African Journal of Biochemistry Research Vol. 5(4), pp. 106-112, April 2011
Available online at http://www.academicjournals.org/AJBR
ISSN 1996-0778 ©2011 Academic Journals

Full Length Research Paper

Radio-protective chelating agents against DNA oxidative damage

M. H. Awwad[2], Samy A. Abd El-Azim[1], F. A. M. Marzouk[3]*, E. A. El-Ghany[3], and M. A. Barakat[1]

[1]Faculty of Pharmacy, Cairo University Cairo, Egypt.
[2]Faculty of Science, Benha University Benha, Egypt.
[3]Labelled Compounds Department, Hot Laboratory Center, Atomic Energy Authority. P. O. Box 13759, Cairo, Egypt.

Accepted 10 November, 2009

The present study aims to investigate the strong neuro and radio-protective activity of chelating agents through inhibition of metal catalyzed Fenton reaction and aims to explore the potential of PCR-RFLP as biomarkers. Polymorphisms of rat brain angiotensin II subtype 2 receptor gene (AT2RG) were investigated as a biomarker for the effect of 6 Gy irradiation with or without prior carnosine and dimer-captosuccinic acid (DMSA) injection alone or in combination. AT2RG was separated into purified form (≈2950 bp) using PCR magnification followed by digestion of AT2RG with restriction endonucleases which gave different polymorphism profiles which illustrated the following, strong antioxidant activity of carnosine alone and moderate antioxidant activity of DMSA. Maximum protection was achieved by the dual action of both carnosine and DMSA. Antioxidants prevented oxidative stress induced mutations of AT2RG that confer stability to its function such as neuroprotection and blood pressure osmoregulation. PCR-RFLP analysis explored the possibility of using of AT2RG polymorphisms restricted by *Xba*I, *Acc*65I restriction endonucleases as characteristic of new biomarkers for non radiation exposed individuals.

Key words: New biomarkers, PCR-RFLP, radio-protective, AT2RGen, chelating agents.

INTRODUCTION

The steadily increasing uses of advanced technologies are paralleled by increasing sources of oxidants such as ionizing radiation. Radiation effects in exposed tissues are the consequence of damage to various critical cell systems. Brain is the one of the highest sensitive organ for radiation induced oxidative stress because of its high O_2 utilization rate, the brain accounts only for a small percentage of the body weight, but consumes about 20% of basal O_2 metabolism which leaks reactive oxygen species as a by product of oxidative metabolism. Having higher metabolism rate, the developing brain are more susceptible to oxidative stress lesions than the mature one. Many of these lesions induced by ionizing radiation are chemically similar to those induced as by-product of oxidative metabolism. Radiation and oxidative impaired metabolism in the brain can lead to excess extracellular glutamate levels which lead to a rising of intracellular Ca^{2+} to pathological levels (Halliwell, 2001; Dai et al., 2007). In addition to its high content of free ferrous ions and autoxidisable neurotransmitters (L-DOPA, epinephrines, encephalins and endorphins) leak free radicals in the brain (Halliwell, 2001; Halliwell and Gutteridge, 1999). Antioxidant defenses in the brain are modest in action and not all antioxidants can penetrate blood brain barrier (Colton et al., 1996; Sherki et al., 2001).

Brain is enriched with iron-containing proteins and irradiation damage to brain tissue readily releases iron and copper ions in forms that are capable of catalyzing such free radical formation, lipid peroxidation, and autoxidation of neurotransmitters (Spencer et al., 1994). Iron chelators were found to alter metal iron catalyzed oxidation. Iron atoms that are close to DNA can interact with highly diffusible, but weak free radical H_2O_2 in a metal-catalyzed Fenton-type reaction to produce $OH^{\cdot}$-mediated DNA damage, strand breaks and DNA mutations (Henle and Linn, 1997).Chelating agents may

*Corresponding author. E-mail: fawzymarzouk@yahoo.com. Tel: 002018-144-3344.

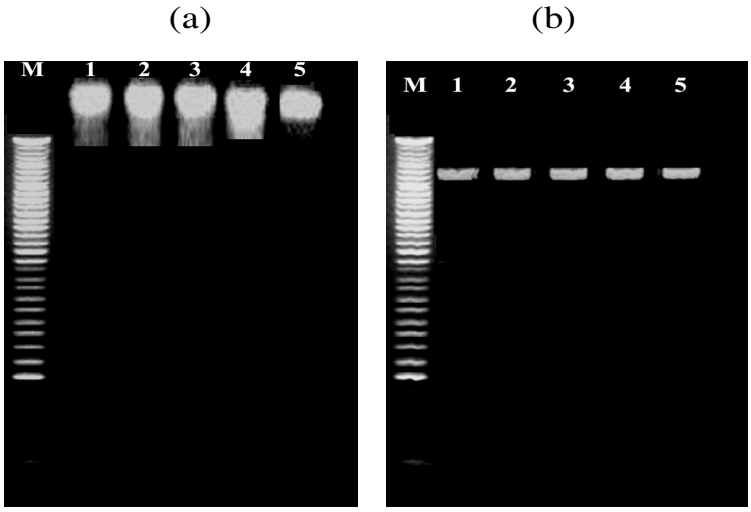

Figure 1. Lane M represents DNA marker; 1 to 5 represent 1 kb ladder lanes in (a) DNA genome (b) AT2R Gene in the studied groups.

protect DNA from hydroxyl radical and prevent enzymes damage which are responsible for DNA enzymatic repair system.

The brain owns a complete rennin-angiotensin system, including both AT_1 and AT_2-receptor subtypes (Reineke et al., 2006; Gasparo et al., 2000; Steckelings et al., 1992). But under pathological conditions such as stroke and oxidative stress as irradiation (Li et al., 2005), increased expression of the AT_2-receptor in brain tissue has been noticed with the involvement possibility of this receptor in maintaining anti-inflammatory functions and neuroprotection of the central nervous system (Li et al., 2005; Schulz and Heusch, 2006). In addition, polymorphisms in the rennin-angiotensin system genes were shown to be associated with immunoglobulin A and diabetic nephropathy, an increased risk of myocardial infarction, and the response to treatment with ACE inhibitors (Steckelings et al., 2005).

This study aims is to evaluate the antioxidant neuro-protective and radio-protective properties of chelating agents as antioxidants such as DMSA and carnosine alone or in combination against irradiation exposure. Also the present study aims is to explore new biomarkers for different radiation exposed groups using PCR-RFLP analysis of AT2R gene and to evaluate possible oxidative stress related pathological conditions for AT2R gene.

MATERIALS AND METHODS

Animals and irradiation protocol

In this study thirty mature male albino rats (*Rattus rattus*) aged approximately 3 months and weighing 100±10 g were used. Rats were bred in the animal house of the Atomic Energy Authority. The animals were kept in the same conditions living and nourishment. All experiments were carried out with the permission of the Egyptian Atomic Energy Authority. The animals were divided into five groups each group consists of six rats. The first group (1) injected with saline as a negative control group while the second were injected with saline before irradiation by one hour as a positive control one (2). The third group was injected with dose equivalent to 200 mg/kg of carnosine before irradiation by one hour (3). The fourth group was injected with both DMSA and carnosine before irradiation by one hour (4). The fifth group was injected with 50 mg/kg of DMSA before irradiation by one hour also (5). The last four groups were γ-irradiated with 6 Gy. Whole body irradiation was performed with ^{60}Co - γ-cell at dose rate of 2 Gy/min. All animals were sacrificed and dissected after one day of irradiation and whole brain were taken for DNA extraction.

DNA preparation

The whole brain was homogenized then one gram from each sample of the five groups was mixed in 500 µl of isotonic saline solution and centrifuged at 5,000 rpm for 5 min. The cell pellet was re-suspended in 500 µl of UNSET (Lysis solution; 8 M urea, 2% sodium dodecyl sulfate, 0.15 M NaCl, 0.001 M EDTA, 0.1 M Tris pH 7.5). The aqueous lysate was repeatedly extracted with 500 µl Phenol-chloroform-isoamyl alcohol several times until protein interface disappeared to separate the organic and aqueous phases. To precipitate the nucleic acid, iced absolute ethanol was added (2:1 v/v), and left to incubate at -20°C for 24 to 48 h. The nucleic acids were recovered by centrifugation at 5,000 rpm for 15 min. The pellet was dried and then re-suspended in 40 µl of sterile H_2O (Awwad, 2003). 1 µl of the resuspend pellets was checked by gel electrophoresis for the presence of DNA (Figure 1a).

Preparation of 0.8% agarose gel

0.8 gm of agarose (sigma) was dissolved in 100 ml TAE buffer (242 gm tris, 3.72 gm EDTA, 700 ml H_2O, 57 ml of glacial acetic acid and the volume brought to 5 L).

Gene amplification and purification using the standard polymerase chain reaction (PCR)

Polymerase chain reaction amplification: A fragment of

angiotensin II subtype two receptor gene of approximately 2950 base pairs length was amplified using the primers:, 5' TTTGGTATGCATTAAGCCTTTTCT 3'; as a forward primer and , 5' GAATTCATTTCCGACATATGCT 3' as a reverse primer. Primers were designed from an alignment of the angiotensin II subtype two receptor gene sequences of rat. The standard PCR reaction mixture was done as mentioned (Kissing et al., 1989). The standard polymerase chain reaction program for amplification of angiotensin II subtype two receptor gene was: 30-53 cycles each cycle included three steps; 1 min, 94°C; 2-3 min, 45°C; and 3 min, 72°C. Glass milk DNA purification was used to purify the gene from the agarose gel. 1 µl of the resuspend pellet was checked by agarose gel electrophoresis for the presence of angiotensin II subtype two receptor gene (Figure 1b) (Kissing et al., 1989).

Restriction fragment length polymorphism (RFLP) protocol

Restriction enzymes were used in this study; these are *Dra*I, *Ava*I and *Acc*65I (Toyobo Biochemicals); *Xba*I and *Bst*XII (Boehringer-Mannheim) and *Bsm*I and *Dra*I (Sigma).Restriction endonucleases were used to digest the AT_2RG of all groups. The digestion was performed for 3.5 h at 37ºC, and the products were evaluated on 2% TAE-agarose gels, stained with ethidium bromide, and bands were detected upon ultraviolet transillumination and photographed (35 mm Kodak Film, England). RFLP profiles were obtained according to the following steps: 1- The restriction buffer (5 µl) was transferred to the Labeled 0.5 µl tubes, 2- 5 µl of each PCR product was added to the labeled tubes. 2- The tubes was centrifuged and placed in water bath (37ºC) for 1.5 h. 3- The tubes were placed on an ice after digestion (Vidigal et al., 1998).

RESULTS

The study dealt with antioxidant activity donated by chelating agents of both carnosine and DMSA alone or in combination against DNA irradiation induced oxidative damage. Radio protective and neuroprotective activities of both chelators were estimated by using PCR-RFLP analysis of AT2RG polymorphic fragments as biomarkers.

The resulting fragments of rat brain of different groups, which have been separated by agarose electrophoresis as photos enable us to identify their different alleles. DNA genome has a high molecular weight about, so it remained in the field of the agarose gel electrophoresis (Figure 1).

The anti-inflammatory angiotensin II subtype II receptor gene (AT2RG) was separated into a purified fragment form by using PCR. The size of AT_2RG was found to be approximately 2950 bp for rats (Figure 1b).

*Bsm*I restriction endonuclease fragmented AT2RG into approximately identical four restriction cuts (200,600,650 and 1500 bp; Figure 2. *Dra*I restriction enzyme cut AT_2RG for all groups into five restriction fragment (150, 300, 350,600 and 1550 bp; Figure 3). X*ba*I restriction endonuclease isolated the non-irradiated group in quite different profile from other groups.

*Xba*I restriction endonuclease clustered the five groups of rat brain AT_2RG for all groups into two groups, group 1 in the first and group 2, 3, 4 and 5 in the second cluster (Figure 4). *Xba*I restriction enzyme digested AT2RG for group A into thee restriction cuts with unique one (400, 1150 and 1400 bp; lane 1). The rest for other groups of rat brain AT2RG were restricted into five bands (50, 400, 450,650 and 1400 bp; lanes 2, 3, 4 and 5).

As shown in Figure 5 *Ava*I restriction enzyme differentiated group 2 into five restriction fragments with two unique ones (100, 250, 600, 700 and 1300 bp ; lane 2). The same enzyme restricted AT2R Gene of groups 3 and 5 into four restriction fragments (350, 600, 700 and 1300 bp; lanes 3 and 5). Groups 1 and 4 were fragmented into three bands by the action of the same enzyme (600, 700 and 1650 bp; lanes 1 and 4).

*Bst*XI restriction endonuclease clustered AT_2R Gene groups into three groups (Figure 6). Group 2 of AT2R Gene isolated into five bands with two characteristic ones (50, 200, 800, 850 and 1050 bp; lane 2).groups 1 and 4 showed three restriction fragments (200, 1000 and 1750 bp; lanes 1 and 4) while groups 3 and 5 showed four restriction fragments (200,850,900 and 1000 bp; lanes 3 and 5).

Rat brain of group A was differentiated when its AT_2RG was digested by *Acc*65I restriction endonuclease. As shown in Figure 7 *Acc*65I restriction enzyme cuts groups 2, 3 and 5 into four similar bands (250, 500, 1000 and 1200 bp; lanes 2, 3 and 5). *Acc*65I restriction endonuclease digested the AT_2RGene of group A into two different bands with characteristic one (500 and 2450 bp; lane 1). The same enzyme restricted AT2R Gene of groups 4 into three restriction fragments (250, 500 and 2200 bp; lanes 1 and 4). So some antioxidant activity was observed by the dual action of carnosine and DMSA (group D) that concluded from less number of mutations with the respect of group 2 (irradiated one). Individual treatment of either carnosine or DMSA (groups 3 and 5) showed quite similar profile with the respect of group 2 (irradiated one) by using *Acc*65I enzyme which means no detectable antioxidant activity by using this enzyme.

DISCUSSION

Reactive oxygen species are formed as a by pass products during cellular metabolism or during stress conditions. Also reactive oxygen species were used to propagate through auto-oxidation steps using transition metal as a catalyst. Irradiation causes oxidative stress which in turn increases free iron that leads to oxidative stress propagation and exaggeration. It is well known that the brain is one of the most sensitive organs for oxidative stress because of its high metabolic rate and high iron content. So chelating agents considerably have potent antioxidant property on the brain especially which can penetrate blood brain barrier.

Since biochemical studies have mainly focused in the last few years on oxidation-reduction biochemistry using molecular tools and chelating agents considerably have potent antioxidant property on the brain especially that

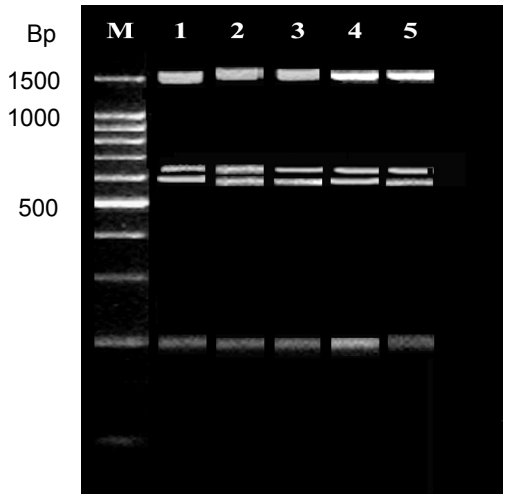

Figure 2. 1 kb ladder in lane M. Lanes 1,2,3,4 and 5 represent the length of AT_2R Gene fragments, which restricted with the *BsmI* endonuclease in the rat groups.

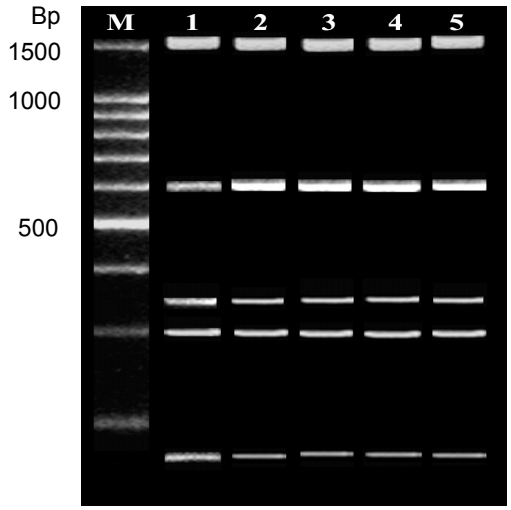

Figure 3. Lane M represents 1 kb ladder; lanes 1 to 5 represent the length of AT2R Gene fragments, which restricted with the endonuclease DraI in the studied groups.

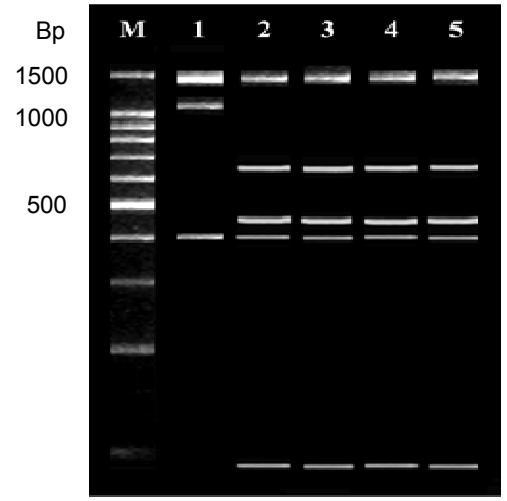

Figure 4. 1 kb ladder in lane M; Lanes 1, 2, 3, 4 and 5 represent the length of AT2R Gene fragments, which restricted with the XbaI endonuclease in the rat groups.

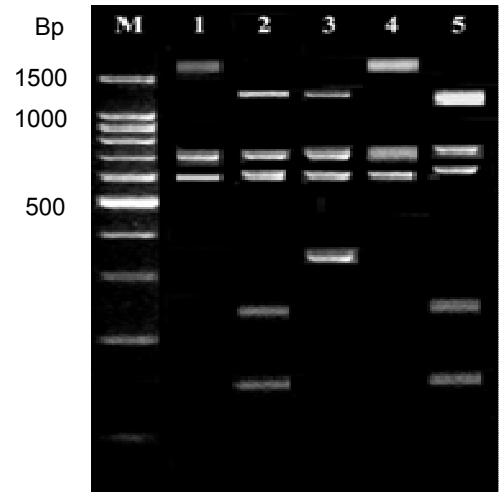

Figure 5. 1 kb ladder in lane M; Lanes 1, 2, 3, 4 and 5 represent the length of AT2R Gene fragments, which restricted with the AvaI endonuclease in the rat groups.

can penetrate blood brain barrier, molecular techniques were used to evaluate oxidative stress and to be as a diagnostic tool for certain stress condition (Collins et al., 1997; Dib, 1996; Dainiak, 2002).

So the present study aims to investigate the neuro-protective and radio-protective properties of either carnosine or DMSA alone or in combination as an antioxidant chelating agents after 6 Gy of gamma irradiation. Also this aims to explore new biomarkers for different groups using PCR-RFLP analysis of AT2RG.

In the present work it was found that polyphylogenecity between all groups which are diagnostic biomarkers for these groups by DNA analysis. The data obtained revealed that normal control group has a characteristic polymorphic bands with *Acc65I* and *Xba*I restriction endonucleases .While irradiated rats group showed a diagnostic polymorphic markers with *Ava*I and *Bst*XII restriction enzymes respectively. Also the polymorphism of all irradiated groups showed three characteristic bands of due to gene induction. All irradiated groups treated with AOs have diagnostic cuts by using *Acc*65I and *Bst*XII restriction endonucleases respectively. So DNA analysis of the AT_2R gene polymorphism for different groups showed high specificity as a diagnostic tool that is

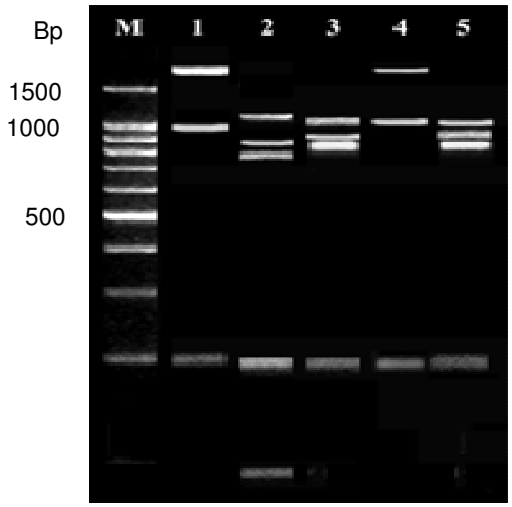

Figure 6. Lane M represents 1kb ladder lanes 1 to 5 represent the length of AT2R Gene fragments, which restricted with the endonuclease BstXI in the studied groups.

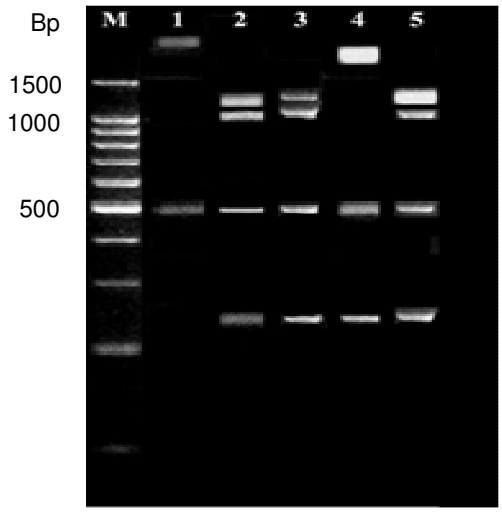

Figure 7. Lane M represents 1kb ladder lanes 1 to 5 represent the length of AT2R Gene fragments, which restricted with the endonuclease Acc65I in the studied groups.

confirmed with Collins et al. (1997).

One of the important roles of chelators is to detoxify metal ions and prevent poisoning. Iron chelation therapy leads to low cytosolic iron concentrations that facilitate resistance by protecting proteins, more than DNA, from IR-induced oxidative damage. By decreasing iron content in some bacteria species by three folds may lead to increasing radio-resistance up to 2000 times than human lethal dose. *D. radiodurans* resistance was attributed to DNA protection from hydroxyl radical and also attributed to enzymes protection which involved in base DNA repair system. Radiation induced DNA mutations can undergo enzymatic repair involving base excision repair or recombination (Coleman and Stevenson, 1996; Oleinick, 1990; Miller et al., 1995).

Ionizing radiation as oxidative stress inducer has sufficient energy to eject an electron from molecules, with the critical target being DNA to give DNA radical (Oleinick, 1990; Powell and McMillan, 1990). Ionizing radiation deposits energy in a bio-distribution model that gives closely spaced damage area, termed "locally multiply damaged sites (Coleman and Stevenson, 1996). Several types of DNA base damage and cross-links are produced that giving rise to both single and double-strand breaks (Coleman and Stevenson, 1996; Oleinick, 1990; Powell and McMillan, 1990). When an electron is ejected, an unstable DNA radical (DNA°) is produced, which is exposed to rapid biochemical reactions, and results in the formation of potentially lethal single and double strand breaks in the DNA. The DNA lesion can be biochemically repaired by reducing species (such as thiol -SH containing groups such as DMSA and other AOs species such as carnosine) or be made more permanent by combining with oxygen species. The accumulation of unrepaired double strand break correlates closely with the loss of cell integrity (Coleman and Stevenson, 1996; Powell and McMillan, 1990).

From oxidative stress point of view AT_2R gene fragments showed high variations among normal control, irradiated (positive control) and all irradiated rat groups treated with AOs according to the difference of the profile obtained with *Xba*I*, Ava*I*, Acc65I* and *Bst*XII restriction endonucleases. On the other hand *Bsm*I and *Dra*I restriction endonucleases did not clarify any difference between all rat groups based on the similar of profiles obtained with these restriction endonucleases. From all previous, the digestion of AT_2R gene for all groups by different restriction enzymes showed polyphylogenetic relationship between AT_2R structures for all groups.

Irradiated control rats which exposed to 6 Gy of γ-irradiation showed different AT_2R gene cuts from normal control group in all restriction endonucleases except for *Bsm*I and *Dra*I ones which indicates DNA fragmentation induced oxidative stress. Results obtained with *Bsm*I and *Dra*I restriction endonucleases did not clarify the difference between all rat groups of AT_2R gene based on the similarity of profiles obtained with these restriction endonucleases so if we exclude them we will find the followings: Carnosine treated group before γ-irradiation exposure showed the same cuts with two restriction enzyme endonucleases (*Sty*I *and Bst*EII) for normal control group and two similar restriction cuts profile of *Acc*65I *and XbaI* restriction enzymes with irradiated control group but there are four different PCR/RFLPs profiles from non irradiated control rat but with less number of mutations in relation to irradiated control with *Ava*I and *BstX*II restriction endonucleases. All of that indicates the strong antioxidant activity of carnosine but not enough to completely block radiation induced oxidative stress which compatible with published data (Dizdaroglu, 1994; Halliwell,

1998).

Irradiated carnosine and DMSA treated rats gave the same restriction cuts for two enzymes which are *Ava*I, and *Bst*XII restriction endonucleases in compared to normal control group of rats. It was found that one similar restriction cut profile by using *Xba*I restriction enzyme with irradiated control group and three different ones in relation to normal control by *Acc*65I, restriction endonuclease with less number of mutations compared to irradiated control one. So it can be concluded that maximum protection can be assessed through dual action of both DMSA and carnosine investigated by better gene profile which is confirmed with Kannan and Flora who declared that the dual action of DMSA with other AOs (Vit. E) are more effective (Kannan and Flora, 2004).

Irradiated DMSA treated rats exhibited similar profile with *Sty*I restriction enzyme only when compared to normal control rats in other study for us under processing. In contrast to the above more similarity with irradiated control rats were shown with gene cuts by *Acc*65I, *Xba*I and *BstE*II restriction endonucleases. In addition there are four different PCR/RFLPs profiles from non irradiated control group with less number of mutations in relation to irradiated control one with *PstX*II and *Ava*I restriction endonucleases. This in turn means the moderate antioxidant activity of DMSA through free iron chelation and chelation of iron released through oxidative stress. Also DMSA may gives its antioxidant effect through reduction of formed DNA radical because of its content of free thiols (Powell and McMillan, 1990; Kannan and Flora, 2004).

DMSA and carnosine are tetra-chelating agents that mean every two atoms of free iron require three molecules of either chelating agents in stoichiometric chelation. In this study these chelating agents were found to alter iron mediated DNA oxidative damage leading to improvement in the picture of PCR-RFLP profile. According to PCR/RFLPs profiles Carnosine alone may have stronger antioxidant property than DMSA because it may exceed iron chelation by forming zinc and copper chelates that may have SOD-mimic action and its alkaline buffering action. But the dual action of both carnosine and DMSA may have maximum brain protection through free metal chelation, SOD-mimic action and direct reducing power for both chelates.

Angiotensin II receptor were found to have two subtypes of receptors with antagonistic action to each other, angiotensin II subtype 1 receptor (AT1R) over expression increases Na/water retention, inflammation, proliferation, vasoconstriction and oxidative stress while the AT2R expression have the reverse action so it represents one of the body protective mechanism. AT1R blocker increases expression of AT2R with modulating action on blood lipids and possible role of antioxidant activity (Steckelings et al., 2005;**,** Steckelings et al., 1992; Baykal et al., 2003). AT1R blockers screening for many patients showed possible lowering action on incidence rate of diabetes mellitus. In the present study antioxidants may prevent or decrease AT2R gene mutation induced oxidative stress that elicits beneficial effect on vascular system (Li et al., 2005; Schulz and Heusch, 2006). Thus antioxidants may decrease incidence rate of diabetes mellitus, hypertension and hyperlipidaemia. Antioxidants may have a beneficial effect on glucose level, blood pressure and total lipid profiles which may be through inhibition of AT2R gene mutation as a possible mechanism.

Conclusion

PCR-RFLP biomarkers were found to be an advanced, simple and rapid tool for evaluation of radio-protecting and antioxidant activity of chelating agents alone or in combination against oxidative stress irradiation induced. Also it may be used as a new biomarker for the diagnosis of radiation exposure at different conditions with respect to antioxidant treatment. PCR-RFLP analysis cleared that the possibility of using AT2RG polymorphisms fragmented by *Xba*I *Acc*65I restriction enzymes as a characteristic new biomarkers for non radiation exposed individuals. Carnosine was shown to have a stronger antioxidant activity than that of DMSA. Maximum protection was achieved by the dual action of both chelating agents' carnosine and DMSA. Chelating agents prevented oxidative stress induced mutations of AT2RG that confer stability to its function such as neuroprotection and blood pressure osmoregulation.

REFERENCES

Awwad MH (2003). Molecular identification of *Biomphalaria alexandrina* and *Bulinus tranctus* using PCR-RFLP of actin gene. J. Egypt. Acad. Soc. Environ.Dev., 3(1): 39-52.

Baykal Y, Yilmaz M, Celik T, Gok F, Rehber H, Akay C,Kocar H (2003). Effect of antihypertensive agents, alpha receptor blockers, beta blockers, angiotensin converting enzyme inhibitors, angiotensin receptor blockers and calcium channel blockers on oxidative stress. J. Hypertens., 21(6): 1207-1211.

Coleman CN, Stevenson AM (1996). Advances in Cellular and Molecular Radiation Oncology. Urol. Oncol., 2: 3-13.

Collins FS, Guyer MS, Chakravarti A (1997). Variations on a theme cataloging human DNA sequence variations. Science, 278: 1580-1581.

Colton C, Wilt S, Gilbert D (1996). Species differences in the generation of reactive oxygen species by microglia. Mol. Chem. Neuropathol., 28: 15-20.

Dai X, Sun Y, Jiang Z (2007). Protective Effects of Vitamin E against Oxidative Damage Induced by Abeta (1-40) Cu (II) Complexes. Acta Biochim. Biophys. Sin. (Shanghai), February, 39(2): 123-130.

Dainiak N (2002). Hematologic consequences of exposure to ionizing radiation. Exp. Hematol., 30: 513-528.

Dib C (1996). A comprehensive genetic map of the human genome based on 5264 microsatellites. Nature, 380: 152-154.

Dizdaroglu M (1994). Chemical determination of oxidative DNA damage by gas chromatography-mass spectrometry. Methods Enzymol., 234: 3-16.

Gasparo M, Catt K, Inagami T, Wright JW, Unger T (2000). International Union of Pharmacology. XXIII. The angiotensin II receptors. Pharmacol. Rev., 52: 415-472.

Halliwell B (1998). Can oxidative DNA damage be used as a biomarker of cancer risk in humans? Problems, resolutions and preliminary results from nutritional supplementation studies. Free Radicals Res., 29: 469-486.
Halliwell B (2001). Role of Free Radicals in the Neurodegenerative Diseases: Therapeutic Implications for Antioxidant Treatment. Drugs Aging, 18(9): 685-716.
Halliwell B, Gutteridge JMC (1999). Free radicals in biology and medicine. 3rd ed. Oxford: University Press.
Henle ES, Linn S (1997). Formation, prevention, and repair of DNA damage by iron/hydrogen peroxide. J. Biol. Chem., 272: 19095-19098.
Kannan GM, Flora SJ (2004). Chronic arsenic poisoning in the rat: Treatment with combined administration of succimers and an antioxidant. Ecotoxicol. Environ. Saf., 58(1): 37-43.
Kissing B, Croom H, Martin A, McIntosh C, McMillan W, Palumpi S (1989). The simple fools guide to PCR version1.0. Department of Zoology, University of Hawaii, Honolulu.
Lees-Miller SP (1995). Godbout R, Chan DW, Absence of p350 subunit of DNA-activated protein kinase from a radiosensitive human cell line. Science, 267: 1183-1185.
Li J, Culman J, Hortnagl H, Zhao Y, Gerova N, Timm M (2005). Angiotensin AT_2 receptor protects against cerebral ischemia-induced neuronal injury. FASEB J., 19: 617-619.
Oleinick NL(1990). Ionizing radiation damage to DNA: Molecular aspects. Rad. Res., 124: 1-6.
Powell S, McMillan TJ (1990). DNA damage and repair following treatment with ionizing radiation. Radiother. Oncol., 19: 95-108.
Reineke C, Steckelings UM, Unger T (2006). Angiotensin receptor blockers and cerebral protection in stroke. J. Hypertens., 24: 116-119.
Schulz R, Heusch G (2006). Angiotensin II type 1 receptors in cerebral ischaemia-reperfusion: initiation of inflammation. J. Hypertens., 24: 124-128.
Sherki Y, Melamed E, Offen D (2001). Oxidative stress induced-neurodegenerative diseases: The need for antioxidants that penetrate the blood brain barrier. Neuropharmacology, 40: 959-975.
Spencer JP, Jenner A, Aruoma OI (1994). Intense oxidative DNA damage promoted by L-dopa and its metabolites: Implications for neurodegenerative disease. FEBS Lett., 353: 246-250.
Steckelings UM, Bottari SP, Unger T (1992). Angiotensin receptor subtypes in the brain. Trends Pharmacol. Sci., 13: 365-368.
Steckelings UM, Kaschina E, Unger T (2005). The AT2 receptor - A matter of love and hate. Peptides, 26: 1401-1409.
Vidigal T, Dias E, Spattz LN, Pires R, Simpson A, Carvalho O (1998). Genetic variability and identification of the intermediate snail host of Schistosoma Mansoni. Mem. Inst. Oswald Cruz., 93: 103-110.