Potential Anti-carcinogenic Effect of Acetazolamide, a Carbonic Anhydrase Enzyme Inhibitor, in Diethylnitrosamine - Induced Hepatocarcinogenesis in Rats

Thesis

Submitted for Partial Fulfillment of Master Degree in Hepatology and Gastroenterology

By

Muhammad Saleh Abdul-Rasheed Hussain
M.B.B.CH

Under supervision of

Prof. Dr. Moataz Mohamed Sayed
Professor of Internal Medicine
Faculty of Medicine – Ain Shams University

Dr. Mohamed Lotfy Soliman
Assistant Professor of Internal Medicine
Faculty of Medicine – Ain Shams University

Dr. Yomna Mohamed Tamim
Lecturer of Clinical Pharmacology
Faculty of Medicine – Ain Shams University

Faculty of Medicine
Ain Shams University
2020

بسم الله الرحمن الرحيم

قالوا

سبحانك لا علم لنا
إلا ما علمتنا إنك أنت
العليم الحكيم

صدق الله العظيم

سورة البقرة الآية: ٣٢

Acknowledgment

First and foremost, I feel always indebted to **ALLAH,** *the Most Kind and Most Merciful.*

I'd like to express my respectful thanks and profound gratitude to **Prof. Dr. Moataz Mohamed Sayed,** *Professor of Internal Medicine - Faculty of Medicine- Ain Shams University for his keen guidance, kind supervision, valuable advice and continuous encouragement, which made possible the completion of this work.*

I am also delighted to express my deepest gratitude and thanks to **Dr. Mohamed Lotfy Soliman,** *Assistant Professor of Internal Medicine, Faculty of Medicine, Ain Shams University, for his kind care, continuous supervision, valuable instructions, constant help and great assistance throughout this work.*

I am deeply thankful to **Dr. Yomna Mohamed Tamim,** *Lecturer of Clinical Pharmacology, Faculty of Medicine, Ain Shams University, for her great help, active participation and guidance.*

I would like to express my hearty thanks to all my family for their support till this work was completed.

Last but not least my sincere thanks and appreciation to all patients participated in this study.

Muhammad Saleh Abdul-Rasheed

List of Contents

List of Tables

List of Figures

List of Figures (Cont...)

Fig. No.	Title	Page No.

List of Abbreviations

Abb.	Full term
2-AAF	*2-acetylaminofluorene*
8-OHdG	*8-hydroxy-2'-deoxyguanosine*
ACT	*Acetazolamide*
ATP	*Adenosine triphosphate*
AFB1	*Aflatoxin-B1*
MASRI	*Ain shams medical research center*
Alt	*Alanine aminotransferase*
ALP	*Alkaline phosphatase*
AFP	*Alpha feto protein*
AASLD	*American association for the study of liver diseases*
AEs	*Anion exchangers*
AQPs	*Aquaporins*
Ast	*Aspartate aminotransferase*
ATM	*Ataxia telangiectasia mutated kinase*
AIH	*Autoimmune hepatitis*
AZA	*Azathioprine*
BCLC	*Barcelona Clinic Liver Cancer*
BSC	*Best supportive care*
BW	*Body weight*
CCl4	*Carbon tetrachloride*
CAs	*Carbonic anhydrases*
CP	*Child-Turcotte-Pugh*
CT	*Computed tomography*
CDK1	*Cyclin-dependant kinase 1*
DNA	*Deoxyribonucleic acid*
DM	*Diabetes mellitus*
DM	*Diabetes mellitus*

d.	*Diet*
DEN	*Diethylnitrosamine*
DMSO	*Dimethylsulfoxide*
DAAs	*Direct acting antiviral agents*
d.w	*Drinking water*
ER	*Estrogen receptor*
pHe	*Extracellular pH*
FLR	*Future liver remnant*
FLRF	*Future liver remnant function*
i.g.	*Gavage*
GEM	*Genetically engineered mouse*
GLUT1	*Glucose transporter 1*
Gdf15	*Growth differentiation factor 15*
BTs	*$HCO3^-$ transporters*
H&E	*Hematoxylin and eosin*
HBV	*Hepatitis B virus*
HCV	*Hepatitis C virus*
HDV	*Hepatitis D virus*
HCC	*hepatocellular carcinoma*
HIFU	*High-intensity focused ultrasound*
HIV	*Human immunodeficiency viruses*
HIF-1 α	*Hypoxia-inducible factor 1-alpha*
IFN γ	*Interferon gamma*
pHi	*Intracellular pH*
ICC	*Intrahepatic cholangiocarcinoma*
IP	*Intraperitoneal*
i.p.	*Intraperitoneal injection*
IRE	*Irreversible electroporation*
LDH	*Lactate dehydrogenase*
LR	*Liver resection*
LT	*Liver transplant*
MRI	*Magnetic resonance imaging*

List of Abbreviations (Cont...)

Abb.	Full term
MMPs	*Matrix metalloproteinases*
MVI	*Microvascular invasion*
MWA	*Microwave ablation*
MAPK	*Mitogen-activated protein kinase*
MELD	*Model for end-stage liver disease*
mRECIST	*Modified Response Evaluation Criteria in Solid Tumors*
MCTs	*Monocarboxylate transporters*
m.d.	*Multiple doses*
NHE	*Na^+/H^+ exchanger*
NBCs	*$Na^+/HCO3^-$ co-transporters*
NDCBE	*Na^+-dependent $Cl^-/HCO3^-$ exchangers*
NK	*Natural killer cells*
NAD	*Nicotinamide adenine dinucleotide*
DMN	*N-nitrosodimethylamine*
NMOR	*N-nitrosomorpholine*
NAFLD	*Nonalcoholic fatty liver disease*
NASH	*Nonalcoholic steatohepatitis*
N/C	*Nuclear/cytoplasmic ratio*
OLT	*Orthotopic Liver Transplantation*
OS	*Overall survival*
PEI	*Percutaneous ethanol injection*
PB	*Phenobarbital*
PDGFR	*Platelet-derived growth factor receptor*
PVT	*Portal vein thrombosis*
PSL	*Prednisolone*
PR	*Progesterone receptor*

List of Abbreviations (Cont...)

Abb.	Full term
PFS	*Progression-free survival*
RFA	*Radiofrequency ablation*
RCT	*Randomized controlled trial*
ROS	*Reactive oxygen species*
s.d	*Single dose*
STRT	*Stereotactic body radiotherapy*
s.c.	*Subcutaneous injection*
SVR	*Sustained virologic response*
TMZ	*Temozolomide*
EASL	*The European association for the study of the Liver*
ECM	*The extracellular matrix*
IARC	*The International Agency for Research on Cancer*
mTORC1	*The mammalian target of rapamycin complex 1*
WHO	*The world health organization*
TAA	*Thioacetamide*
Y-90	*Transarterial radioembolization with Yttrium-90*
TACE	*Transcatheter arterial chemoembolization*
TNF-α	*Tumor necrosis factor alpha*
TNM	*Tumor-node-metastasis*
VEGF	*Vascular endothelial growth factor*
pVHL	*Von Hippel-Lindau tumor suppressor protein*

INTRODUCTION

Hepatocellular carcinoma (HCC) is a major global health problem that accounts for more than 90% of primary liver malignancies ***(Wang et al., 2016).*** It is the 6th most common cancer, and the fourth cause of cancer-related deaths around the world, accounting for 7% of all cancers ***(Shiha et al., 2020).*** Its incidence in men is 2-3 times than women, representing the 5th commonest malignancy in men and the 9th commonest cancer in women ***(Monga, 2020)***.

Liver cancer major risk factors include viral hepatitis infection, food additives, alcohol abuse, and toxic exposure: aflatoxin-B1 (AFB1) intake from contaminated food, environmental and industrial toxic chemicals, and air and water pollutants. Besides obesity and metabolic diseases, starting from nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH), diabetes and autoimmune reactions also contribute to HCC development ***(Arboatti et al., 2018).***

Animal models of more advanced HCC have appeared to be crucial for investigating the genetic alterations, signaling pathways and microenvironment interactions implemented in HCC evolving and progression. Furthermore, they have given the chance for the evaluation of potential novel therapeutic strategies and drugs in preclinical trials ***(Liu et al., 2020)***.

Nitrosamines have very high carcinogenic properties. Therefore, application of these substances, especially DEN (Diethylnitrosamine), has become highly attractive for inducing liver tumorigenesis in rodents as an experimental model of human hepatocarcinogenesis ***(Tolba et al., 2015)***.

On primary metabolic activation, DEN produces the promutagenic adducts, O6-ethyl deoxy guanosine and O4- and O6-ethyl deoxy thymidine that can produce DNA chain damage, depurination or binding to DNA and often generating a miscoding gene sequence, paving the way to initiate liver carcinogenesis ***(Janani et al., 2010).***

The pH of solid tumors is acidic due to increased fermentative metabolism and poor perfusion. It has been hypothesized that acid pH promotes local invasive growth and metastasis. The hypothesis that acid mediates invasion proposes that H+ diffuses from the proximal tumor microenvironment into adjacent normal tissues where it causes tissue remodeling by permitting degradation of the adjacent normal tissue extracellular matrix by proteinases, increases angiogenesis and inhibits the immune response to tumor antigens ***(Estrella et al., 2013).***

Carbonic anhydrases (CAs) are a family of enzymes responsible for the reversible hydration of carbon dioxide to carbonic acid, being key molecules in two vital processes: cell metabolism and pH regulation. This family of enzymes comprises 15 members that are classified according to their cell location into: membrane-associated, cytosolic, mitochondrial or secreted ***(Granja et al., 2017).***

Membrane-bound CAs have an extracellular active site and can provide the H^+ or HCO_3^- ions formed during catalytic turnover for various physiological/pathological processes, among which is extracellular acidification. It has been shown that two CA isozymes (hCA IX and hCA XII) are prominently associated with and over expressed in many tumors, where they are involved in crucial processes connected with cancer microenvironment acidification, progression and response to chemotherapy ***(Ozensoy et al., 2008).***

Acetazolamide (2-acetylamino-1, 3, 4-thiadiazole-5-sulfonamide), with a molecular formula of C4H6N4O3S2, belongs to the family of sulfonamide drugs is a CA inhibitor that mediates its therapeutic effect through modulation of the activity of the membrane and cytosolic CAs. It is a weak acid (pKa 7.2) and at pH 8.45 most of its molecular population contained a negative charge resulting in inactivation of the enzyme ***(Safarian et al., 2007)***.

Acetazolamide is used for the treatment of glaucoma, acute mountain sickness, seizure disorders, edema and periodic paralysis ***(AHFS DI Essentials, 2018).***

AIM OF THE WORK

This animal study was designed to evaluate the effect of acetazolamide on tumor growth and viability in Diethylnitrosamine (DEN)-induced HCC rat models.

30 male Wister rats were divided into 3 groups (normal control group, HCC-induced group, and Acetazolamide- treated HCC induced group).

After induction of HCC in the second and third groups using DEN at the dose of 70mg/kg via intraperitoneal injection once per week over 10 weeks, acetazolamide was tested at the dose of 60mg/kg as a single daily dose intragastricaly over 3 weeks in the third group.

- **Outcome measures**

I- Lab values:

1- AST

2- ALT

3- Total bilirubin

4- Direct bilirubin

5- Albumin

6- Alpha feto protein

II- Histopathological examination:

1- Tumor grading

2- Tumor necrosis