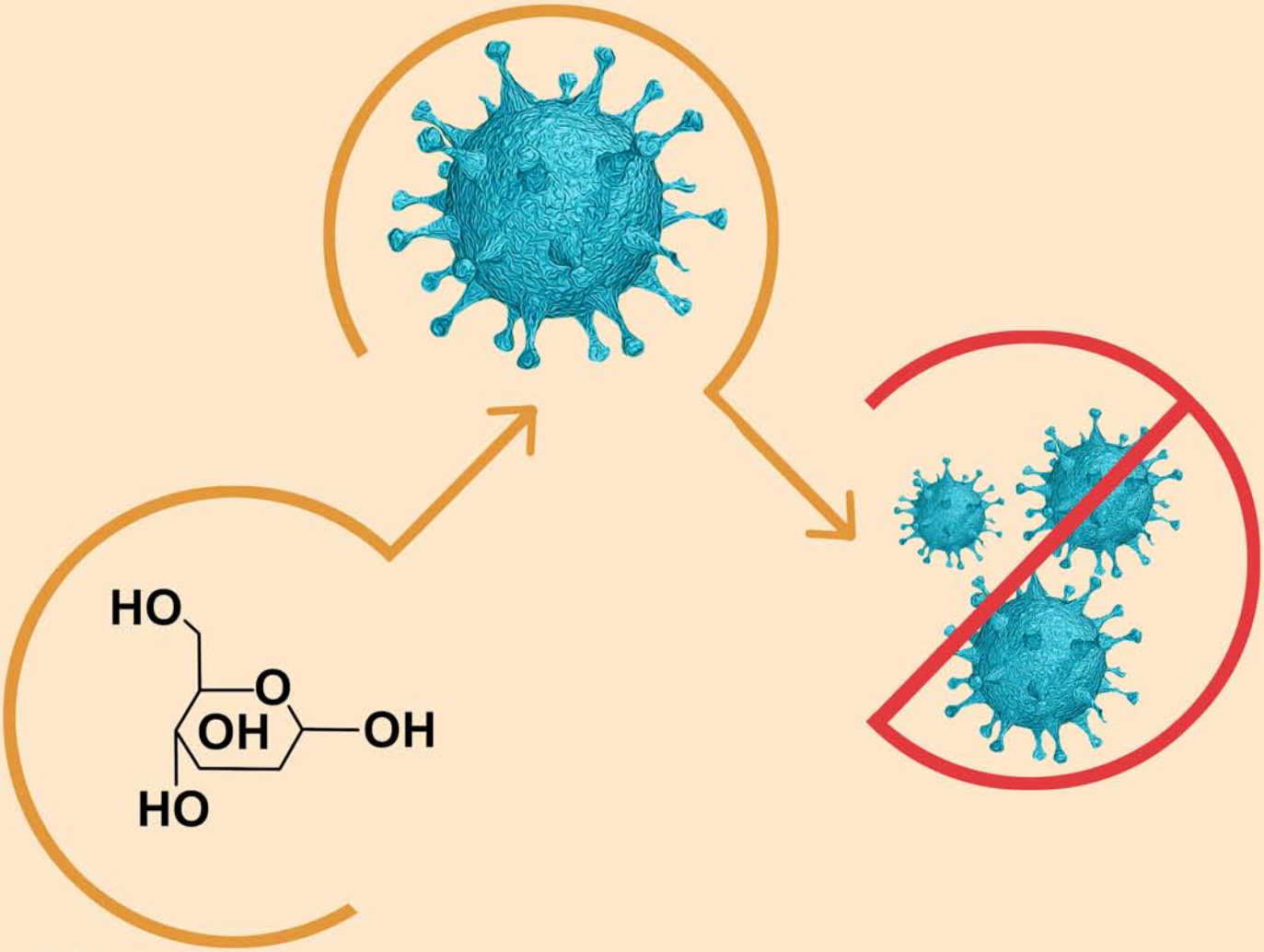
2-DEOXY-D-GLUCOSE:

CHEMISTRY AND BIOLOGY



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2-Deoxy-D-Glucose: Chemistry and Biology

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2-Deoxy-D-Glucose: Chemistry and Biology

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CONTENTS

FOREWORD

It is with great pleasure that I compose the Foreword for this timely and insightful publication on the 'Chemistry and Biology of 2-Deoxy-D-Glucose (2-DG)'. As comprehensively outlined throughout the chapters, 2-DG is emerging as a versatile molecule with vast therapeutic potential in the fields of antiviral, anti-cancer, and neurological research.

This publication provides an exceptional overview of the medicinal chemistry that underlies 2-DG, encompassing its structure, synthesis, analytical characterization, and pharmacological actions along with its role in diagnostics and therapeutics. The chapters delve into the various synthetic pathways for producing 2-DG, elucidating the advantages and limitations of each method. Additionally, the text thoroughly discusses spectroscopic, chromatographic, and optical techniques for the analysis of 2-DG. These sections offer invaluable insights into optimizing the production and analysis of this crucial pharmaceutical intermediate.

Of particular interest are the mechanistic investigations into the biological effects of 2-DG. As explained, 2-DG acts as a glycolytic inhibitor and glycosylation modulator, selectively disrupting pathological metabolism in viruses, cancers, and seizures. The publication compiles compelling evidence from preclinical and clinical studies that highlight the therapeutic potential of 2-DG in these disease contexts. Furthermore, ongoing research on enhancing the delivery and efficacy of 2-DG through medicinal chemistry approaches is also prominently featured.

In conclusion, this publication provides a comprehensive and well-rounded overview of an exciting molecule that bridges the realms of chemistry and biomedicine. The chapters draw upon essential perspectives from synthetic organic chemistry, analytical methods, pharmacology, and molecular medicine to shed light on the diverse aspects of 2-DG research and applications, including prospects for cancer diagnosis, treatment, and surveillance. This interdisciplinary knowledge equips readers with a solid foundation to advance the potential of 2-DG and related compounds as diagnostic and therapeutic agents.

I commend the editors and authors for creating an outstanding reference that will educate, inspire, and guide future interdisciplinary endeavors in this medically relevant field.

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PREFACE

2-Deoxy-D-glucose (2-DG) is a modified molecule of glucose that has garnered significant interest in research in recent years due to its potential for therapy in various diseases. As outlined in this book, 2-DG and its derivatives have shown promise as agents against viruses, cancer, seizures, and COVID-19.

The book provides a comprehensive overview of the chemistry and biology of 2-DG. It delves into the structure, properties, and methods of synthesis of 2-DG, providing important insight into this pharmaceutical intermediate. Analytical techniques for characterizing and establishing the purity of 2-DG are also discussed.

A key highlight of the book is the exploration of the mechanisms and applications of 2-DG in biomedicine. As an inhibitor of glycolysis, 2-DG displays broad antiviral activity by disrupting the supply of energy and replication of viruses. Chapters outline evidence for the effectiveness of 2-DG against herpes simplex virus, influenza, Ebola, and SARS-CoV-2. The book also extensively covers the use of 2-DG and its analogs in cancer therapy, given their ability to selectively target tumor metabolism.

Beyond its roles in antiviral and anticancer applications, the book examines the emerging potential of 2-DG in the management of seizures and neurological conditions. Contradictory effects as anticonvulsant and proconvulsant are elucidated across different models. Therapeutic possibilities in Alzheimer's, aging, and stroke are discussed.

The book emphasizes how the unique properties of 2-DG and its derivatives enable their dual application in medical diagnostics and therapy. Radio-labeled 2-DG offers enhanced imaging, while functionalized analogs may improve drug delivery. This integrated approach can pave the way for more precise and personalized medicine.

Overall, this book provides a comprehensive reference on the chemistry and biology of 2-DG. It compiles essential insights from interdisciplinary research to offer a well-rounded perspective on this versatile molecule. The collective knowledge presented here will equip readers to further explore the therapeutic applications of 2-DG and related compounds. I hope this book serves as a valuable addition to the scientific literature, inspiring further advancement in this medically relevant field.

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CHAPTER 1

2-Deoxy-D-Glucose: Chemical Structure and Properties

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Abstract: 2-Deoxy-D-glucose (2DG) is a variant of glucose lacking the 2-hydroxyl group. This minor alteration has significant biological and pharmacological implications, enhancing its therapeutic value and necessitating evaluations of its safety and efficacy in clinical environments. This chapter delves into the chemical composition of different deoxy-D-glucose molecules, focusing on the structure and characteristics of 2DG.

Keywords: Analysis, Deoxy-D-glucose, Property, Structure, Toxicity.

1. INTRODUCTION

Deoxy sugars are sugars in which a hydroxyl group (-OH) on the carbon ring is substituted with a hydrogen atom. Deoxyribose, a prominent deoxy sugar, constitutes the sugar-phosphate backbone of DNA, bearing the molecular formula $C_5H_{10}O_4$. Other notable deoxy sugars are detailed in Table **1**. Sugars undergoing the replacement of two hydroxyl groups with hydrogen are classified as dideoxy sugars, with colitose and abequose being instances [1].

1.2. Nomenclature

Traditionally, many deoxy sugars were given trivial or common names. However, a systematic nomenclature system has been developed to name these compounds more precisely. This system uses the prefix 'deoxy' followed by the position number indicating which carbon atom has the hydroxyl group replaced by a hydrogen. The stem name of the parent sugar is then included, along with any necessary configurational prefixes to specify the stereochemistry at the remaining chiral centers of the deoxy sugar molecule.

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Table 1. Examples of deoxy sugars.

Entry	Trivial Name	Systematic Name	Use/Presence
1.	L-fucose	6-deoxy-L-galactose	Primary constituent of fucoidan found in brown algae and occurs within N-linked glycans.
2.	D-Quinovose	6-deoxy-D-glucose	Component of the sulfolipid known as sulfoquinovosyl diacylglycerol.
3.	L-Rhamnose	6-deoxy-L-mannose	Present in plant glycosides [2].
4.	Deoxyribose	2-deoxy-D-ribose,	Forms the sugar-phosphate backbone of DNA.
5.	Fuculose	6-deoxy-L-tagatose	A significant component of avian influenza virus particles.
6.	L-Pneumose	6-deoxy-l-talose	-
7.	Colitose	3,6-dideoxy-L-xylo-hexose	Present in the O-antigen of certain Gram-negative bacteria.
8.	Abequose	3,6-Dideoxy-D-xylo-hexos	Found within the O-specific chains of lipopolysaccharides present in specific serotypes of Salmonella and Citrobacter bacteria [3].

2. DEOXY-GLUCOSE

When a hydroxyl group in D-glucose is substituted with a hydrogen atom, the result is deoxy-D-glucose, a molecule that possesses one less oxygen atom than D-glucose. Various deoxy-D-glucose types can be produced based on which carbon's attached oxygen is eliminated. Illustrations of these molecular structures are provided in Fig. (**1**) and Table **2**.

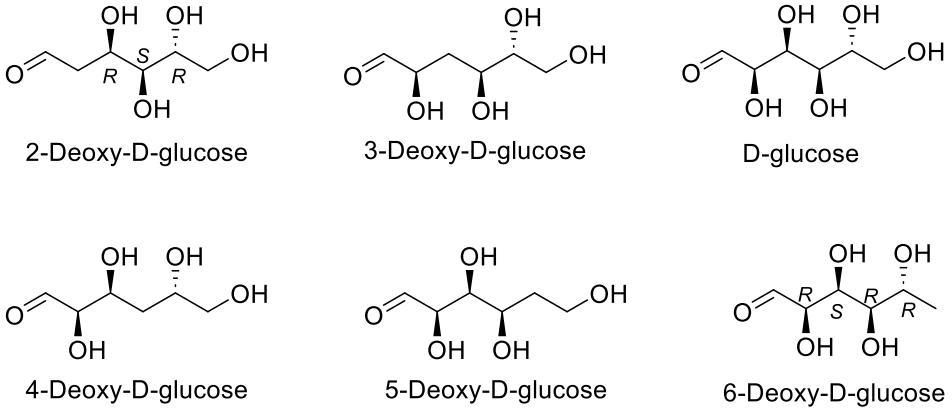

Fig. (1). Various deoxy sugars originating from D-Glucose.

Table 2. Different deoxy sugars derived from D-Glucose.

Entry	CAS	Deoxy-D-glucose	MP (°C)	$[\alpha]^{T}_{D}$
1.	154-17-6	2-Deoxy-D-glucose	146.5 [4]	$[\alpha]^{23}_{D}$ + 48.8° (c 0.13, water) [4].
2.	4005-35-0	3-Deoxy-D-glucose	120 [5]	$[\alpha]^{23}_{D}$ + 6.3° (c 1.2, water) [5].
3.	7286-46-6	4-Deoxy-D-glucose	131-132 [6]	$[\alpha]^{23}_{D}$ + 60.3° (c 2.4, water) [6].
4.	7640-19-9	5-Deoxy-D-glucose	Pail-yellow oil	$[\alpha]^{18}_{D}$ + 24.1° (c 7.8, water) [7].
5.	7658-08-4	6-Deoxy-D-glucose D-quionovose	139.5 [8]	-

Based on data available at AS Common Chemistry. CAS, a division of the American Chemical Society. https://commonchemistry.cas.org/

2-Deoxy-D-glucose (2DG) is the most common but other deoxy positions and substitutions can alter potency, metabolism, and effects. 2DG has become an important research tool and leads to potential therapeutic applications.

3. 2-DEOXY-D-GLUCOSE (2DG)

2DG is a synthetic compound [9], however, α and β D-glucopyranose forms (2-deoxy-α-D-*arabino*-hexopyranose, and 2-deoxy-β-D-*arabino*-hexopyranose) were extracted from the carbohydrate fraction of the solid-state fermentation product of *Actinosynnema pretiosum* ssp. *auranticum* ATCC 31565 [10, 11]. 2DG, has been assigned with the CAS registry number 154-17-6, and its structure has been depicted in Fig. (**2**). This compound is also known by the synonyms 2-deoxy-D-arabino-hexose and D-arabino-2-deoxyhexose. The α-pyranose form of the reducing aldose 2-deoxy-D-arabino-hexose (2-deoxy-D-*arabino*-hexopyranose) adopts a $^{4}C_{1}$ conformation, where the anomeric hydroxyl group is positioned axially, and the remaining substituents align equatorially. In the crystalline state, the four hydroxyl groups participate in an intricate three-dimensional hydrogen-bonding network, each acting as an intermolecular hydrogen-bond donor [12].

4. PHYSICAL PROPERTIES

2-Deoxy-D-glucose (2DG) exists as a crystalline solid with a white to off-white appearance [13]. Its melting point has been reported as 146°C [13]. However, when recrystallized from methanol, it forms colorless needle-like crystals with a melting range of 151-154°C and a specific optical rotation value of +43.0° (c=1.0, H_2O, 15°C) [11]. Under different conditions, such as in a 0.13 concentration aqueous solution, the specific rotation value for 2DG has been documented as +48.8° at 23°c [4]. These physical properties, including the melting behavior and optical activity, can aid in characterizing and identifying the 2DG compound.

CHAPTER 2

Methods and Procedures for the Synthesis of 2-Deoxy-D-Glucose

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Abstract: Many synthetic procedures for preparing 2-deoxy-D-glucose (2DG) are available in the literature. The synthesis of 2DG involves the modification of glucose at 2-position. Several methods to synthesize 2DG include glucose nitrosation and reductive amination of 2-deoxy-D-arabinose. These methods are highly efficient and produce high yields of 2DG. This chapter discusses various methods for synthesizing 2DG and their advantages and disadvantages. This chapter describes the different approaches for synthesizing 2DG and how the choice of method affects its purity, yield, and properties.

Keywords: Deoxy-D-glucose, Synthetic procedures.

1. INTRODUCTION

Although several methods have been published for the synthesis of 2DG, many have significant limitations that constrain their utility [1, 2]. Classical procedures often suffer from issues such as low yields, cumbersome workup or purification steps, and the formation of impure diastereomeric or racemic mixtures. This limits the accessibility, scalability, and stereochemical control of the current 2DG synthetic routes. There is a need for optimized and practical synthetic methods that improve the yield, simplicity, and stereoselectivity to support the research, development, and eventual production of 2DG. Overcoming these challenges in 2DG synthesis will facilitate its study and therapeutic applications. One or more methods could solve some of these problems, and reviewing these methods could provide insight into the issues to be addressed while developing new methods.

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Raman Singh, Antresh Kumar & Kuldeep Singh (Eds.)

2. SYNTHETIC METHODS

This section summarizes the various synthetic methodologies reported in the literature for the preparation of the important compound, 2-deoxy-D-glucose (2DG). As illustrated in Fig. (**1**), 2DG is a C-2 epimer of both D-glucose and D-mannose [3]. Therefore, the deoxygenation reaction at the C-2 position of D-glucose or D-mannose produced the same final product, 2DG. Consequently, synthetic routes starting from D-glucose, D-mannose, or derivatives of these two hexose sugars have been widely explored as potential approaches to accessing 2DG.

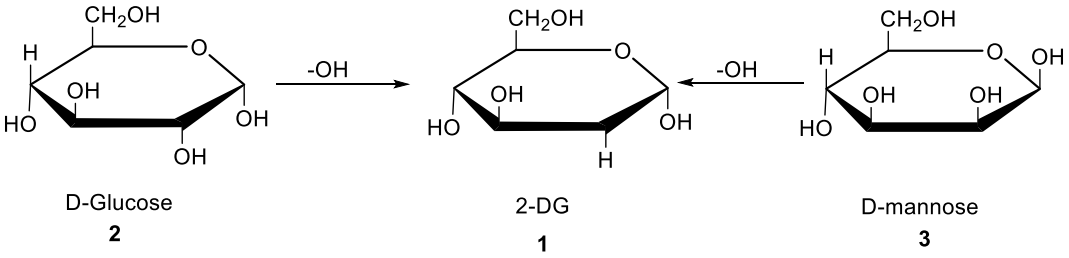


Fig. (1). DG from D-glucose and D-mannose.

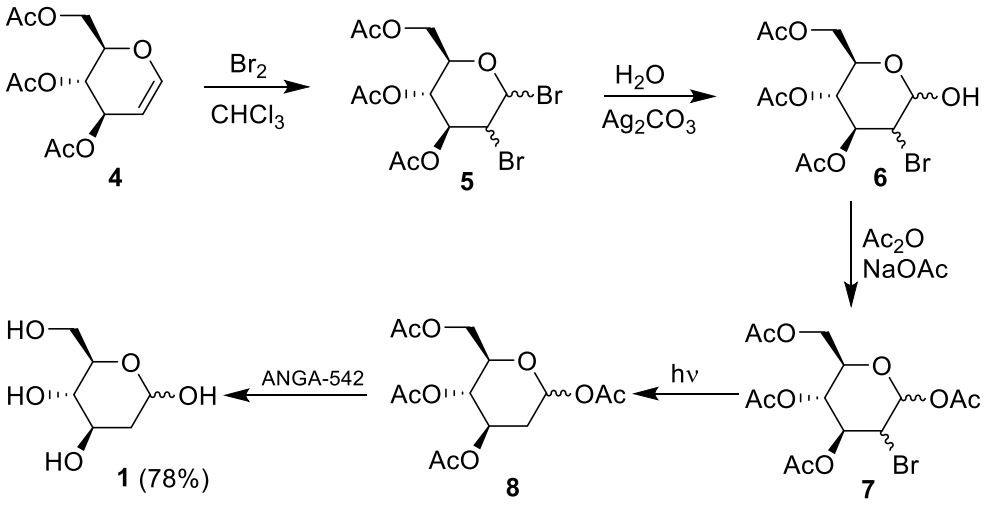
2.1. From Glucal and its Derivatives

Glucal, a glycal derived from glucose, served as the standard starting material for the synthesis of 2DG. A commonly employed method for preparing glucal involves the classical Fischer–Zach reductive elimination reaction, which utilizes zinc dust in acetic acid to reduce 2,3,4,6-tetra-O-acetyl-D-glucopyranosyl bromide [4]. The general conversion process for synthesizing 2DG involves the initial bromination (or halogenation) of glucal at the C-2 position, followed by the subsequent replacement of bromine atom with a hydrogen atom. Bromination is typically carried out in a nucleophilic solvent using molecular bromine. Various reagents have been explored for the second step, which involves substituting the bromine attached to the C-2 carbon with hydrogen, as summarized in Table **1**.

Table 1. Reagents used to replace bromine attached to C-2 with a hydrogen.

S. No.	Catalyst/Reagent or Condition	References
1.	Photolysis	Binkley & Bankaitis, 1982 [5]
2.	Raney nickel/ H_2	Monneret, 1983 [6]
3.	Pd/C	Mereyala & Mamidyala, 2004 [7]
4.	Zn/NaH_2PO_4	Xu *et al.*, 2017 [8]
5.	Benzene, Bu_3SnH, MeCN, Et_2O, KF	Hakamata and co-workers [9]

In addition to chemical methods, enzymatic halohydration of glycals has been reported in the literature as an alternative approach for synthesizing 2-deoxy-D-glucose (2DG) [10]. Binkley and colleagues described a photolytic method involving the treatment of α and β anomers of compound **7** to yield the corresponding α and β anomers of compound 8. Subsequently, compound **8** was subjected to an ion-exchange resin, Baker's ANGA-542, in methanol, resulting in the formation of 2DG with a 78% yield. Precursor compound **7** was synthesized through a multi-Step process involving the nucleophilic bromination of compound **4**, followed by hydrolysis and acetylation reactions (Scheme **1**). This photochemical approach, coupled with the ion-exchange resin treatment, represents an alternative synthetic strategy for obtaining 2DG from glycal-derived intermediates [5].



Scheme (1). Preparation of 2DG by photolysis coupled with ion-exchange resin treatment.

Monneret *et al.* reported a high-yielding synthetic method for producing 2DG from the precursor compound 3,4,6-tri-O-acetyl-1,5-anhydro-2-deoxy-D-*arabino*-hex-1-enitol. Their approach involved two key steps: 1) bromination of the precursor using N-bromosuccinimide as the brominating agent and 2) subsequent debromination achieved by catalytic hydrogenation over Raney nickel under 1 bar of hydrogen pressure. This two-step sequence afforded 2DG with an impressive 95% overall yield, making it an efficient synthetic route for obtaining this important glucose analog [6].

Mereyala and colleagues reported an economical and high-yielding synthetic process for the production of high-purity 2DG starting from (R)-D-Glycal

CHAPTER 3

Characterization of 2-Deoxy-D-glucose

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Abstract: 2-Deoxy-D-glucose is an important pharmaceutical intermediate, and its analytical characterization is critical for establishing its purity and quality. This chapter summarizes spectroscopic techniques, including UV, IR, NMR, and mass spectrometry, along with HPLC and GC studies used for the complete structural elucidation and purity analysis of 2-Deoxy-D-glucose. The UV spectrum of 2-Deoxy-D-Glucose showed no distinct peaks. The IR spectrum displayed characteristic bands for the O-H and C-H functional groups. ^{1}H, ^{13}C, APT, DEPT NMR, HSQC, and HMBC experiments confirmed the nominally proposed structure. ESI-MS revealed an $[M+Na]^{+}$ ion at m/z 187. Specific optical rotation was measured in water. HPLC studies estimated the related substances and assay to be 1.2% and 99.8%, on an anhydrous basis, respectively. The residual solvents, such as methanol, isopropyl alcohol, ethyl acetate, and toluene, were determined by GC headspace and found to be within the limits. The collective analytical evidence confirmed that the test sample met the quality specifications for 2-Deoxy-D-glucose.

Keywords: Assay, Chromatography, Characterization, Karl fisher, Related substances, Spectroscopy, Specific optical rotation, 2-deoxy-D-glucose.

1. INTRODUCTION

2-Deoxy-D-Glucose can be described as a synthesized glucose analog that acts as an antagonist of D-glucose. It is synthesized from D-Glucal; therefore, both compounds may be present during the synthesis. Compared with glucose, the hydroxyl group at position 2 is replaced with hydrogen [1].

This chapter describes the techniques used for the characterization of 2-Deoxy-D-Glucose and the determination of its purity by high-performance liquid chromatographic (HPLC). Glucose and glucose analogs present a very unique set

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of difficulties in HPLC analysis, which is due to the lack of chromophores on the sugar molecules, leading to poor sensitivity and specificity for ultraviolet absorption detection; therefore, HPLC with RI detection is used [2].

2. EXPERIMENTAL

2.1. Chemicals and Reagents

The synthesis of 2-DG has been reported previously [1]. 2-Deoxy-D-Glucose was manufactured by Mankind Pharma Limited. Honeywell Hydranal™ Karl Fisher reagent was used. Gradient-grade methanol and acetonitrile were purchased from Merck (Darmstadt, Germany). N, N-Dimethylacetamide, isopropanol, ethyl acetate, and toluene (AR grade) were purchased from Sigma-Aldrich (Merck, Darmstadt, Germany). Potassium bromide was obtained from Merck (Darmstadt, Germany). DMSO-d6 was procured from Eurisotop (Cambridge Isotope Laboratories, Inc.). Distilled water was obtained using a Milli-Q water purifier system (Milli-Q IX-7015; Millipore, Milford, MA, USA).

2.2. Instrumentation and Methodology

2.2.1. Sulphated ash Content

The sulfated ash content of 2-Deoxy-D-Glucose was determined by ignition of 1 g of the sample at 600ºC for 3 h, along with sulfuric acid treatment of the residue and reignition until a constant weight was achieved.

2.2.2. Water Content

The moisture content of 2-Deoxy-D-Glucose was determined by Karl Fisher titration using a T5 Excellence Titrator (Mettler Toledo). The moisture content of the samples was determined using methanol of known weight (50 mg).

2.2.3. Specific Optical Rotation

Polarimetric measurements were performed using an AUTOPOL® V PLUS scientific polarimeter. The sample cell length was 1 dm and the output LED light source had a wavelength of 589 nm. A 1% w/w solution in water was used for measurements at 25 ± 0.5°C. The Rudolph Research software allows for easy determination of the angle of polarization of the solution.

2.2.4. UV-VIS Spectroscopy

The ultraviolet absorption spectrum of an approximately 10 ppm 2-Deoxy-D-Glucose solution in distilled water was recorded to determine λmax using a

PerkinElmer Lambda 365 UV-Vis spectrophotometer equipped with a quartz cell with a 1 cm path length. The obtained spectrum was processed using the UV WINLAB ES software.

2.2.5. FTIR

The samples were prepared as a potassium bromide dispersion with 0.5%w/w concentration of the test sample or standard by applying a pressure of approximately 8 tons/cm2 (800MPa) using a dye press. The IR absorption spectrum of 2-Deoxy-D-Glucose was obtained using a PerkinElmer Spectrum Two FT-IR spectrometer in the wavelength range(s) of 4000 cm-1 - 400 cm-1, with a calculated resolution of 4 cm^{-1}. Sixteen scans were obtained and processed using the Spectrum ES software.

2.2.6. Nuclear Magnetic Resonance

Nuclear magnetic resonance spectra were recorded on a Bruker Ascend 400 MHz spectrometer with DMSO-d_6 as the solvent, tetramethylsilane (TMS) as the internal standard, and a 5 mm PABBO BB probe. ^{1}H NMR spectra were recorded at a base frequency of 500 MHz at 24° with 10 scans, relaxation delay of 2s, and pulse angle of 90°. ^{13}C NMR had a base frequency of 100 MHz at 24°, with 3000 scans, a relaxation delay of 2s, and a pulse angle of 90°. Heteronuclear single quantum coherence spectroscopy (HSQC) and heteronuclear multiple bond correlation spectroscopy (HMBC) experiments were performed using the same instrument and in the same solvent to assign the correlation. The ^{1}H chemical shift values are reported with respect to that of the TMS peak [δ_H(TMS)= 0.00 ppm], and the ^{13}C chemical shift values are reported with respect to that of the solvent residual peak [δ_C(DMSO-d_6) = 39.52 ppm].

2.2.7. Mass Spectrometry

Mass Spectrometry was conducted on a Waters Xevo TQS-Micro (Waters Corp., Milford, MA, USA) mass spectrometer attached to a Waters Acquity TM Ultra Performance Liquid Chromatography (UPLC) system (Waters, Milford, MA). The analytes were identified using positive electrospray ionization mass spectrometry (ESI). The capillary voltage and collision energy were maintained at 3.5 kV and 20 eV, respectively. Nitrogen was used as desolvation gas at a flow rate of 650 L/h at 350°C. The spectra were recorded in the range of m/z 100-1000 for full scan analysis. The sample was prepared by dissolving approximately 1 mg of the sample in methanol.

CHAPTER 4

[^{18}F]Fluoro Analogue of D-Glucose: A Chemistry Perspective

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Abstract: 2-[^{18}F]fluoro-D-glucose ([^{18}F]FDG) is a versatile molecule in nuclear medicine that has evolved into a vital radiotracer in medical imaging applications *via* positron emission tomography (PET) [^{18}F]FDG is derived from its derivative, 2-deoxy-D-glucose (2-DG), where the triflate group is attached to carbon-2 [^{18}F]FDG serves as a crucial non-invasive diagnostic tool and is prominently utilized in non-invasive imaging of various metastatic diseases, particularly cancer imaging. Its importance as a tracer has been further enhanced by its unexpected attribute of generating a low body background through excretion, leading to its effective application in PET/CT for highly-sensitive and specific tumor detection. This chapter provides insight into the synthesis of [^{18}F]FDG, employing various reaction protocols such as electrophilic and nucleophilic processes. This chapter also summarized the purification and their quality assurance methods and highlighted the distinct challenges associated with each. The nucleophilic technique produces [^{18}F]FDG with a higher yield and purity than the electrophilic method for routine manufacture. Commercially devoted automated modules for FDG production use this method, demonstrating its widespread use in clinical imaging. Nucleophilic reactions of [^{18}F]fluoride ions attacking the C-2 position of mannose triflate to produce FDG are routine in clinical imaging. The final [^{18}F]FDG product satisfies safety, purity, and efficacy standards through rigorous quality control and assurance. The trajectory from glucose discovery to the development of [^{18}F]FDG exemplifies the continuing advancement of medical imaging methods. FDG's accomplishment shows how biology, chemistry, and medical technology are interrelated, providing a better understanding and treatment of complicated diseases like cancer.

Keywords: Cancer, Imaging, Nuclear medicine, PET/CT, [^{18}F]FDG.

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Raman Singh, Antresh Kumar & Kuldeep Singh (Eds.)

1. INTRODUCTION

1.1. Definition and Background of 2-Fluoro-D-Glucose (2-FDG)

Glucose was first identified by Jean Baptiste Andre Dumas, a French chemist, in 1838 [1]. He coined the term glucose for sugars obtained from honey, grapes, starch, and cellulose. The molecular formula of glucose was established 20 years later [1 - 3]. Glucose is a simple sugar that consists of six carbon atoms. It has an open-chain structure, with aldehyde and alcohol functional groups. However, in aqueous solutions, only a negligible amount of the open-chain form of glucose remains. Glucose undergoes a ring formation reaction, forming two isomers, α-*D*-glucopyranose and β-*D*-glucopyranose. These isomers differ in the orientation of the -OH functional groups on carbon-1 [4] (Fig. **1**). Glucose has been extensively studied for its medicinal activities, particularly in anticancer treatment. The 2-deoxy-D-Glucose (2-DG) is an additive of glucose that has been investigated for its potential as an anticancer drug [5].When the -H group at the second position of the derivative of glucose (2-DG) is replaced by a [^{18}F]fluorine ion, termed as [^{18}F]FDG, which is extensively used as a non-invasive biomarker *via* positron emission tomography (PET) [6].

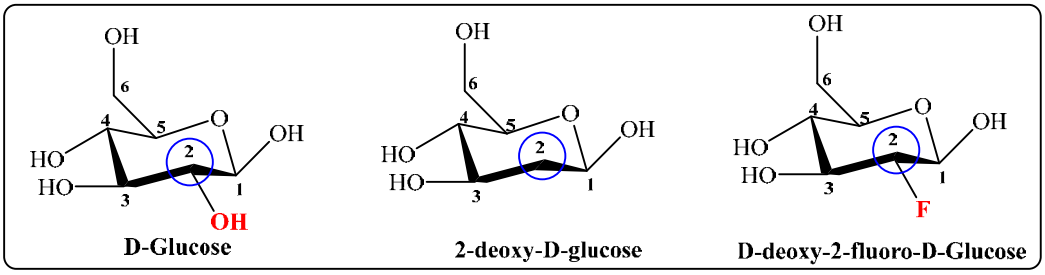

Fig. (1). The chemical skeleton of FDG, 2-DG, and glucose displaying changes at carbon-2.

1.2. Biological Importance of [^{18}F]FDG

FDG was designed based on 2-Deoxy Glucose, a derivative of glucose, wherein the hydroxyl group (-OH) on C-2 was substituted with a hydrogen atom, as shown in Fig. (**1**) [7].

Many researchers have claimed that the biological behaviour of 2-DG closely mirrors that of glucose. In view of cell uptake, the glucose, 2-DG is phosphorylated by hexokinase. The hydroxyl group on C-2 is not a critical factor for this process; however, glucose metabolism stops functioning after phosphorylation because of the essential role of the hydroxyl group on C-2 in the subsequent step involving phosphohexoseisomerase (Fig. **2**) [6 - 8]. Consequently, 2-DG6P was trapped in the cells as a metabolic record. Essentially,

the elimination of the hydroxyl group from C-2 isolates is a hexokinase reaction. This unique property of 2-DG was observed by Sols and Crane in 1954, who noted this characteristic [9]. ^{18}F-FDG is transported into the brain tissue by GLUTs and phosphorylated to ^{18}F-FDG-6-phosphate ([^{18}F]FDG6P) in the presence of hexokinase (HK). [^{18}F]FDG is not further metabolized but remains trapped and can be detected in cells to evaluate brain glucose uptake. These investigations have laid the foundation for the utility of [^{18}F]FDG, which is crucial for exploring human glucose metabolism [8, 9]. Initially, [^{18}F]fluorine radionuclide was chosen because of its high C-F bond energy and adequate half-life of 110 min. The ^{18}F-labeled variant of 2-DG synthesis is centered on substituting ^{18}F at the carbon-2 atom while maintaining the characteristics of the original molecule. The logical choice for [^{18}F]fluorine substitution at C-2 is that modifying this carbon atom would not disrupt the facilitated transport needed to penetrate the blood-brain barrier or the hexokinase reaction. The development of [^{18}F]FDG was further supported by the successful synthesis of unlabeled FDG, which proved its efficacy as a hexokinase substrate. The significance of fluorine substitution on C-2 was evident from the considerable decrease in hexokinase affinity observed for both 3-deoxy-3-fluoro-*D*-glucose and 4-deoxy-4-fluoro-*D*-glucose simultaneously.

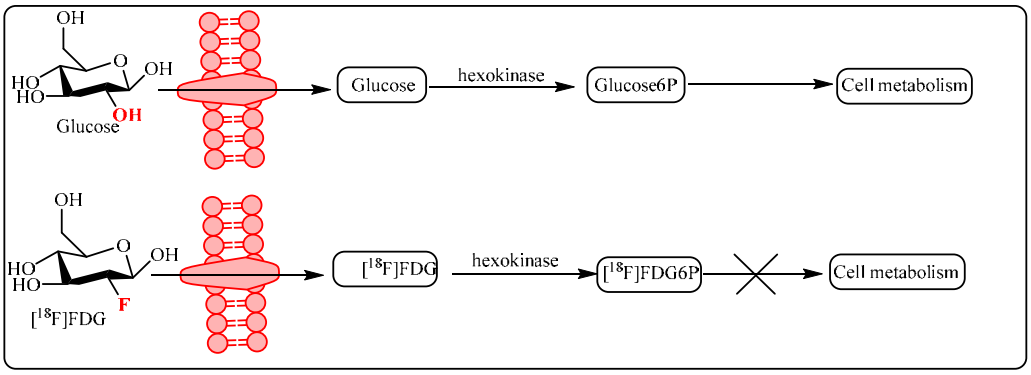

Fig. (2). Reaction pathway for [^{18}F]FDG.

The distinctive feature of [^{18}F]FDG, its heightened absorption in rapidly proliferating tumors owing to enhanced tumor glycolysis and its minimal background in the body, yield an exceptional signal-to-noise ratio for tumor detection (Fig. **3**). The low body background of [^{18}F]FDG is attributed, in part, to its excretion, unlike glucose, which undergoes resorption from the urine to plasma *via* active transport across the renal tubule. Unlike glucose, the absence of a hydroxyl group on C-2 in [^{18}F]FDG prevents active transport, contributing to its low background [7 - 10]. This unexpected property of a low body background

CHAPTER 5

Antiviral Potential of 2-DG Used in Different Viral Infections

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Abstract: The evolution of viral infections has pushed researchers constantly to find new approaches to disseminate these infections. One such promising finding in this aspect is 2-deoxy-D-glucose (2-DG), a glucose analogue that gained attention for its potential as an antiviral agent effective against a variety of viral infections. The antiviral properties of 2-DG are due to its ability to interfere with viral replication within host cells, hence reducing the severity of infections. 2-DG is easily taken up by cells as it mimics glucose-like structure but interferes with glycolysis and other metabolic pathways. It also acts as a glycosylation inhibitor that helps in the disruption of viral assembly. Viruses are obligate and utilize the host cell machinery for proliferation. 2-DG mechanistically disrupts the energy supply by inhibiting the glycolysis cycle and providing an unfavourable environment for viral replication. 2-DG elicits broad-spectrum antiviral activity as it was found to be very effective against different families of viruses. By interfering with this process, 2-DG not only interferes with viral replication but also with the ability of the virus to enter host cells and evade the immune system. Although 2-DG has shown some promising antiviral potential, it also possesses some side effects as well. All the attributes related to the antiviral potential of 2-DG have been discussed in this chapter.

Keywords: Antiviral, Glycosylation, Viral infections, 2-deoxy-D-glucose, 2-DG analogs.

1. INTRODUCTION

Viruses are well-known opportunistic, and obligate pathogens that entirely depend on the host cell machinery for their progression and clinical implications. Their survival is accomplished by the synthesis of viral components such as nucleic acids, protein, glycans, and lipid membranes, using the host cell machinery. The progression of the viral particle entirely depends on the metabolic profile of the

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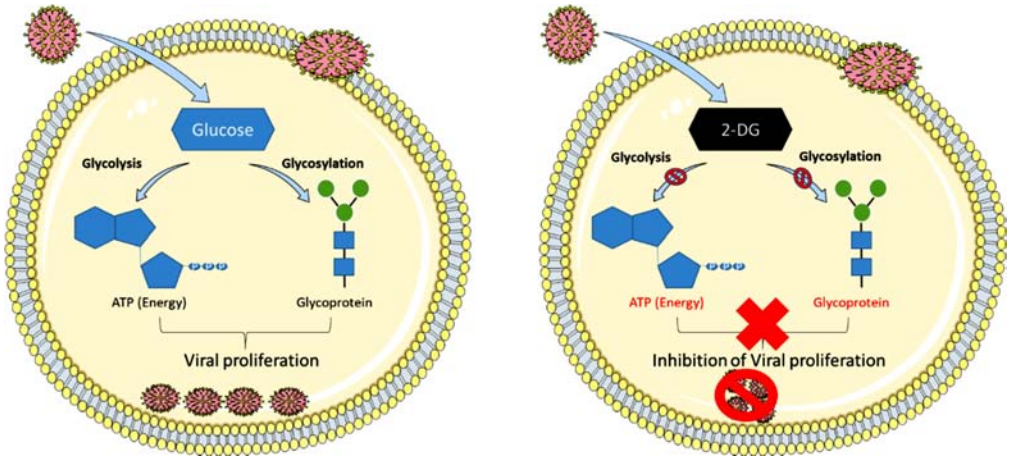
Raman Singh, Antresh Kumar & Kuldeep Singh (Eds.)

host cell. Thereby, viruses can modulate the metabolic profile of the host cells in multiple ways. Both retro or non-retro viruses, one way or another, regulate different metabolic pathways such as glycolysis, and pentose phosphate pathway, and induce amino acids and lipids synthesis [1]. For instance, the human cytomegalovirus (HCMV), herpesvirus-1 (HSV-1), and adenovirus upregulate the glycolysis and tricarboxylic acid (TCA) cycle and also increase nucleotide and lipid synthesis [2]. As a general notion, viral infection more often affects the glycolysis pathway. The virus-infected cells adapt metabolic alterations that aid to meet the high anabolic demand required for viral progression. Upregulation of the glycolysis cycle is the most common metabolic shift that occurs during viral infections. However, other metabolic alterations were also well reported which are often virus-specific. Previous studies reported that during viral infection, the most common enzyme induced is phosphatidylinositol-3-kinase (PI3K) or protein kinase (PKB/Akt) signaling that drives the expression glucose transporters 1 or 4 (GLUT1/ GLUT4) [3 - 5]. The expression of GLUT1/ GLUT4 glucose transporter resulted in inducing glucose uptake, which helps them to overcome the increased energy demand.

In the recent past, the entire global community faced a COVID-19 pandemic situation that affected millions of lives. This pandemic infection left no part of the world untouched and openly challenged health agencies for the development of therapeutics against the same. The COVID-19 pandemic also caused different coinfections with epidemiological impacts. Mucormycosis is a common example that rapidly emerged as a secondary infection in COVID-19 infected patients with a significant morbidity and mortality rate [6]. Different efforts have been devised to find new or repurposed existing drugs during that period and have been tested against such infections. Ivermectin, dexamethasone, ritonavir, favipiravir, remdesivir, hydroxychloroquine, *etc.* are such common examples [7]. During the same course of time, a glucose analog termed as 2-Deoxy-D-glucose (2-DG) was also employed to test the efficacy against the COVID-19 infection. 2-DG is basically, a non-metabolizing glucose analog that specifically acts up on the glycolysis cycle and blocks the functionality of enzymes involved in the glycolysis cycle along with the inhibition of viral protein glycosylation leading to the inhibition of viral replication (Fig. **1**). Apart from other inhibitory effects related to glucose metabolism, 2-DG also acts on phosphoglucoisomerase that obstructs the conversion of glucose-6-phosphaet to fructose-6-phosphate. It disturbs the metabolic ability of cells and dictates the cells toward apoptosis. 2-DG is a synthetic analog of glucose where –OH (hydroxyl group) of the second carbon position is replaced with a hydrogen atom. Similar to glucose,2-DG is taken up by the cells through glucose transporters by facilitated diffusion mainly by GLUT1 and GLUT4; and active uptake by the SGLT transporter [8, 9]. 2-DG inhibits the catalysis of hexokinase and phosphoglucoisomerase involved in the

conversion of glucose to phosphoglucose, given the situation when there is a sufficient amount of 2-DG-6P generated, by the virtue of product inhibition.



A B

Fig. (1). (**A**) Viral proliferation in the presence of glucose, (**B**) Inhibition of viral proliferation in the presence of 2-DG.

2. HOST GLYCOSYLATION: A NECESSITY FOR VIRAL REPLICATION

Glycosylation is a process in which a carbohydrate is attached to hydroxyl or other functional groups of protein and lipids. Such modifications made macromolecules serve several cellular and physiological functions. Protein glycosylation is a process where glycan is attached to the amide nitrogen of the asparagine. This process occurs in the early stage of protein synthesis, followed by oligosaccharide trimming and remodeling during transit from ER to the Golgi apparatus. It is well established that glycosylation is primarily required for the progression of viral replication in which glycosylated viral proteins tend to promote gene expression, fusion, binding of viral particles to the cell surface receptors, and prevention of antibody neutralization. The glycosylation of the viral proteins is carried out using N-glycans synthesized in the host ER through carbohydrate metabolism [10, 11]. As virus particles invade the host cell, they tend to hijack the glycosylation pathway for modifications of their proteins. N-linked glycosylation of viral envelope or surface protein enhances its folding and subsequent trafficking. Viruses often employ calnexin and calreticulin for the facilitation of function folding of the viral proteins [12]. During the progression of viral infection, glycosylation plays a very crucial role in the modification of the proteins that help in viral particle multiplications and transmission [13]. Glycosylation patterns also tend to regulate the communication with receptors,

CHAPTER 6

2-Deoxy-D-Glucose and its Derivatives: Dual Role in Diagnostics and Therapeutics

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Abstract: This chapter delves into the multifaceted applications of 2-Deoxy-d-Glucose (2-DG) and its derivatives as versatile tools in diagnostics and therapeutics. Highlighting their dual role in the medical landscape, this chapter provides a comprehensive overview of the diverse functions and mechanisms by which these compounds contribute to both diagnostic assessments and therapeutic interventions. The first section examines the use of 2-DG and its derivatives in diagnostics, detailing their efficacy in various imaging techniques, diagnostic assays, and investigative procedures. Their unique properties and specific interactions in these contexts were explored to elucidate their significance in the accurate detection and visualization of specific physiological conditions or anomalies. The subsequent segment shifts the focus towards the therapeutic realm, where the book chapter investigates the potential and current applications of 2-DG and its derivatives in treating a spectrum of diseases and conditions. From their roles in cancer therapy to neurological disorders and severe acute respiratory syndrome-related coronavirus-2 (SARS-CoV-2) treatment, the chapter outlines the mechanisms and clinical advancements where these compounds show promise as therapeutic agents. Throughout this discussion, the chapter emphasizes the evolving landscape of 2-DG and its derivatives, touching upon ongoing research, challenges, and future prospects in harnessing their dual attributes for enhanced healthcare outcomes. The exploration of these compounds in both diagnostic and therapeutic realms not only illuminates their versatility but also underlines the potential for innovative and integrated medical approaches.

Keywords: Cancer therapy, Neurological disorders, SARS-CoV-2 treatment, Warburg effect, 2-Deoxy-D-Glucose.

1. INTRODUCTION

2-Deoxy-D-glucose (2-DG), a synthetic analog of glucose, has been captivated in the scientific field because of its various biological properties and potential therapeutic applications. 2-DG has been widely employed as a research tool in

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various biological and medical fields [1]. The lack of a hydroxyl group at the 2'-position on the pyranose ring distinguishes 2-DG from glucose and prevents it from continuing further glycolysis after being phosphorylated by hexokinase (HK), the first enzyme of the glycolytic pathway [2]. As a result, 2-DG acts as an inhibitor of pathways utilizing glucose and mannose, affecting multiple enzymes and cellular processes through various mechanisms. This multifaceted disruption interferes with the production of energy, nucleotides, and other essential macromolecules, thereby impacting cell growth and survival [1, 2]. Other cellular activities that rely on glucose, such as N-glycosylation, pentose phosphate pathway (PPP), and hexosamine biosynthesis pathway, can be influenced by 2-DG [3]. The same glucose transporters (GLUTs) mediate glucose absorption by transferring 2-DG into the cell. As a result, it preferentially accumulates in cells that consume large amounts of glucose, such as cancer cells, neurons, and immunological cells [2, 3].

Using techniques such as autoradiography, nuclear magnetic resonance, and positron emission tomography (PET), 18F-2-deoxyglucose, a derivative of 2-DG has been employed as a tracer molecule to analyze the distribution and activity of glucose metabolism in living organisms [4]. 2-DG has also been employed as a pharmaceutical agent against various illnesses such as cancer, epilepsy, neurodegeneration, and infection [5, 6]. 2-DG has shown promising results in preclinical and clinical trials as an adjuvant therapy for cancer and COVID-19 by exploiting the metabolic vulnerabilities of these diseases [6]. However, 2-DG has some limitations and challenges such as toxicity, specificity, and delivery [7]. In this chapter, we review the history, chemistry, biology, and applications of 2-DG, as well as the current status and prospects of 2-DG as a diagnostic and therapeutic tool.

Glucose serves as the predominant cellular energy source and functions as a substrate for various metabolic processes. It is primarily derived from consumed dietary carbohydrates, but it can also be produced within the body *via* gluconeogenesis [8]. Because glucose is hydrophilic, it requires specific GLUT proteins to allow cellular uptake [9]. Tumor cells require the overexpression of GLUT transporters to boost glucose uptake–20-30 times compared to normal cells [9, 10]. Glucose is the primary reservoir of energy for most cells, predominantly in the brain, muscles, and tumors. Glucose metabolism involves a series of biochemical processes that transform glucose into pyruvate or lactate, generating adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide hydrogen (NADH) as energy carriers [11]. The first step of glucose metabolism is glycolysis, which occurs in the cytoplasm and does not require oxygen. Glycolysis consists of ten enzymatic reactions that split one molecule of glucose (6-carbon), yielding two pyruvate molecules (3-carbon) and producing a pair of

ATP molecules and a pair of NADH molecules [11, 12]. The fate of pyruvate relies on the availability of oxygen and the cell type. Under aerobic conditions, pyruvate is carried into the mitochondria and oxidized into acetyl-CoA, which enters the tricarboxylic acid (TCA) cycle and electron transport chain (ETC), producing more ATP and NADH [13].

Glucose metabolism in cancerous cells involves the conversion of glucose, the main source of energy for most cells, into pyruvate or lactate [12]. Cancer cells exhibit a distinct glucose metabolism compared to normal cells, characterized by increased glucose uptake and the conversion of glucose to lactate through fermentation, even when oxygen and functional mitochondria are present. This phenomenon is termed the Warburg effect, which allows cancer cells to sustain and multiply in hypoxic environments. Cancer cells rely on acquiring carbon for cell proliferation. Complete oxidation of glucose to CO_2 may hinder this process by depriving cancer cells of the necessary carbon required for producing new cells [14]. Glucose metabolism in cancer cells is regulated by multiple factors such as oncogenes, tumor suppressors, signaling pathways, and microenvironmental cues [15].

Glucose metabolism in cancer cells also affects their plasticity, which is the ability to switch between different phenotypes and behaviors such as stemness, differentiation, invasion, and metastasis [14]. Tumor cells adapt to low oxygen levels by changing gene expression and signaling pathways. In response to hypoxic stress, tumor cells undergo a series of metabolic adaptations to maintain their survival and growth. These adaptations include elevated expression of protooncogenes, such as c-Myc, the instigation of signaling pathways, such as PI3K/Akt, and the triggering of specific transcription factors, such as hypoxia-inducible factor-1α (HIF-1α) [16]. One significant alteration is the activation of HIF-1α, a transcription factor that controls the expression of many genes related to glucose metabolism. HIF-1α helps tumor cells switch from oxidative metabolism to aerobic glycolysis, which produces energy and reduces oxidative stress in the absence of oxygen. HIF-1α also regulates the intake of glucose, functioning of glycolytic enzymes, function of mitochondria, and removal of damaged mitochondria by autophagy. HIF-1α balances oxygen demand and supply as well as ATP and reactive oxygen species (ROS) production in tumor cells [17]. Shifting oxidative metabolism to aerobic glycolysis is a key strategy for tumor cells to thrive and proliferate in hypoxic environments [18].

Glycolysis occurs in the cytoplasm and does not require oxygen. Glycolysis begins when glucose enters the cell through GLUT transporters and is phosphorylated by HK to glucose 6-phosphate (G6P) (Fig. **1**) [19]. This molecule has the option to enter the PPP for NADPH and ribose-5-phosphate production or

CHAPTER 7

2-Deoxy-D-Glucose as a Potential Antiviral and Anti-COVID-19 Drug

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Abstract: The search for effective therapeutics has been unyielding in the relentless battle against the COVID-19 pandemic. A potential drug candidate is 2-deoxy-D-glucose (2-DG), which has been evaluated as a polypharmacological agent for antiviral therapy due to its influence on the glycolytic pathway. This chapter delves into the promising role of 2-deoxy-D-glucose (2-DG) as a potential anti-viral drug. With a focus on the biochemical and pharmacological aspects, this chapter explores how 2-DG may disrupt the viral life cycle and modulate host immune responses. An in-depth analysis of the current scientific evidence, including preclinical studies and clinical trials, will be highlighted to shed light on the drug's efficacy, safety, and potential as a treatment option. Furthermore, the challenges and prospects of 2-DG in the context of COVID-19 management will be elaborated. The COVID-19 pandemic has posed unprecedented challenges to global healthcare systems, demanding swift and innovative approaches to combat the virus. Amid this backdrop, the utilization of 2-deoxy-D-glucose (2-DG) as an anti-COVID-19 drug has emerged as a promising avenue for research and therapeutic development. This chapter offers an exhaustive exploration of the potential of 2-DG in the context of COVID-19 treatment. Additionally, action mechanisms and safety concerns associated with administering 2-DG in treating COVID-19 will be reviewed. This chapter aims to equip readers with a comprehensive understanding of 2-DG's role in the fight against COVID-19 and its place in the evolving the landscape of antiviral therapeutics.

Keywords: Antiviral therapy, Glycolytic, Polypharmacological agent, SARS-CoV-2, 2-DG.

1. INTRODUCTION

Energy is a basic need for all living things to continue to exist in the universe. Glucose serves as the primary energy source for all living things (including

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Raman Singh, Antresh Kumar & Kuldeep Singh (Eds.)

plants), from primordial forms to multicellular, structured creatures. Cancer, viral infections, and COVID-19 are diseases that have been shown to rewire cell metabolism. As a result, the cell uses glycolysis primarily to meet the high energy requirement necessary for the rapid growth of diseased cells or viruses. While glycolysis does produce ATP, which is a form of energy, the primary reason cells, especially cancer cells or cells infected by viruses, rely heavily on glycolysis might be more related to their need for carbon. 2-Deoxy-D-glucose, commonly called, is a synthetic substitute for glucose. 2-Deoxy-D-glucose (2-DG) was discovered as a competitive inhibitor of glycolysis that is non-metabolizable and contains a hydrogen atom instead of the hydroxyl group found in C-2 carbon of glucose (Fig. **1**). Competitive inhibition is a specific kinetic term that refers to competition for the substrate binding site. It is not really applicable to a metabolic pathway with many enzymes.

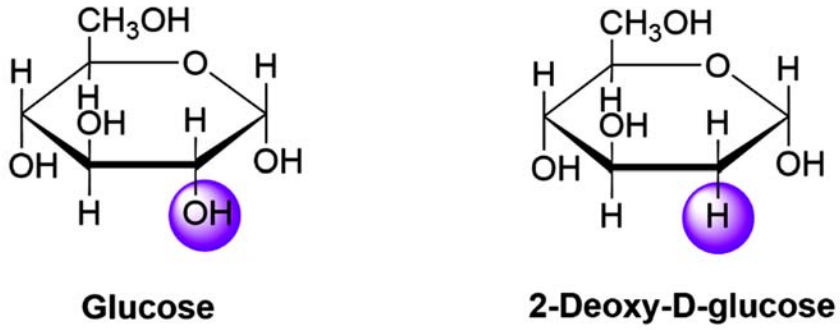

Fig. (1). Structure of Glucose and 2-Deoxy-D-glucose (2-DG) [1].

In the glycolysis process, glucose gets converted into pyruvate, which depends on enzymes such as hexokinase and phosphohexose isomerase, the structural difference in 2-DG inhibits both. Hexokinase readily phosphorylates 2DG to give 2DG-6P. This 2DG-6P can inhibit hexokinase at high concentrations. Consequently, 2-DG's suppression of glycolysis and subsequent adenosine triphosphate (ATP) depletion confirm 2-DG's status as an antimetabolite of glucose. Apart from its ability to inhibit glycolysis, 2-DG also exhibits its anticancer, anti-inflammatory, antiviral, and calorie restriction mimetic properties by modulating the adenosine monophosphate-activated protein kinase (AMPK), and tumour suppressor gene (p53), and protein N-glycosylation in the endoplasmic reticulum (ER) [1].

Despite the extensive use of 2-Deoxy-D-glucose (2-DG) under various situations and physiological applications, the role of this synthetic analogue of glucose has simply been speculated based on a poor metabolic rate and impaired protein glycosylation. In order to understand its role in biological systems, various

studies, such as interference with glycolysis, nutritional deprivation conditions, and energy depletion like feeding cells with low or no glucose, and simulation *via in-vitro* culture or in animals by adding 2-deoxy-D-glucose to the medium, have been carried out [2].

2. BIOLOGICAL EFFECTS OF 2-DG

The 2-Deoxy-D-Glucose has a diverse biological effect on metabolic processes. For example, oxidative stress is caused by various metabolic processes of 2-DG, such as the inhibition of 2-DG on glycolysis and ATP generation, disruption of protein N-glycosylation, reduction in energy metabolism and NADPH levels, and interference with cellular thiol metabolism. By impression of this metabolic disruption, 2-DG is identified as an effective cytotoxic agent and has little influence on the viability of healthy cells. The molecule possesses multiple functions, encompassing antiviral, anti-inflammatory, anticancer, and antiepileptic properties, among others. Additionally, it serves as a tracer and a hypoglycemic agent, functioning as both a D-glucose and D-mannose mimic [3]. Its adverse effects stem from its capability to inhibit glycosylation while leaving glycolysis unaffected. Reported experimental results revealed that 2-DG is relatively harmless at lower doses but can lower the blood pressure and slow breathing under higher doses [4].

2-DG can consult neuroprotection to the central nervous system (CNS) of the neurons but sources increased cell death in the existence of hypoxia [4]. In the CNS, microglia cells serve as the macrophages. In *in-vitro* models, 2-DG inhibits the microglia's glycolysis, resulting in ATP depletion and causing cell death. The reason behind the selective impact of 2-DG on microglia is their heightened reliance on glycolysis during activation. In contrast, neurons primarily rely on mitochondria to fulfil their ATP needs. Therefore, in animal models of disorders such as Parkinson's disease, Alzheimer's disease, stroke, meningitis, trauma, and epilepsy that are associated with microglial activation-related neuronal loss, neurons are mostly unaffected by the actions of the treatment. Neuroprotection is not absolute, and pathological conditions linked to hypoxia, such as trauma, vascular dementia, and stroke, have been linked to increased neuronal death. Most studies indicate that a clinically tolerable dose of 2-DG is up to 63 mg/kg/day [3, 5 - 7].

3. 2-DG IN VIRAL REPLICATION

Since the late 1950s, experimentation with 2-DG in viral replication was reported [8]. Kilbourne's initial publication in 1959 noted a considerable decrease in influenza virus load in the chorioallantoic membrane of embryonated chicken eggs [8]. Following that, 2-DG was investigated as an antiviral agent against a

CHAPTER 8

Prospects for Cancer Diagnosis, Treatment, and Surveillance: [^{18}F]FDG PET/CT and Innovative Molecular Imaging to Direct Immunotherapy in Cancer

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Abstract: Positron Emission Tomography (PET), a noninvasive technique, is most suitable for quantitative evaluation of *in vivo* tumor biology. Based on its metabolic activity, the accumulation of F-18 fluorodeoxyglucose ([^{18}F]FDG), a positron emitter radionuclide, is most explored indicative of tumor features. Quantitative evaluation of FDG uptake is frequently used for treatment monitoring following chemotherapy or chemoradiotherapy. Several investigations showed that FDG PET, which measures metabolic change, was a more sensitive marker than CT or MRI, which measures morphological change. [^{18}F]FDG is now frequently used to assess tumor metabolism as well as to track the effectiveness of immunotherapy, which is a useful treatment for several malignancies. With the use of *in vivo* whole-body CD^{8+} T cell and PD-L1 expression imaging, for instance, radiopharmaceuticals that are novel in nature offer the rare chance to characterize the immunological tumor microenvironment (TME) and more accurately forecast which patients may react to therapy. Longitudinal molecular imaging may also aid in clarifying potent changes, especially in instances of resistance that occurred during immunotherapy, and aid in guiding a more individualized therapeutic strategy. To categorize, forecast, and track treatment response and molecular dynamics in areas of therapeutic need, this review focuses on new and existing uses of [^{18}F]FDG for imaging.

Keywords: Checkpoint inhibitors, Immunotherapy, Positron emission tomography (PET), Tumor metabolism, [^{18}F]FDG.

1. INTRODUCTION

An essential tool for detecting and treating cancer, the glucose analog PET radiotracer [^{18}F]FDG detects glucose consumption [1, 2]. Modern clinical and

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Raman Singh, Antresh Kumar & Kuldeep Singh (Eds.)

research applications encompass all major disciplines of human health, including neurology, cancer, cardiology, and infection/inflammatory diseases as hybrid PET/computed tomography (PET/CT) is commercially available. For instance, [^{18}F]FDG PET imaging is helpful in the early staging of lung, colorectal, and esophageal malignancies and in detecting recurrences [1]. Furthermore, preclinical and clinical research indicates that modifications in the amount of [^{18}F]FDG accumulated in tumors following specific treatments may be highly predictive and serve as an early biomarker of treatment effectiveness [3, 4]. The therapeutic efficacy of [^{18}F]FDG PET in the treatment of cancer has been established, and the metabolic process by which [^{18}F]FDG detects glucose consumption is well characterized. Nevertheless, there is still a dearth of comprehensive knowledge on the signaling pathways that govern glucose consumption and [^{18}F]FDG accumulation in cancer. By comprehending these signaling pathways, it may be possible to link the amount of [^{18}F]FDG accumulation to specific and potentially targetable changes in a given cancer type. This could also help identify additional therapies for which [^{18}F]FDG PET could be used as an early predictor of therapeutic efficacy.

The present scenario of [^{18}F]FDG PET/CT in systemic and tumoral immune response monitoring is the primary focus of this chapter. This review recognizes the limitations of morphological imaging in the systemic monitoring of immunotherapy response, with an elaboration of investigation of the incremental benefit of [^{18}F]FDG PET/CT as a molecular biomarker.

2. CURRENT STATUS OF [^{18}F]FDG AND ITS MECHANISM OF ACTION IN VARIOUS DISEASES

Positron emission tomography (PET) is used in conjunction with 2-deoxy-2-[^{18}F]fluoro-D-glucose ([^{18}F]FDG), a positron-emitting radiotracer, to diagnose and track a variety of disorders. Standard imaging technologies like CT, MRI, and X-rays enable very detailed observation of both healthy and unhealthy tissue. Functional imaging methods such as PET scans can be used in addition to structural modalities to address some of these shortcomings. [^{18}F]FDG is therefore a well-recognized biomarker with further advantages for a range of biological research.

2.1. Neurology

The FDA has authorized the use of FDG in neurology to locate aberrant glucose metabolism foci connected to epileptic seizure foci. Additionally, FDG can aid in the visualization of different brain regions for variations in glucose metabolism, which aids in the diagnosis of a variety of neurological disorders such as brain damage and other neurological disorders like dementia, Parkinson's disease, Alzheimer's disease, *etc* [5 - 7] (Fig. **1**).

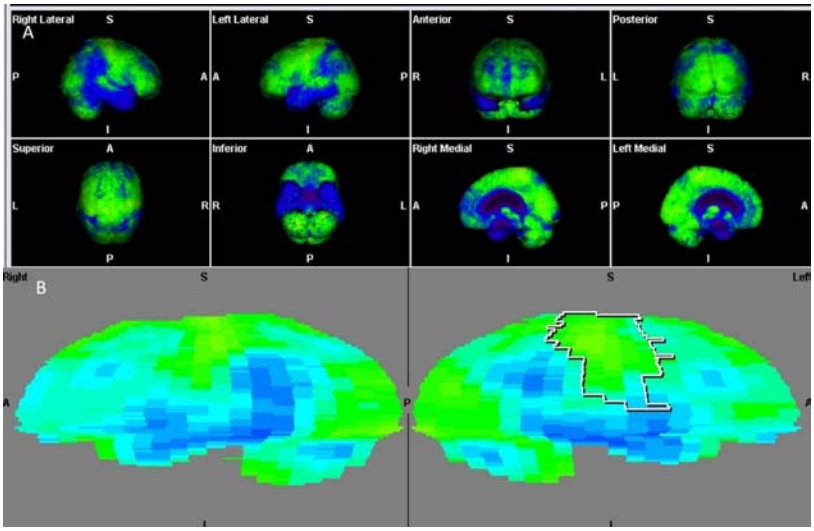

Fig. (1). 56 year male presented with progressive forgetfulness for 1 year. (**A**) Brain FDG PET/CT 3-D volumetric images revealed hypometabolism in the temporoparietal region. (**B**) SPM images revealed significant symmetrical hypometabolism in the temporoparietal region. The findings were consistent with Alzheimer's Disease.

The main fuel for the brain is glucose, which can help diagnose some pathologic disorders by showing differences in glucose usage from the normal metabolic rhythm and FDG can help in identifying the locations of seizure foci. FDG-PET can not only pinpoint the locations of seizure foci but also reveal details regarding the overall functional state of the brain [8].

2.2. Cardiology

When coupled with myocardial perfusion imaging, FDG shows which left ventricular myocardium has residual glucose metabolism and how much left ventricular dysfunction is present. The buildup of macrophages and myocardial ischemia that causes atherosclerosis can also be seen using FDG [9]. A heart that is in good health uses free fatty acids as its primary energy source, but anaerobic glucose metabolism replaces fatty acid metabolism in ischemia myocardial tissue. The myocardium that is hibernating among patients with coronary artery disease and left ventricular dysfunction is identified by FDG-PET in nuclear medicine when the patients are scheduled for coronary revascularization. The principle behind its use is that while myocytes that have suffered reversible injuries can utilize glucose, those that have suffered irreversible injuries cannot. Systolic function in the area with reduced perfusion can be reversed if blood flow is restored, as indicated by the accumulation of FDG in such places. A perfusion-metabolism mismatch is a term used to describe the observed pattern of reduced

CHAPTER 9

2-Deoxy-D-Glucose as an Emerging Chemotherapeutic Agent in Cancer Management

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Abstract: Cancer cells have a unique property of uncontrolled growth and thus they require a constant supply of energy. Warburg observed that tumor cells prefer glycolysis even under oxygenic conditions and the process is known as aerobic glycolysis. Hence, cancerous cells show an enhanced glucose-to-lactate conversion rate. As cancerous growth is accompanied by enhanced glucose uptake, this feature is best suited for the management of unwanted cell proliferation by blocking the glucose metabolism of cancer cells. 2-deoxy-D-glucose (2DG), a glucose antimetabolite is considered a competitive inhibitor of glucose transport and glucose phosphorylation. It inhibits the glycolytic pathway primarily due to the inhibition of phosphohexose isomerase by 2-deoxy-D-glucose-6-phosphate (2DG- 6P). Its chemical resemblance to 2-deoxymannose causes interruption in the initial steps of N-linked glycosylation leading to the misfolding of proteins resulting in endoplasmic reticulum stress. In addition to the two properties of 2DG namely, the prevention of glycolysis and selective storage in the tumor cells, there are several other attributes of 2DG apart from the ones mentioned above that make it an attractive target for use as an antitumor agent. Some properties include the capability of inducing autophagy in tumor cells, inhibiting genomic replication as well as mRNA expression of viral genes responsible for Omit the induction of oncogenesis, blocking pathological angiogenesis while being cautious towards established endothelial tubes and prominent anti-metastatic effect. In the present chapter, various aspects of the use of 2DG in cancer management have been discussed.

Keywords: Anticancer, Competitive inhibition, Chemotherapeutic drug, Glycolysis, Warburg effect, 2-deoxy-D-Glucose.

1. INTRODUCTION

Unicellular organisms have Omit the tendency to initiate their reproductive phase as soon as possible when nutrients are available. They possess a system that senses the nutrient supply and accordingly channelizes the metabolites to fulfill

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Raman Singh, Antresh Kumar & Kuldeep Singh (Eds.)

their requirement of carbon, nitrogen, and free energy for preparing the building blocks required to produce a new cell. However, biomass production ceases when there is a scarcity of nutrients. Under such circumstances, organisms readjust their metabolism in such a way that maximum free energy is conserved in order to survive through the starvation period. Also, the cells prefer not to enter the proliferative stage without undergoing proper cell cycle checkpoints. For this, they possess a cellular mechanism to prevent the entry of excessive nutrients, if available, so that they may not cause unnecessary induction of cell proliferation. Mammalian cells utilize the nutrients present in the external environment only when stimulated by growth factors but the process of cell division in cancer cells becomes independent of growth factors due to some genetic mutations.

Dividing cells require replication/synthesis of all the chemical constituents that are essential to build macromolecules required for the synthesis of all cellular components such as DNA, RNA, proteins, glucose, and lipids. Precursors for cell division are provided in some form or the other through glucose. Therefore, glycolysis is upregulated to maintain the level of glycolytic intermediates needed to support biosynthesis in proliferating cells [1]. Owing to the profound role of glucose in cellular metabolism and proliferation, its analogs, such as 2-deoxy-D-glucose, which have a metabolic fate different from glucose can be employed to regulate widespread diseases such as cancer, epilepsy, aging-related issues, and viral infections.

2. GLUCOSE METABOLISM IN CANCER CELLS

In normal cells, glucose first gets metabolized in the glycolytic cycle to produce NADH and pyruvate. Both the end products of the glycolytic cycle, NADH and pyruvate are transported into the mitochondria where oxidation of pyruvate takes place through the TCA cycle while NADH is oxidized to NAD^+ in the electron transport chain [2]. The reducing equivalents produced in the TCA cycle are used to fuel oxidative phosphorylation to maximize the synthesis of adenosine triphosphate (ATP) [3].

When glucose is subjected to oxidation through the Krebs cycle and the transfer of electrons occurs through the electron transport chain, several-fold higher amounts of ATP are generated in terms of ATP generated per molecule of glucose oxidation as compared to aerobic glycolysis. Even then aerobic glycolysis is preferred over oxidative phosphorylation as it provides ATP at a faster rate. Analysis of data generated from a study on 31 cancer cell lines/tissues suggested that the average percentage of ATP contribution from glycolysis is 17% [4]. It is evident through a study on the reduced flux-balance model by Vazquez *et al*.

(2010) [5] that synthesizing ATP through aerobic glycolysis is of normal occurrence in a cell that tends to have more mitochondria than its threshold.

Since cancer cells have a peculiar feature of sustained proliferation, they overcome the feedback regulatory mechanisms to meet their anabolic demands. For the continuation of glycolysis, a constant supply of NAD^+ and ADP is required for the continuation of glycolysis. NAD^+ from NADH is generated during the conversion of pyruvate to lactate by lactate dehydrogenase. Enhanced rate of glycolysis that is encountered in cancer cells is fueled by this enzymatic activity [6]. Growth-regulating factors stimulate proliferation in normal cells of healthy tissues by binding to their respective transmembrane receptors. Nutrients and oxygen required for this purpose are made available through the blood supply [7]. In contrast to normal cells, which require mitogenic growth signals for their propagation, cancer cells depend on their growth factors so they have reduced requirement for extracellular growth factors. This implies that instead of the normally occurring paracrine mode of operation of growth factors, cancer cells mainly rely on the autocrine mode of growth factors utilization. Mutations in receptor-associated signaling molecules and insensitiveness towards anti-growth stimuli lead to independent cellular proliferation in tumor cells [8]. Unrestrained cellular proliferation of cancer cells places newly formed tumor cells away from the blood vessels in the area where the tumor is proliferating. Therefore, the availability of blood and glucose to the core of the tumor cells gets impaired. Partial oxygen pressure declines to extremely hypoxic values ($\leq$ 0.5mm Hg) in avascular areas in a tumor [9]. Tumor cells experiencing hypoxia secrete a transcription factor known as Hypoxia-inducible factor-Iα (HIF-Iα), which, in turn, regulates tumor pH and angiogenesis.

The term angiogenesis is used for the formation of new blood vessels and is often found exacerbated in tumors. Vascular endothelial growth factor (VEGF) and Ang-2 are the two critical factors that are secreted in response to hypoxia and are involved in angiogenesis. HIF-Iα induces the expression of VEGF by binding to its promoter and thereby promotes tumor angiogenesis [10]. Transcriptional regulation of VEGF also takes place by Ras-ERK and PI(3)K–AKT pathways [11].

One of the enzymes of the glycolytic pathway-hexokinase (HK), which is involved on phosphorylation of glucose to produce glucose-6-phosphate by utilizing an ATP is also a target of HIF-Iα and hence it is responsible for high glycolytic rate in hypoxic solid tumors [12]. There are four types of HKs found in mammalian tissues that are encoded by different genes – *HK1*, *HK2*, *HK3,* and *HK4* [13]. Biochemical attributes of the above-mentioned hexokinases are comparable to each other but they show unique tissue localization and enzymatic

CHAPTER 10

2-Deoxy-D-Glucose: A Glycolysis Inhibitor in the Treatment of Cancer

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Abstract: Cancer involves abnormal and rapid cell growth, which requires an increased energy supply for proliferating cells. As the demand for glucose rises in cancer cells, the expression and activity of glucose transporters (GLUTs) also increase to facilitate higher cellular glucose uptake. Cancer cells tend to shift their glucose metabolic pathway from mitochondrial oxidative phosphorylation towards aerobic glycolysis. 2-Deoxy-D-glucose competes with glucose and involves aerobic glycolysis. It leads to the inhibition of HK and PGI, diminishes ATP production, and induces apoptosis. Further, the increase in the AMP/ATP ratio promotes the AMPK signaling, downregulating VEGF, and leading to angiogenesis inhibition and autophagy. As the structural mimic of mannose, 2-DG interferes with the N-linked glycosylation, leading to ER stress, and triggering the mitochondrial apoptotic pathway. 2-DG has been employed as an antiproliferative, antiangiogenic, and antimetastatic drug by being involved in the energy metabolic pathway. Combination therapy shows improved results and reduces chemotherapeutic drug resistance. In this chapter, we will discuss the Warburg effect, the role of 2-DG in the inhibition of aerobic glycolysis, and how 2-DG inhibits the various other cancer hallmarks in energy metabolic pathway. Also, reports on cancer treatment as well as cancer cell-imaging and risks associated with chronic exposure are discussed.

Keywords: Angiogenesis, Autophagy, Apoptosis, Cancer, Glycolysis.

1. INTRODUCTION

Glucose, a monosaccharide sugar molecule, is the primary source of the energy metabolism of living cells. The human body acquires its energy needs *via* food containing carbohydrates, proteins, *etc*. Among these, the carbohydrate is oxidized to ATP (adenosine triphosphate), stored as glycogen, converted into lipids, or utilized to synthesize cellular constituents. There are two processes by

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Raman Singh, Antresh Kumar & Kuldeep Singh (Eds.)

which glucose formation occurs. The first is glycogenolysis (glucose formation from glycogen), and the second is gluconeogenesis (formation of glucose from non-carbohydrate sources like lactate, amino acids, and glycerol). The liver is majorly involved in this glucose formation, and the renal cortex of the kidney produces glucose by gluconeogenesis [1]. Glucose is a polar molecule, which means it requires assistance from specialized transporters to cross the lipid bilayer of cell membranes. In the human body, there are fourteen different glucose transporter (GLUT) isoforms. Among these, GLUT1 (present in various tissues including pancreatic beta cells), GLUT2 (found in the intestine, kidney, and liver cells), GLUT3 (expressed in the brain), and GLUT4 (present in the heart, kidney, adipose tissue, and brain) are particularly noteworthy for their roles in glucose uptake and metabolism in different tissues and cell types [2]. After food intake, blood glucose level increases and the GLUTs start their functions. This is how the regular energy metabolism begins [3].

The energy requirement will be higher than expected during malignant tumourigenesis as they have a high proliferation rate. This energy need stimulates more GLUTs to upregulate glucose metabolism [4]. The energy metabolism of cancer cells differs from that of normal cells. It will be discussed in detail in the later part of this chapter under the Warburg Effect. The elevated glucose metabolism in cancer cells can be proven well with the reports of cancer progression under hyperglycemic and hypoglycemic conditions. Cancer patients with chronic hyperglycemia show an elevated level of cell proliferation, angiogenesis, and metastasis than the ones who do not have hyperglycemia [5]. Furthermore, high blood glucose levels can decrease the sensitivity of cancer cells to chemotherapeutic drugs, reducing the drug's effectiveness [6]. In contrast, inducing low blood glucose levels (hypoglycemia) has been shown to inhibit various processes in metastatic breast cancer cells studied in laboratory experiments, including cell differentiation, metastasis, formation of new blood vessels (angiogenesis), and the ability to form colonies [7]. The drug Pioglitazone is used for the treatment of type 2 diabetes and also inhibits breast cancer cell growth [8]. At this point, one can understand the importance of glucose in the survival and progression of cancer cells.

2-DG gets attention in cancer treatment. 2-DG has a vast therapeutic window, and its pharmacokinetics were well studied [9]. Like glucose, 2-DG gets into the cell by GLUTs, crosses the blood-brain barrier, and involves all metabolic pathways [10]. In the succeeding titles, the effect of 2-DG in cancer therapy will be discussed against its natural analogs, glucose, and mannose.

2. WARBURG EFFECT: ENERGY METABOLISM IN CANCER CELLS

The process of glucose metabolism starts in the cytoplasm as soon as GLUTs absorb glucose inside the cell [11]. Glycolysis produces two molecules of pyruvate and two molecules of ATP from one molecule of glucose. When there is enough oxygen present, pyruvate in mitochondria undergoes oxidative phosphorylation, or OXPHOS, which produces thirty molecules of ATP and carbon dioxide. Thirty-two ATP molecules are thus the net energy gained from glycolysis, which is followed by OXPHOS. In case of oxygen scarcity, pyruvate takes the anaerobic glycolysis pathway (lactic acid fermentation pathway). This produces two molecules of ATP and lactate. This is how glucose metabolism happens in normal cells [12].

Otto Heinrich Warburg, a German scientist, discovered that tumor cells affect how glucose is metabolized. Tumor cells are susceptible to hypoxia, which triggers the activation of hypoxia-inducible factor 1-alpha (HIF-1α), which is essential to the energy metabolism of cancer cells [13]. The glucose metabolism shifted from mitochondrial OXPHOS to aerobic glycolysis even when there was adequate oxygen tension. The "Warburg effect" is the name of this mechanism. There is a marked decrease in mitochondrial oxidation, and two molecules of ATP are produced as pyruvate is converted into lactate. Thus, four ATP molecules are the net energy gained during aerobic glycolysis [14]. Warburg effect causes diminished ATP production and the secretion of lactic acid [15]. Fig. (**1**) explains the glucose metabolic pathway of normal and cancer cells.

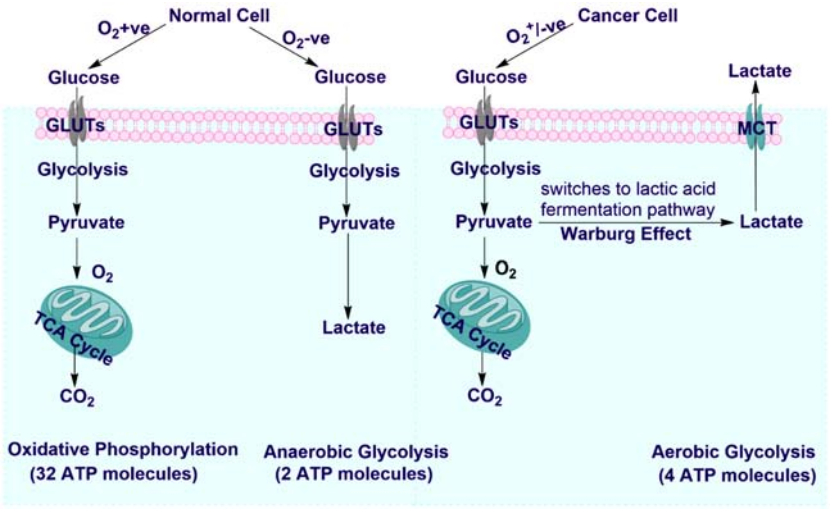

Fig. (1). Glucose Metabolism in Normal Cells *vs* Cancer Cells.

CHAPTER 11

Dual Role of 2-Deoxy-D-Glucose in Seizure Modulation

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Abstract: 2-Deoxy-D-glucose (2-DG) is a glucose analog that inhibits glycolysis. Conflicting evidence exists regarding the effects of 2-DG on seizure activity. The effects of 2-deoxy-D-glucose (2-DG) on seizures and epileptogenesis have been a subject of interest in the field of neuroscience and epilepsy research. In the 6-Hz seizure threshold test, 2-DG significantly increased the seizure threshold, indicating anticonvulsant properties. However, in other models, such as the mouse electroshock seizure threshold test, intravenous pentylenetetrazol test, and intravenous kainic acid test, 2-DG decreased the seizure threshold and exhibited proconvulsant effects. Similarly, the related compound 3-methylglucose reduced seizure threshold when administered intravenously with pentylenetetrazol. In contrast, 2-DG administered chronically retarded the progression of kindled seizures in rats, suggesting antiepileptic effects. The anticonvulsant actions of 2-DG may be mediated through the inhibition of glycolysis and diversion of glucose metabolism towards the pentose phosphate pathway. Meanwhile, its acute proconvulsant effects are likely due to reduced glucose uptake. In summary, 2-DG displays both anticonvulsant and proconvulsant actions on seizures, which depend on the model system and mechanisms involved, including glycolytic inhibition and decreased glucose uptake. Further study is needed to fully elucidate the contradictory effects of 2-DG on seizure activity in different experimental models.

Keywords: Anticonvulsant, Epilepsy, Proconvulsant, Seizure, Synaptic excitation, Seizure threshold, 2-Deoxy-D-glucose.

1. INTRODUCTION

Epilepsy is a chronic non-communicable neurological disorder of the brain that affects over 50 million people worldwide. The defining features of epilepsy include a brief period of uncontrollable movement of a part of or the whole body. Such sudden abnormal behavior of the patients is termed a seizure. The recurrent

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Raman Singh, Antresh Kumar & Kuldeep Singh (Eds.)

epileptic seizure is due to impulsive neuronal excitability at the synapse, resulting in the loss of consciousness and control over bladder or bowel movements. Various structures and processes related to neurons, ion channels, receptors, glia, and inhibitory and excitatory synapses are associated with the development of a seizure. Antiseizure drugs are used to regulate neural excitability for the treatment of epilepsy. Different groups of studies have revealed that various metabolic factors also affect neural excitability, which gained more attention for targeting a promising source for the prevention of epileptic episodes. The clinical observations suggest that ketogenic diets containing high fat, low-carbohydrate, and rich proteins are proven to control seizures and prevent epilepsy. However, the underlying mechanism of the ketogenic diet's role in controlling seizures is still not well-defined. The clinical studies noted that a low carbohydrate diet remarkably controls seizures which led to the hypothesis that restriction in the glycolysis and carbohydrate metabolism might have an anticonvulsant activity. The purpose of this chapter is to review the evidence on 2-DG to explore the action mechanism and its therapeutic activity both anticonvulsant and pro-convulsant agents.

2. ROLE OF 2-DG IN EPILEPSY

2-deoxy-D-glucose (2-DG) is a glucose analog that inhibits glycolysis which has been extensively studied for its potential as a chemotherapeutic agent against different health disorders. The detailed mechanism of 2-DG in glycolysis inhibition has been discussed earlier in this book. Interestingly, 2-DG has also demonstrated conflicting effects on seizures in different experimental models. The effects of 2-deoxy-D-glucose (2-DG) on seizures and epileptogenesis have been the subject of intense interest in the field of neuroscience and epilepsy research. The effects of 2-DG involved different mechanisms, such as the inhibition of glycolysis *versus* reduced glucose uptake. The anticonvulsant and proconvulsant actions of 2-deoxy-D-glucose (2-DG) have been extensively studied in various experimental models. These findings highlight the need for further investigation to elucidate the precise mechanisms underlying its actions and to explore its therapeutic potential in various neurological and oncological conditions. Elucidating the contradictory impacts of 2-DG on seizures in different models may provide insights into glucose metabolism in epilepsy and alternative glycolytic inhibitors for treatment. In the 6-Hz seizure threshold test, 2-DG elevates the seizure threshold and displays anticonvulsant properties. However, in other models like the mouse electroshock seizure threshold test and pentylenetetrazol or kainic acid models, 2-DG reduces the seizure threshold and acts as a proconvulsant. Clinical studies on kindling models also suggest that 2-DG can retard the progression of kindled seizures and have antiepileptic effects. The research on 2-DG's anticonvulsant and proconvulsant actions, its potential as

a chemotherapeutic agent, and its applications in neuroimaging collectively demonstrate the diverse effects of this glycolytic inhibitor. The anti and pro-convulsant properties of 2-DG are determined by its effects on glucose metabolism and its subsequent impact on seizure susceptibility. The metabolic changes due to this antimetabolite lead to both anticonvulsant and proconvulsant effects, depending on specific seizure models. In some tests, 2-DG has been shown to elevate the seizure threshold indicating its anticonvulsant activity. However, in other seizure models, it has been found to reduce the seizure threshold, demonstrating the proconvulsant properties [1].

3. ANTICONVULSANT EFFECTS

2-DG, a glycolytic inhibitor, has shown promising anticonvulsant effects in the rat kindling model of temporal lobe epilepsy [2]. It effectively suppresses spontaneous neuronal firing and epileptiform bursts in hippocampal slices, indicating its potential as an anticonvulsant agent [3]. Additionally, acute anticonvulsant actions of 2-DG were observed in experimental models of seizures and epilepsy, suggesting its potential therapeutic use in these conditions [2]. 2-Deoxy-d-glucose (2-DG) is being developed as a potential anticonvulsant and disease-modifying agent for patients with epilepsy. The anticonvulsant role of 2-DG is based on its ability to interfere with glucose metabolism by inhibiting the glycolytic enzymes. This interference leads to anticonvulsant actions. 2-DG is avidly taken up into the cells by GLUT transporters and is phosphorylated by hexokinase to form 2-DG-6-phosphate. This compound competitively inhibits the downstream process in glycolysis, preventing the progression of glycolytic pathway, since 2-DG-6-P cannot undergo the downstream steps of the pathway. Consequently, 2-DG shunts glucose metabolism into the pentose phosphate pathway (PPP), limiting glycolysis and increasing the glucose flux through the PPP. This metabolic alteration is believed to contribute to the anticonvulsant activity of 2-DG. 2-DG has shown suppressive effects on interictal-like epileptiform discharge (IED) but at the same time, promotes the induction of seizure-like epileptiform discharge (SLE). It has been shown that acute exposure to 2-DG or severe hypoglycemia decreases spontaneous IED induced by certain conditions, while also inducing seizure-like discharge. Findings suggest that the inhibitory effect of 2-DG on IED is linked to the impairment of glycolysis, while the proconvulsant effects are caused by an excitatory mechanism that depends on the impairment of mitochondrial oxidative phosphorylation [4]. The dual nature of 2-DG on seizure susceptibility suggests a complex interplay between its metabolic actions and the specific mechanisms underlying different seizure models. Additionally, 2-DG has been found to elevate the seizure threshold in the 6-Hz seizure test, indicating its anticonvulsant action. Among various studies, 2-DG has demonstrated acute anticonvulsive and chronic antiepileptic properties, both *in*

ANNEXURE

Lipinski's Rule of Five

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Abstract: Christopher A. Lipinski formulated a rule based on his observation that most drugs administered orally are relatively small and moderately lipophilic molecules. This rule serves as a guideline to determine whether a molecule with specific pharmacological or biological activity possesses properties that would make it a viable orally active drug in humans. Lipinski's Rule of Five functions as a powerful screening tool in the initial phases of drug discovery, enabling researchers to pick molecules with ideal attributes for subsequent development and testing.

Keywords: Absorption, Acceptors, Biological activity, Donors, Exceptions drugability, Lipinski's rule of five.

1. INTRODUCTION

Lipinski's Rule of Five, also known as the Rule of Five (RO5), is an approach utilized in drug discovery that aids in assessing the drug-likeness of chemical compounds. In 1997, Christopher A. Lipinski, a scientist at Pfizer, formulated this rule based on his observation that most drugs administered orally are relatively small and moderately lipophilic molecules [1].

Lipinski and his coworkers at Pfizer analyzed a database of compounds that successfully passed Phase I clinical trials and entered into Phase II studies. They correlated the computed physicochemical properties of these molecules to their observed aqueous solubility, permeability, and oral bioavailability using graphing software JMP [2].

Lipinski used JMP to generate plots of Pfizer compounds that successfully cleared the clinical trial hurdle between Phase I and Phase II. Through this analysis, he noted some common trends in the physicochemical properties of these successful

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drug candidates [2]. This led him to propose the Rule of Five as a guideline to determine whether a molecule with specific pharmacological or biological activity possesses properties that would make it a viable orally active drug in humans [3].

2. LIPINSKI'S RULE OF FIVE

The name "rule of five" comes from the fact that all the conditions have multiples of five as the determinant conditions. According to Lipinski's rule, an orally active drug should have no more than one violation of these four conditions [4, 5].

The Rule of Five states that, for a compound to have a reasonable probability of being membrane permeable and easily absorbed, it should meet the following criteria [4, 6]:

1. Molecular Weight < 500 Da
2. logP < 5
3. Hydrogen Bond Donors < 5
4. Hydrogen Bond Acceptors < 10

Poor absorption or permeation is more likely when a compound violates two or more of the following:

1. Molecular weight > 500 Da
2. Calculated logP > 5
3. More than 5 hydrogen bond donors
4. More than 10 hydrogen bond acceptors

This set of criteria helps filter out compounds with poor oral absorption and low permeation, ensuring potential drug candidates have favorable physicochemical properties for effective delivery and action within the body. The Rule of Five is a valuable tool in drug discovery for assessing chemical libraries and prioritizing promising drug candidates [7].

3. ROLE OF LIPINSKI'S RULE OF FIVE IN DETERMINATION OF DRUG-LIKENESS

Lipinski's Rule of Five has been instrumental in the design and evaluation of various classes of compounds, ranging from antimicrobial agents to anticancer drugs [8]. The rule provides a straightforward framework for determining the drug-likeness of molecules, enabling researchers to prioritize compounds with higher probabilities of success in terms of absorption, distribution, metabolism, and excretion (ADME) properties [9]. By considering parameters such as

molecular weight, hydrogen bond donors, hydrogen bond acceptors, and lipophilicity, Lipinski's Rule of Five aids in the rational selection of compounds with favourable pharmacokinetic profiles [10].

The most critical aspect in drug development is to verify that potential drug candidates exhibit the requisite physicochemical qualities for effective therapeutic action. The majority of drug candidates fail while moving clinical trial phase 1 to phase 2. When there are multiple violations of Lipinski's Rule of Five, it might result in issues with the bioavailability of a substance. This demonstrates the significance of these parameters in determining the suitability of a chemical compounds for oral delivery. Therefore, RO5 functions as a powerful screening tool in the initial phases of drug discovery, enabling researchers to pick molecules with ideal attributes for subsequent development and testing [11].

4. EXCEPTIONS OF THE RULE OF FIVE

The RO5 is derived from a distribution of estimated qualities among thousands of medications. Consequently, certain medications will inherently fall outside of the rule's parameter cutoffs. Most USAN (United States Adopted Name) medications with characteristics not covered by the Lipinski criterion can be grouped into a relatively small number of therapeutic groups. These oral active medicinal classes include cardiac glycosides, vitamins, antifungals, and antibiotics.

Because of the molecular characteristics of these molecules, the medications can function as substrates for naturally occurring transporters. There won't be many instances of chemicals that break the RO5 if these classes are removed from the USAN library [4, 6]. Drugs or clinical candidates having good bioavailability may be termed as Beyond Rule-of-5 (bRo5) compounds [12]. RO5 should not be hard and fast. It is important to note that to break a rule it is necessary to understood why there is a rule [2].

CONCLUSION

Lipinski's rule of five plays a pivotal role in drug discovery by providing a set of criteria to assess the drug-likeness of chemical compounds. By considering parameters such as molecular weight, lipophilicity, and hydrogen-bond donors and acceptors, this rule aids researchers in identifying potential drug candidates with favorable pharmacokinetic properties. Adherence to Lipinski's rule of five helps select compounds that are more likely to exhibit optimal oral absorption and bioavailability, thereby enhancing the efficiency of the drug development process. Although there are many exceptions, this rule helps to flag compounds.

SUBJECT INDEX

A

B

C

D

E

F

G

H

I

K

L

M

N

O

P

R

S

T

U

V

Z

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Kuldeep Singh

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