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THE EFFECT OF BENZO(a)PYRENE ON MALE MOUSE *MUS MUSCULUS*

PARICHEHR HANACHI

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THE EFFECT OF BENZO(a)PYRENE ON MALE MOUSE *MUS MUSCULUS*

By

PARICHEHR HANACHI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

June 2002

DEDICATION

Dedicated to my husband, Mahmood Abaie, and my daughter Somayeh for their devotion, understanding and support during all difficulties and to my parents for their true love, constant trust and principles that guided my life and to many researchers whose have not given due recognition for the many hours spent in the laboratory and friends to provide humanity with solutions to better life

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

THE EFFECTS OF BENZO(a)PYRENE ON MALE MICE *MUS MUSCULUS*

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PARICHEHR HANACHI

June 2002

Chairman: Assoc. Prof. Dr. Nor Aripin Shamaan

Faculty: Science and Environmental Studies

A study was carried out to determine the effect of benzo(a)pyrene (BaP) on selected enzyme activities , the pathology of lung, liver and kidney and cellular aspects of the mice *Mus musculus*. A suppression or change in the activities of several enzymes in these tissues can be used as a potential technique for the diagnosis of carcinogenesis in the early stage.

The initial work involved the evaluation of lethal dose and the threshold dose required for induction of carcinogenesis in adult mice. Subsequent work involved the determination of glutathione S-transferase (GST) and glutathione peroxidase (GPx) activities over a time period during which the mice were treated with benzo(a)pyrene. Finally, induction of GST and GPx was achieved by long-term treatment with BaP. The GST was purified partially by affinity chromatography. Determination of the effect of BaP on the histology of liver, lung, kidney of mice

were also carried out. Results obtained showed that GST and GPx activities were induced by BaP in a dose-dependent manner (100-250 mg/kg body weight). The mice were injected with BaP at a dose of 200 mg/kg body weight once at the start of the short term study, GST activity was induced at maximum after 4 days, and after that the activity dropped to almost normal values.

The effect of BaP on GST and GPx activities in the liver, lung , kidney and blood of male mice were studied in long-term study. The mice with injected 200 mg/kg BaP once a week for 8 weeks. The GST activity was significantly increased ($P<0.05$) after 2 weeks in liver, lung, kidney and blood while GPx activity was significantly increased ($p<0.05$) in liver and lung with hydrogen peroxide as the substrate after 4 and 8 weeks, GPx activity did not change when cumene hydroperoxides was used as a substrate.

Histological changes in the liver and lung was observed after 2 weeks and in the kidney after 4 weeks of treatment. The kidney showed mild inflammation after 4 weeks. Liver histology of mice treated after 2 weeks showed some cells with binucleation and after 4 weeks showed degeneration and necrosis and hepatocytes were slightly enlarged. The lung cells showed severe acute inflammation after 2 weeks and after 4 weeks showed sever epitelization and the cells lost their normal shape and arrangement and the nuclei become hyperchromatic after 8 weeks.

Attempts to purify GST in the mice were carried out using GSH-sepharose affinity matrix. The cytosolic GST purified in this study resolved into three discrete molecular species of approximate molecular weight 25271 D, 23478 D and 25839 D respectively comparable to the previously designated isoforms MI, MII and MIII. The GST in the BaP treated sample exhibited electrophoretic migration on SDS-PAGE closely similar to the normal control. Purified liver GST had higher specific activity than the lung and the subunits were of comparable size. This may indicate the existence of common GST isoform in both organs. The main finding in this research related to result of IEF. It showed three activity peaks in the normal control and BaP treated samples livers with different pIs and substrate specificities. The GST activity toward ethacrynic acid in the treated mice was significantly higher than normal control indicating BaP induced the GSTMII (class Pi). In this study, laboratory trials with biochemical measurements supported by toxicity and histological studies were tested as tools for the assessment of the environmental hazard of BaP to target organisms.

Abstrak tesis dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan bagi Ijazah Doktor Falsafah

KESAN BENZO(a)PIREN TERHADAP TIKUS JANTAN *(MUS MUSCULUS)*

Oleh

Parichehr Hanachi

Jun 2002

Pengerusi : Prof. Madya Dr. Nor Aripin Shamaan

Fakulti : Sains dan Pengajian Alam Sekitar

Suatu kajian telah dijalankan mengenai kesan benzo(a)piren terhadap aktiviti beberapa enzim, patologi paru-paru, hati dan buah pinggang, dan aspek sel tikus *Mus musculus*, serta potensi memperolehi suatu teknik diagnostik untuk mengesan karsinogenesis pada peringkat awal.

Kerja selidik peringkat permulaan melibatkan penilaian dos maut dan dos ambang (threshold dose) yang diperlukan untuk mengaruh karsinogenesis bagi tikus dewasa. Kerja selidik berikutnya melibatkan penentuan/penganggaran aktiviti glutation S-transferase dan glutation peroksidase dalam jangka masa tikus tersebut menerima dos benzo(a)piren. Pada peringkat akhir kerja selidik, pengaruhan/induksi GST dan GPx dicapai melalui pemberian dos BaP dalam jangka masa panjang. GST telah ditulen separa dengan kaedah kromatografi afiniti.. Kerja selidik tambahan juga dilakukan untuk menentukan kesan BaP terhadap histologi tisu hati, paru-paru dan buah pinggang tikus tersebut. Keputusan yang diperolehi

menunjukkan aktiviti GST dan GPx telah diaruh oleh BaP dan pengaruhan bergantung kepada dos yang diberi (100-250 mg/kg berat badan). Tikus tersebut telah disuntik dengan BaP sekali pada dos 200 mg/kg berat badan pada permulaan kajian jangka pendek. Aktiviti GST diaruh ke tahap maksimum selepas 4 hari, dan selepas itu aktiviti enzim menurun ke tahap normal/lazim.

Kesan BaP terhadap GST dan GPx tisu hati buah pinggang dan darah tikus dewasa telah dikaji secara pengajian jangka panjang. Tikus tersebut telah disuntik dengan 200mg/kg BaP sekali seminggu selama 8 minggu. Aktiviti GST meningkat secara signifikan ($P<0.05$) selepas 2 minggu bagi tisu hati, paru-paru, buah pinggang dan darah. sementara aktiviti GPx meningkat secara signifikan ($p<0.05$) dalam hati dan paru-paru menggunakan H_2O_2 sebagai substrat. Selepas 4 dan 8 minggu, aktiviti GPx tidak berubah apabila cuCOOH digunakan sebagai substrat.

Perubahan histologi pada hati dan paru-paru dapat diperhatikan selepas 2 minggu rawatan dan pada ginjal selapas 4 minggu rawatan. Ginjal menunjukkan berlakunya sedikit keradangan selepas 4 minggu rawatan. Selepas 2 minggu rawatan, histologi pada hati tikus menunjukkan sesetengah sel mengalami binukleasi dan selepas 4 minggu, ia menunjukkan kemerosotan manakala nekrosis dan hepatosit menjadi besar sedikit demi sedikit. Sel paru-paru pula menunjukkan berlakunya serangan radang akut selepas 2 minggu. 4 minggu selepas itu ia menunjukkan

berlakunya pemotongan epitelization dan seterusnya sel mengalami kehilangan bentuk dan susunan asalnya. Nukleus pula menjadi hiperkromatik selepas 8 minggu.

Percubaan untuk menulenkan GST pada tikus telah dilakukan menggunakan matrik afiniti GSH-sepharose. GST sitosol yang ditulen dalam kerja selidik ini dapat dipisah kepada tiga spesis molekular yang berasingan, dan bersaiz 25271D, 23478 D dan 25839 D. Ia adalah dahulunya bersamaan dengan designasi MI, MII dan MIII. GST dalam sampel yang telah dirawat mempamerkan migrasi/ pergerakan elektroforesis SDS-PAG seperti kawalan normal. GST yang telah ditulenkan dari hati mempunyai aktiviti spesifik yang lebih tinggi berbanding dengan paru-paru Subunit GST dari organ tersebut merupakan saiz yang setanding. Ini menunjukkan kewujudan isoform yang biasa di dalam kedua-dua organ tersebut Keputusan dari kaedah IEF menunjukkan tiga puncak aktiviti bagi sampel kawalan dan rawatan yang mempunyai pI berbeza dan spesifisiti substrat yang berbeza. Aktiviti GST terhadap asid ethacrynic didalam tikus telah meningkat berbanding tikus kawalan. Dalam kerja selidik ini, percubaan menggunakan pengukuran biokimia disokong dengan kajian toksiti/keracunan dan histologi telah diuji sebagai alat untuk menilai ancaman persekitaran BaP terhadap organisma sasaran.

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And finally, my loudest thanks to my daughter, Somayeh. Without her encouragement and uncanny and ability to keep me grounded in reality, I certainly would not have had the energy to complete this journey.

I certify that an Examination Committee met on 17th June 2002 to conduct the final examination of Parichehr Hanachi on her Doctor Philosophy thesis entitled " The Effect of Benzo(a)pyrene on Male Mouse *Mus musculus*" in accordance Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The committee recommends that candidate be awarded the relevant degree. Members of the Examination Committee are as follows.

Nor Aripin Shamaan, Ph.D.
Associate Professor
Faculty of Science and Environmental Studies,
Universiti Putra Malaysia
(Chairman)

Juzu Hayati Arshad, Ph.D.
Associate Professor
Faculty of Science and Environmental Studies,
Universiti Putra Malaysia
(Member)

Mohd. Arif bin Syed, Ph.D.
Associate Professor
Faculty of Science and Environmental Studies,
Universiti Putra Malaysia
(Member)

Johari bin Ramli, Ph.D.
Associate Professor
Faculty of Science and Environmental Studies,
Universiti Putra Malaysia
(Member)

Abdolamir Allameh, Ph.D.
Associate Professor
Faculty of Medical Sciences Tarbiat Modares University
(Independent Examiner)

SHAMSHER MOHAMAD RAMADILI, Ph.D.
Proffessor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia
Date: 1 AUG 2002

This thesis submitted to the senate of Universiti Putra Malaysia has been accepted as fulfillment of the requirements for the degree of Doctor Philosophy.

AINI IDERIS, Ph.D.
Professor/Dean
School of Graduate Studies,
Universiti Putra Malaysia

Date: 12 SEP 2002

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institution.

PARICHEHR HANACHI

Date: 1.8. 2002

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LIST OF ABBREVIATIONS

AAF	2-Acethylaminofluorene
APS	Ammonium persulphate
a.a	Amino Acid
BSA	Bovine Serum Albumin
BaP	Benzo(a)pyrene
BPB	Bromophenol blue
Bp	Boiling point
C	Control
CV	Coefficient of variation
CDNB	1-chloro-2,4-dinitrobenzene
cuOOH	Cumen hydroperoxides
2DE	2 Dimensional Electrophoresis
DCNB	1,2-Dichloro-4-nitrobenzene
DEN	Diethylnitrosamine
DNA	Deoxyribonucleic acid
DEAE	Diethylaminoethyl
e.u.	Enzyme per unit
EA	Ethacrynic acid
EDTA	Ethylenediaminetetra acetic acid
GST	Glutathion S-transfrase
GPx	Glutathion peroxidase
GSH	Glutathione (reduced form)
GSSG	Glutathione disulphate (oxidised)
g	Gram
HCl	Hydrogen chloride
IEF	soelectric focusing
K_2HPO_4	Potassium hydrogen phosphate
KH_2PO_4	Potassium dihydrogen phosphate
KCl	Potassium chloride
L	liter
mA	Miliampere
mg	Milligram
mL	Millilitre
mM	Millimolar
mw	Molecular weight
mp	Melting point
M	Molar
ME	Mercaptoethanol
NADH	Reduced nicotinamide adenine dinucleotide