"Pharmacological study on the potential effect of piceatannol on cisplatin- induced nephrotoxicity in rats"

Thesis presented by

Sara Abdel Moneim Abdel Zaher Wahdan

M.Sc., Ain Shams University (2014)

Assistant Lecturer of Pharmacology and Toxicology,

Faculty of pharmacy, Ain Shams University.

Submitted for partial fulfillment of PhD degree in Pharmaceutical Sciences to Department of Pharmacology and Toxicology, Faculty of Pharmacy, Ain Shams University.

Under the supervision of

Dr. Ebtehal El-Demerdash Zaki

Professor and head of Pharmacology and Toxicology Departement

Faculty of Pharmacy, Ain Shams University.

Dr. Samar Saad El-Deen Azab

Associate Professor of Pharmacology and Toxicology,

Faculty of Pharmacy, Ain Shams University.

Dr. Doaa Ahmed El-Sherbini

Lecturer of Pharmacology and Toxicology,

Faculty of Pharmacy, Ain Shams University.

Faculty of Pharmacy

Ain Shams University

(2017)

"دراسة فارماكولوجية للتأثير المحتمل لمركب بيساتنول فى تسمم الكلي المحدث بدواء سيسبلاتين فى الجرذان"

رسـالة مقدمـة مـن الصيدلانية

سارة عبد المنعم عبد الظاهر وهدان

ماجستير علوم صيدلية . جامعة عين شمس (2014)

مدرس مساعد بقسم الأدوية والسموم . كلية الصيدلة . جامعة عين شمس

كجزء للحصول علي درجة الدكتوراة فى العلوم الصيدلية

(علم الأدوية والسموم)

تحت إشــراف

أ.د/ إبتهال الدمرداش زكي

أستاذ و رئيس قسم الأدوية والسموم . كلية الصيدلة .

جامعة عين شمس

د/ سمر سعد الدين عزب

أستاذ مساعد علم الأدوية والسموم . قسم الأدوية والسموم . كلية الصيدلة .

جامعة عين شمس

د/ دعاء احمد مختار

مدرس علم الأدوية والسموم . قسم الادوية والسموم، كلية الصيدلة .

جامعة عين شمس

كلية الصيدلة

جامعة عين شمس2017

Abstract

Cisplatin induced nephrotoxicity is a major side effect limiting its chemotherapeutic use. We investigated whether piceatannol (PIC)can protect against cisplatin-induced nephrotoxicity in rats and the potential underlying mechanisms. A single dose of cisplatin (7mg/kgi.p.) was used to induce nephrotoxicity and PIC was given in different daily doses (5, 10 and 20 mg/kg i.p.), for seven days starting two days before cisplatin injection. Cisplatin significantly increased blood urea nitrogen and serum creatinine levels and induced tubular degeneration, hemorrhage and cast formation. PIC at dose 10 mg/kg was effective in preventing these alterations. In subsequent mechanistic study, PIC (10 mg/kg i.p.) reversed cisplatin-induced kidney injury molecule 1 and neutrophil gelatinase associated lipocalin and ameliorated cisplatin-induced oxidative stress, inflammation and apoptosis. PIC inhibited cisplatin-induced lipid peroxidation and glutathione depletion. Additionally, PIC reversed cisplatin-induced reduction in the gene expression of nuclear factor E2-related factor 2 and the relative mRNA level of antioxidant related genes; hemeoxygenase-1, cysteine ligase catalytic and modifier subunits, as well as superoxide dismutase and glutathione-S-transferase activities. Furthermore, cisplatinproinflammatory response was reduced by PIC treatment as evidenced by decreased tissue levels of interleukin-1β, tumor necrosis factor-α, cyclooxygenase-2 and inducible nitric oxide synthase, as well as reversing nuclear factor kappa-B activation. Moreover, PIC suppressed cisplatin-induced apoptosis by decreasing p53 and cytochrome C expression and caspase-3 activity. Furthermore, we also investigated PIC ability to modify cisplatin cytotoxicity on two different cancer cell lines; Human hepatocellular carcinoma and Prostate carcinoma cell lines. PIC enhanced cisplatin cytotoxic effects by lowering its IC_{50} values in both cell lines. Therefore PIC has the potential for ameliorating cisplatin-induced renal injury while improving its cytotoxic activity.

Key wards: Cisplatin, piceatannol, nephrotoxicity, oxidative stress, inflammation, apoptosis.

Acknowledgements

Most heartfelt thanks are due to ***Dr. Ebtehal El Demerdash****, Professor of Pharmacology and Toxicology, Department of Pharmacology and Toxicology, Faculty of Pharmacy, Ain Shams University, who has made this work possible by her great effort, continuous guidance, support and indispensable help in practical work and thesis writing. In fact, she was more than a supervisor, she never stopped supporting and encouraging me. Her precious advices were always pushing me forward.*

I am greatly thankful to ***Dr.SamarSaadElDeenAzab****, Associate Professor of Pharmacology and Toxicology, Department of Pharmacology and Toxicology, Faculty of Pharmacy, Ain Shams University. I am deeply grateful for her continuous support, guidance as well as tremendous effort and indispensable help in the practical workand thesis writing.*

I wish to express my appreciation and gratitude to ***Dr.Doaa Ahmed El-Sherbini,*** *Lecturer ofPharmacology and Toxicology, Department of Pharmacology and Toxicology,,Ain Shams University, for her guidance, kind cooperation and discussion throughout the work.*

I would like to thank ***Dr. Ahmed Esmat and Dr. EmanMantawy,*** *lecturers of Pharmacology and Toxicology,* ***MSc PharmacistReemTarek****Assistant lecturer of Pharmacology and Toxicology, Department of Pharmacology and Toxicology, Faculty of Pharmacy, Ain Shams University, for their help in the practical work.*

It is my great pleasure to thank all members of Pharmacology and Toxicology Department, Faculty of Pharmacy, Ain Shams University and every person in my Faculty who supported me and helped me in my way.

Finally, but of great importance, I wish to express my deep gratefulness and thanks to my family; for their continuous moral and emotional support.

List of Contents

ARE	Antioxidant response elements
AIF	Apoptosis inducing factor
ATP	Adenosine triphosphate
BAD	Bcl-2-associated death promoter
Bak	Bcl-2 homologous antagonist/killer
Bax	Bcl-2 associated X protein
BCA	Bicinchoninic acid
Bcl-2	B-cell lymphoma 2
Bcl-xl	B-cell lymphoma-extra large
BSA	Bovine serum albumin
BUN	Blood urea nitrogen
CAT	Catalase
CK	Creatinine kinase
COX-2	Cyclooxygenase- 2
Ctr-1	Cupper transporter-1
Ct	Cycle threshold
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DSS	Dextran sodium sulfate
DTNB	5,5'-dithio-bis (2-nitrobenzoic acid)
DW	Distilled water
ECL	Enhanced luminol based chemiluminesence

ER	Endoplasmic reticulum
ERα	Estrogen receptor α
ERβ	Estrogen receptor β
EDTA	Ethylene-diaminetetraacetic acid
GGT	Gamma-glutamyltranspeptidase
GFR	Glomerular filtration rate
GLUT-4	Glucose transporter-4
GCLC	Glutamate-cysteine ligase catalytic subunit
GCLM	Glutamate-cysteine ligase modifier subunit
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GPx	Glutathione peroxidase
GR	Glutathione reductase
GSH	Reduced glutathione
GSSG	Oxidized glutathione
GST	Glutathione-S-transferase
HO-1	Hemeoxygenase-1
HMG	High motility group
H_2O_2	Hydrogen peroxide
HRP	Horseradish peroxidase
HepG2	Human hepatocellular carcinoma
IgE	Immunoglobulin E
IFN-γ	Interferon-γ

IκB	Inhibitor of kappa B
IKK	IκB kinase
IL	Interleukin
iNOS	Inducible nitric oxide synthase
ICAM-1	Intracellular adhesion molecule-1
IP	Intra-peritoneal
ICP-OES	Inductively Coupled Plasma Optical Emission Spectroscopy
JAK-1	Janus kinase-1
Keap-1	Kelch-like ECH-Associated Protein 1
KIM-1	Kidney injury molecule 1
LDH	Lactate dehydrogenase
LPS	Liopopolysaccahride
Maf	Musculoaponeuroticfibrosarcoma
MDA	Melanocyte stimulating hormone
MSC	Mesenchymal stem cells
mRNA	Messenger RNA
MAPK	Mitogen-activated protein kinase
NADH	Nicotinamide adenine dinucleotide
NGAL	Neutrophil gelatinase associated lipocalin
Nrf-2	Nuclear factor E2-related factor 2
NF-κB	Nuclear factor kappa B
NO	Nitric oxide
OCT-2	Organic cation transporter 2

PIC	Piceatannol
Pt	Platinum
PPAR-α	Peroxisome proliferator-activated receptor alpha
Puma	P53 upregulated modulator of apoptosis
PIDD	P53 inducible protein with death domain
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RQ	Relative quantity
RT-PCR	Real time polymerase chain reaction
STAT	Signal transduction and activator of transcription
SYK	Spleen tyrosine kinase
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SOD	Superoxide dismutase
TBARS	Thiobarbituric acid reactive substances
TLR	Toll like receptor
TGF-β1	Transforming growth factor β1
TBS	Tris-buffered saline
TBST	Tris-buffered saline with tween
TNF-α	Tumor necrosis factor-alpha
TNFR	Tumor necrosis factor receptor

. Cisplatin

Cisplatin is one of the most potent chemotherapeutic drugs, widely used for cancer treatment. Cisplatin is used alone or in combination with other chemotherapeutic agents for the treatment of a spectrum of specific cancers, including testicular, ovarian, bladder, head and neck, esophageal, small and non-small cell lung. It is also used for breast, cervical, stomach and prostate cancers, as well as Hodgkin's and non-Hodgkin's lymphomas, neuroblastoma, sarcomas, multiple myeloma, melanoma, and mesothelioma **(Miller et al., 2010).**

1.1. Discovery and history

Cisplatin was first synthesized by M. Peyrone in 1844 while its chemical structure was first elucidated by Alfred Werner in 1893. However, the compound gained scientific attention in 1960s, when **Rosenberg et al. (1965)** at Michigan State University,identifiedcertain electrolysis products of platinum electrodes which were able to inhibit cell division of Escherichia coli. These initial observations created much interest in the possible use of these products in cancer chemotherapy.In 1968, cisplatin was injected at the non-lethal dose to mice bearing a standard murine transplantable tumor, and caused marked tumor regression**(Rosenberg et al., 1969).**

In 1971, cisplatin was taken for clinical trials by the US National Cancer Institute (NCI). By the end of the 1970s it had earned a place as the key ingredient in the systemic treatment of germ cell cancers. It was the first FDA-approved platinum compound for cancer treatment in 1978 **(Kelland, 2000).**The discovery of cisplatin is considered a corner stone which triggered the interest in platinum and other metal-containing compounds as potential anticancer drugs (**Florea and Büsselberg, 2011)**.

1.2. Chemistry

Cisplatin [(CAS No. 15663-27-1), cisplatinum, cis-diamminedichloroplatinum(II)], is a metallic (platinum) compound with a chemical formula: $H_6Cl_2N_2Pt$.It is a white or deep yellow to yellow-orange crystalline powder at room temperature. It is slightly soluble in water (1 in 1000) and N,Ndimethylformamide, insoluble in alcohol; sparingly soluble in dimethylformamide. A 0.1% solution in 0.9% saline has a pH of 4.5-6.0. The injectable preparation has a pH of 3.5-6.2**(Dasari and Tchounwou, 2014).**

Cisplatin is stable under normal temperatures and pressures but transform slowly over time to the trans-isomer. Cisplatin has a molecular weight of 301.1 gm/mol, a density of 3.74 g/cm^3 and a melting point of 270°C**(Dasari and Tchounwou,2014).**

Cisplatin exists as an electro-neutral, four coordinate platinum complex. It has two chloride ion ligands situated adjacent to one another, and two ammonia ligands, in a square (tetragonal) planar structure**(Fig.I).** The anticancer activity of cisplatin is due to the relative ease of substitution of the chlorine ligands with nucleophilic species like nucleic acid bases of a DNA strand.The cis isomer has antitumor activity, while the transisomer of cisplatin, transplatin, showed no antitumor activity**(Johnstone et al., 2015)**