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Preparation of ^{99m}Tc-Carnosine and ^{99m}TcO-(V)-DMSA Complexes, Biological Distribution, and Estimation of Their Gene Anti- Polymorphisms Induced by γ-Irradiation

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Abstract

Background: Two chelating agents (Carnosine and DMSA) were used to study their labeling conditions with technetium-99m followed by biological distribution investigation. Molecular studies were done via PCR/RFLP analysis of angiotensin II subtype II receptor gene for monitoring their antioxidant activity through free iron chelation leading to inhibition of Fenton reaction.

Material and methods: Carnosine was labeled by mixing 4 mg with 30 mg glucose and 25 μg $SnCl_2.2H_2O$, followed by pertechnetate and stand at room temperature for 60 minutes. Minor modification was done to prepare ^{99m}Tc(V)-DMSA tracer in one step, by adding pertechnetate solution to the lyophilized kit contains 1mg DMSA, 0.1 mg $SnCl_2.2H_2O$, and 30 mg glucose at pH 9. The biodistribution of the two tracers in normal and tumor-induced mice. The molecular investigation of the anti-oxidant activity of both carnosine and DMSA in 6 Gy γ-irradiated rats using the anti-inflammatory angiotensin II subtype II receptor gene (AT_2RG) as indicator.

Results: Carnosine and DMSA were labeled with Technetium-99m yielding 85% and 97%, respectively the ability of both tracers to localize in tumor sites but the priority to the ^{99m}Tc (V)-DMSA. Molecular studies showed strong antioxidant activity of carnosine but not enough to block radiation induced oxidative stress and Moderate antioxidant activity of DMSA was achieved by chelating free iron and iron released through oxidative stress. Maximum protection was achieved through the dual action of both DMSA and carnosine.

Conclusion: moderate and high labeling yield were achieved for both ^{99m}Tc(V)DMSA and ^{99m}Tc-canosine respectively with higher selectivity of the former to tumor sites and maximum protection were achieved by the dual action of both chelating agents.

Key Words: *Carnosine / DMSA / Anti-oxidant / Technetium-99m Labeling*

Introduction

The steadily increasing use of nuclear and radiation technologies in medicine, industry, agriculture and scientific research, has been paralleled by increasing potential risk for radiation over acute exposure. This may be intended as in the case with medical and occupational exposure or accidental as a shot dose as in the case with accidental exposures. *Invivo* effects of radiation on biological systems are obviously so complicated to be fully analyzed. So most of the investigators have undertaken *invitro* studies in order to minimize the role played by many interfering parameters as encountered *invivo* experimentation (Moseley and Belin, 1984). DNA is an important target for the damaging effects of ionizing radiation and endogenously induced reactive oxygen species. Ionizing radiation produces through direct and indirect effects a variety of DNA lesions, such as single strand breaks (SSBs), double-strand breaks (DSBs), a variety of base modifications, sugar modifications) and DNA-DNA and DNA- protein cross-link (O'Neill and. Fielden, 1993; Wallace, 2002 and von Sonntag, 1987s. The DSB is generally thought to be the main lesion involved in cell killing and formation of chromosomal aberrations (Johnston *et al.,* 1998; Jeggo, 1990 and Pfeiffer, 1998). Many of the lesions induced by ionizing radiation are

chemically similar to those induced as by-products of oxidative metabolism; however, ionizing radiation also induces complex damage known as non-DSB clustered DNA damage sites (Gulston *et al*., 2002; Sutherland *et al*., 2000 and Jenner *et al*., 2001) It has been hypothesized that lesions within these complex DNA damage sites induced by ionizing radiation would be less readily repaired than when present as isolated lesions Nikjoo *et al*., 2001). Indeed the efficiency of repair of DSB induced in mammalian cells is critically dependent upon the ionization density of the radiation (Jenner *et a*l., 1993; Prise *et al*., 1998 and Blocher, 1988), which influences the complexity of DSB and loss of genome stability. More recently, it has become evident that processing of lesions within non-DSB clustered DNA damage sites based on *invitro* biochemical approaches is indeed retarded (Dianov *et al* .,2001; Chaudhry, and Wienfeld, 1995; Harrison, *et al* .,1999; Harrison *et al* .,1998; David-Cordonnier *et al.*,2000; David-Cordonnier *et al*.,2001a; David-Cordonnier *et al*., 2001b; David-Cordonnier *et al*., 2001c; David-Cordonnier *et al*.,2002;Chaudry, and Weinfeld,. 1997 and Lomax *et al* .,2004).

The prediction that radiation induces non-DSB clustered DNA damage sites initially arose from biophysical models (Nikjoo *et al*., 1997 and Goodhead, 1994). However, more recently experimental evidence has shown that non-DSB clustered damage sites are indeed induced in mammalian cells by γ-radiation in yields that are four to eight times that of prompt DSB (Gulston *et al*., 2002; Sutherland *et al*.,2000 and Sutherland *et al* .,2002). γ-Radiation also produces a type of non-DSB clustered damage, which contains heat-labile sites, in `naked' DNA (Gulston *et al*., 2002; Pryor and Stone, 1993; Hunninghake and Crystal, 1983) and cellular DNA (Bandy and Davison, 1990 and Shimoda-Matsubayashi *et al*., 1996).

Reactive oxygen species (ROS) have been implicated in many diseases, including cancer, by causing DNA damage and spontaneous chromosomal breakage and activating procarcinogens (Wang *et al.,* 2004). The body has a very effective network of antioxidants serving as scavengers of superoxide and hydrogen peroxide, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). SOD is one of the key enzymes that detoxifies the superoxide radical (O_2^-) and generates hydrogen peroxide (H_2O_2), which in turn is detoxified by catalase and GPx. Of the three forms of SOD in humans, mitochondrial manganese SOD (MnSOD) may be particularly important for antioxidant defense as the mitochondria are the major site for cellular metabolism and the main site for the production of ROS (Ambrosone *et al.,* 1999).

Polymorphisms of genes leading to a reduction of antioxidant activities have been postulated to be a possible mechanism. There is a polymorphic site identified in the mitochondria targeting sequence of human MnSOD gene (Lifton 1996). In addition, polymorphisms in the angiotensin-converting enzyme (ACE) gene were shown to be associated with an increased risk of myocardial infarction, immunoglobulin A (IgA) nephropathy, the renal complications of diabetes, hypertension, and the response to treatment with ACE inhibitors.

Carnosine (beta-alanyl-L-histidine) is a dipeptide commonly present in mammalian tissue, and in particular in skeletal muscle cells; it is responsible for a variety of activities related to the detoxification of the body from free radical species and the by-products of membrane lipids peroxidation, but recent studies have shown that this small molecule also has membrane-protecting activity, proton buffering capacity, formation of complexes with transition metals, and regulation of macrophage function. It has been proposed that carnosine could act as a natural scavenger of dangerous reactive aldehyde from the degradative oxidative pathway of endogenous molecules such as sugars, poly unsaturated fatty acids (PUFAs) and proteins. In particular, it has been recently demonstrated that carnosine is a potent and selective scavenger of alpha, beta-unsaturated aldehyde, typical by-products of membrane lipids peroxidation and considered second messengers of the oxidative stress, and inhibits aldehyde-induced protein-protein and DNA-protein cross-linking in neurodegenerative disorders such as Alzheimer's disease, in cardiovascular ischemic damage,

in inflammatory diseases. The research for new and more potent scavengers for alpha, beta-unsaturated aldehyde has produced a consistent variety of carnosine analogs (Guiotto *et al*., 2005).

During this study, Carnosine and DMSA were labeled with technetium-99m via direct labeling technique to prepare carnosine-Technetium-99m complex and an oxo-core technetium-99m complex of DMSA in heptavalent coordination form. The produced ^{99m}Tc-carnosine and 99mTcO-(V)-DMSA were evaluated biol-ogically in mice via intravenous applic-ation. In addition, the antioxidant activities of carnosine and DMSA single and in combination were evaluated in rats induced a gene polymorphism via γ-irradi-ation (6 Gy) using angiotensin II subtype II receptor gene (AT_2RG) amplification and restriction as indicator.

Experimental

Carnosine, DMSA, and stannous chloride dihydrate were purchased from Sigma-Aldrich, Germany. Technetium-99m was eluted from moly generator purchased from "ELUTEC" Brussels, Belgium. Deoxynucleotide triphosphates (dNTP, dATP, dGTP, dTTP, and dCTP) were obtained from Perkin Elmer Cetus, USA. The tag DNA polymerase used for AT_2R gene amplification was obtained from Boehringer Mannheim Biochemica, Germany and Gibco/BRL, Gaithersburg, Md., USA. Restriction enzymes *StyI, AspI,* and *BstEII* were purchased from Boehringer-Mannheim while SspI from Sigma-Aldrich. Mice were used for biodistribution studies.

Technetium-99m Labeling of Carnosine and DMSA

The labeling of DMSA with technetium-99m was done as follow:

1- One mg of DMSA was dissolved in double distilled water in the presence of 0.5 M sodium bicarbonate sufficient to bring the reaction mixture to pH 9 ± 0.2 after the addition of the acidic solution of stannous chloride dihydrate 0.1 mg.
2- Thirty mg of glucose was added to the above solution.
3- The required amount of pertechnetate (740-1480 MBq) was added and the reaction mixture was incubated at the ambient temperature (25 ºC) for 15 minutes.

The labeling of carnosine with technetium-99m was done using a simple procedure, just by adding the pertechnetate solution (740-1480 MBq) to the reaction vial contains 4 mg carnosine, 25 µg stann-ous chloride dihydrate, 30 mg glucose, and the pH was adjusted to 8.5 using bicarbonate solution. The reaction mixture was incubated at room temperature for 60 minutes.

Radiochemical Analysis

The radiochemical analysis was done for two complexes using the same system as follow: Spot from the reaction mixture was applied to the ITLC-SG-60 which was developed in ascending manner by a mixture of n-butanol: acetic acid: water (3:2:3). ^{99m}Tc (V)-DMSA was at R_F 0.5 – 0.7, ^{99m}Tc (III)-DMSA was at R_f 0.0, and $^{99m}TcO^-_4$ was at R_f 0.9 –1.0. Acetone was used to separate 99mTc-Carnosine from free pertechnetate, R_F for ^{99m}Tc-Carnosine and colloids was 0.0 – 0.1, while R_f for $^{99m}TcO^-_4$ was 0.9 – 1.0. In addition, saline was used to separate ^{99m}Tc-Carnosine from colloids, R_f for ^{99m}Tc-Carnosine was 0.9 – 1.0 while R_f for $^{99m}TcO^-_4$ was 0.0 – 0.1.

Biodistribution Studies

This experiment was done by diluting the solution of ^{99m}Tc-Carnosine and ^{99m}Tc (V)-DMSA with one ml saline and passed through 0.22 µm Millipore filter for steril-ization and to remove colloids in the case of ^{99m}Tc-carnosine. 200 – 250 MBq of the sterile solution was injected via intravenous into the white mice weighting 25-30 gm (group of 3 mice). The mice were maintained on normal diet in a metabolic cage. The different mice groups were sacrificed at different time intervals corresponding to 0.25, 0.5, 1, 2, and 4 h post-injection respectively for each group. Samples of fresh blood, bone and muscle were collected and counted by gamma counter. The different organs were removed, counted and compared to the standard solution of radioactivity. The

average percent values of the administrated dose/organ were calculated.

Animals and Irradiation Protocol

Thirty female rats mean age 150 – 180 days were used in the experiments (six animals for each group). All experiments were carried out with the permission of the Egyptian Atomic Energy Authority. Animals were divided into five groups. The first group was used as negative control, the second group was used as positive control, the third group was treated with carnosine (200 mg/Kg) one hour before γ-irradiation, the forth group was treated with carnosine and DMSA (200 and 50 mg/Kg, respectively) one hour before γ-irradiation, and the fifth group was treated with DMSA (50 mg/Kg) one hour before γ-irradiation. The last four groups were γ-irradiated with 6 Gy. Rats were killed by neck dislocation after 30 min. Whole body irradiation was performed with ^{60}Co - γ-cell at dose rate of 2 Gy/min.

DNA Preparation

One gram from each sample of the five groups was homogenized in 500 µl of isotonic saline solution and centrifuged at 5,000 rpm for 5 minutes. The cell pellet was re-suspended in 500 µl of UNSET (Lysis solution; 8M urea, 2% sodium dodecyl sulfate, 0.15M NaCl, 0.001M EDTA, 0.1M Tris pH 7.5). Phenol-chloroform extraction was used two to three times 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 ^{o}C for 24 to 48 hours. The nucleic acids were recovered by centrifugation at 5,000 rpm for 15 minutes. The pellet was dried and then re-suspended in 40 µl of sterile H_2O. One µl of the re-suspended pellet was checked by agarose gel electrophoresis for the presence of DNA.

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 liters).

Gene Amplification and Purification Using the Standard Polymerase Chain Reaction (PCR)

To amplify the complete nuclear DNA gene, one µl of whole cell DNA template was used plus oligonucleotide primers complementary to either the 5 or 3 ends of the AT_2RG. To amplify the complete AT_2RG, one µl of whole cell DNA template was used plus oligonucleotide primers complementary either to the 5` and 3` ends of the gene as presented in Table (1). Program for amplification of nuclear DNA was 30 -35 cycles; one minute at 94 ^{o}C, two to three minutes at 45 ^{o}C, and three minutes at 72 ^{o}C. PCR products were isolated after separation by agarose gel electrophoresis, using a 3 mm X 6 mm 12-well comb. Ethidium bromide was used to stain PCR products in the gel (50 mg/100 ml 1X TAE) for ten minutes. The PCR products (bands) were visualized under UV lamp and then cut from the gel. Glass milk DNA purification (Gene clean Kit) was used to purify the gene from the agarose gel. Three micro liters of the amplification products were visualized on 0.8% ethidium bromide stained agarose gel to check the quality of amplification. The remaining 7 µl were mixed with 53 µl double distilled water and divided into 10 µl aliquots for enzyme digestion.

Restriction Fragment Length Polymorphism (RFLP) Protocol

Restriction endonucleases were used to digest the AT_2RG of all groups. The digestion was performed for 3.5 hours at 37 ^{o}C, 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 (5µl) presented in Table (2) was transferred to the Labeled 0.5µl tubes, 2- 5µl of each PCR product was added to the labeled tubes. 2- The tubes was centrifuged and placed in water bath (37 ^{o}C) for 1.5 hours. 3- The tubes were placed on an ice after digestion.

Results

Radiolabeling of Carnosine with Technetium-99m

Many factors can affect the coordination of technetium-99m with carnosine. The most important one is the pH of the reaction mixture. The experiment was carried out by adding pertechnetate to the appropriate buffer solutions contain 4 mg carnosine and 25 μg stannous chloride dihydrate, and the reaction mixture was incubated at ambient temperature for 60 min. As cleared from Figure (1), at acidic pH (3, 5) the radiochemical yields of ^{99m}Tc-carnosine were very low and closely to each other (17 %). Increase the pH of the reaction medium to 9, a dramatic increase in the radiochemical yield of ^{99m}Tc-carnosine was observed (65%). At high alkaline pH value 11, the radiochemical yield of ^{99m}Tc-carnosine began to decrease with an increase in colloids and free pertechnetate was observed.

Carnosine amount plays an important role in the labeling process. As cleared from Figure (2) a maximum radiochemical yield of ^{99m}Tc-carnosine (60 %) was obtained at 4 and 5 mg. At low amounts of carnosine 0.5 and 1.0 mg, the radiochemical yield was very low and not exceeds 25 %. Increasing the amount of carnosine gradually caused increase of the radiochemical yield.

Stannous chloride dihydrate which is the most applied reducing agent in the technetium-99m chelation chemistry played an effective role in this labeling process. As it is clear from Figure (3), low amounts of stannous chloride produced low radiochemical yield. Because the labeling of carnosine with technetium-99m was done at alkaline pH (8.5), an increase in the amount of stannous chloride dihydrate leads to the formation of colloids (reduced hydrolyzed technetium and stannous hydroxide), so it is not recommended to increase stannous chloride. The maximum radiochemical of ^{99m}Tc-carnosine (65%) was obtained using 25 μg of stannous chloride dihydrate.

The addition of glucose to the reaction mixture is of three values, the first one it works as reducing agent. The second as radical scavenger to prevent fast degradation of the labeled carnosine by γ-radiation. The third it act as a filler to improve the physical appearance of the freeze dried disc upon lyophilization of the final product. The effect of addition of glucose (30 mg) to the reaction mixture is presented in Figure (4), which showed a fantastic increase in the radiochemical yield of ^{99m}Tc-carnosine exceed 85%.

Experiments for pentavalant DMSA complex optimization, using the following variations were introduced: (1) the renal DMSA kit was adjusted to pH 8.2 with sodium bicarbonate and labeled at different time periods; (2) the renal DMSA kit was adjusted to pH 9 with sodium bicarbonate and labeled at different time periods; and (3) DMSA formula with 0.1 mg stannous chloride dihydrate contains 30 mg glucose at pH 8.2 and 9.

The results of these experiments are presented in Tables (3- 6). As cleared from Table (3), a maximum percent conversion of 99mTc (III)-DMSA to ^{99m}Tc-(V)-DMSA was obtained at pH 8.2 after 60 minutes conversion time. The presence of 99mTc (III)-DMSA in the reaction mixture interfere with the pentavalant analogue and not altered after 240 minutes. At pH 9, the renal DMSA complex was changed to the pentavalant analogue with 84% within 30 minutes and increased slightly to 88 % at 60 minutes as presented in Table (4). The DMSA formula which contains 0.1 mg stannous chloride dihydrate and 30 mg glucose, the maximum radiochemical yield of ^{99m}Tc(V)-DMSA ((97%) was obtained at the shortest reaction time (15 minutes) at both pH values 8 and 9 as presented in Table (5).

Biological Distribution

The biological distribution of ^{99m}Tc-Carnosine was done in the adult albino mice. The mice were sacrificed at different time periods by decapitation under ether anesthesia. The data of this study is presented in Table (7), and expressed as total injected dose / organs. In concern to the biological distribution of ^{99m}Tc (V)-DMSA in normal mice, the data of these experiments are presented in Table (8).

Molecular Biology Studies

The fragments of rat brain which have been separated by agarose electrophoresis

enabled us to identify their different alleles. DNA genome of rate brain cell has a high molecular weight, so it remained in the field of the agarose gel as shown from Figure (6 a). The anti-inflammatory angiotensin II subtype II receptor gene (AT_2RG) was separated into a purified fragment form using PCR. The size of AT_2RG was found to be approximately 2950 bp for rats as shown in Figure (6 b). ***Ssp*I** restriction enzyme did not differentiate the five groups of rat brain when it digested AT_2R gene into five restriction fragments 50, 250, 450, 500, and 1700 bp as shown in Figures (7 a & b). As shown in Figures (8 a &b), ***AspI*** restriction enzyme cuts the AT_2RG into three restriction fragments for groups 1 and 4 at 250, 850 and 1850 bp, while the same enzyme cuts groups 2, 3 and 5 into five similar bands at 250, 300, 500, 900 and 1000 bp.

BstEII restriction endonuclease clustered the AT_2RG into two groups as shown in Figures (9 a & b). AT_2RG isolates of groups 1, 3 and 4 were digested by ***BstEII*** restriction enzyme into two restriction fragments 450 and 2500 bp, while that of groups 2 and 5 were fragmented into four bands 150, 350, 900 and 1550 bp. ***StyI*** restriction endonuclease clustered AT_2RG groups into two groups as cleared from Figures (10 a & b). Only AT_2RG restriction fragments of group 2 have five bands with characteristic two bands by the action of ***StyI*** restriction enzyme (50, 300,350, 750 and 1500 bp. Groups 1, 3, 4 and 5 showed four bands by the action of ***StyI*** restriction endonuclease (300,350,750 and 1550 bp.

Table 1: The PCR primers sequences used for amplification of AT_2RG

Direction	Sequence
Forward 5` 3` Reverse 5`3`	5`TTTGGTATGCATTAAGCCTTTTCT 3` 5` GAATTCATTTCCGACATATGCT 3`

Table (2): Reagents used in RFLP

Reagent	Volume per sample, µl
Restriction buffer	1.0
Ultra-pure water	3.8
BSA	0.1
Enzyme	0.1
PCR product	5.0

Total reaction volume	10.0

Table (3): Percent conversion of renal DMSA to ^{99m}Tc (V)-DMSA by bring pH of the reaction mixture to 8.2 at different intervals of time

% conversion			
Time/min	^{99m}Tc(V)-DMSA	^{99m}Tc(III)-DMSA	$^{99m}TcO_4^-$ &RH^{99m}Tc
5	57.3 ± 4.9	42.7 ± 4.9	0.22 ± 0.18
15	72.0 ± 5.0	28.0 ± 5.0	0.15 ± 0.05
30	77.0 ± 3.0	23.0 ± 3.0	0.07 ± 0.01
60	83.0 ± 3.6	17.0 ± 3.6	-
120	85.0 ± 2.0	16.0 ± 2.8	-
240	83.0 ± 2.6	17.0 ± 2.6	-

Table (4): Percent conversion of renal DMSA to ^{99m}Tc (V)-DMSA by bring pH of the reaction mixture to 9 at different intervals of time

% conversion			
Time, min	^{99m}Tc(V)DMSA	^{99m}Tc(III)DMSA	$^{99m}TcO_4^-$ & RH ^{99m}Tc
5	65.7 ± 1.5	39.3 ± 2.5	0.05 ± --
15	76.0 ± 1.6	24.0 ± 1.2	0.02 ± --
30	84.3 ± 2.1	15.7 ± 1.1	-
60	87.3 ± 2.3	12.7 ± 2.3	-
120	88.0 ± 2.6	12.0 ± 1.6	-
240	86.7 ± 2.5	10.3 ± 1.2	-

Table (5): Percent radiochemical yield of ^{99mTc} (V) DMSA using 0.1 mg $SnCl_2.2H_2O$ and 30 mg glucose

%Radiochemical yield			
Reagent added	^{99m}Tc(V)DMSA	^{99m}Tc(III)DMSA	$^{99m}TcO_4^-$ & RH ^{99m}Tc
pH 8	97.0 ± 0.86	2.9 ± 0.9	0.03 ± --
pH 9	96.3 ± 1.15	4.3 ± 1.1	0.05 ± --
30 mg glucose at pH 8	96.2 ± 0.70	3.3 ± 0.8	0.60 ± 0.1
30 mg glucose at pH 9	95.3 ± 1.52	4.4 ± 0.6	0.27 ± --

Table (6): *Invitro* stability of ^{99m}Tc (V) DMSA at pH 8.2, 0.1 mg $SnCl_2.2H_2O$ and 30 mg glucose

%Radiochemical yield			
Time, min	^{99m}Tc(V)DMSA	^{99m}Tc(III)DMSA	$^{99m}TcO_4^-$ & RH ^{99m}Tc
5	96.8 ± 2.1	2.4 ± 2.6	0.8 ± 0.2
15	96.7 ± 3.2	2.3 ± 2.9	1.0 ± 0.2
30	96.5 ± 2.2	2.9 ± 2.2	1.6 ± 0.2
60	97.0 ± 0.8	1.6 ± 0.9	1.4 ± 0.2
120	97.2 ± 1.6	2.8 ± 0.8	-
240	97.3 ± 0.6	2.7 ± 0.6	-
360	96.3 ± 1.5	3.7 ± 0.5	-

Table (7): Biological distribution of ^{99m}Tc-Carnosine complex in normal mice organs

% Injected dose / organ at different times post injection					
Organs & body fluids	**15 min**	**30 min**	**60 min**	**120 min**	**240 min**
Blood	36.7 ± 2.5	27.3 ± 2.5	19.9 ± 2.1	14.7 ± 1.8	11.3 ± 1.8
Muscle	9.8 ± 1.1	5.5 ± 0.7	6.0 ± 0.4	8.2 ± 1.3	7.0 ± 1.0
Bone	8.7 ± 1.1	9.2 ± 1.6	6.1 ± 0.7	4.4 ± 0.7	4.1 ± 0.9
Kidney	5.3 ± 0.5	6.4 ± 1.2	6.6 ± 0.5	5.6 ± 0.6	5.2 ± 0.6
Urine	16.3 ± 1.5	19.6 ± 2.1	20.5 ± 2.2	25.1 ± 1.3	29.2 ± 1.7
Liver	5.6 ± 0.8	8.1 ± 1.4	7.5 ± 0.9	5.9 ± 0.7	6.9 ± 1.6
Intestine	6.1 ± 0.6	8.97 ± 1.5	15.7 ± 1.7	17.2 ± 2.5	19.2 ± 1.4
Stomach	3.9 ± 0.4	4.8 ± 1.1	8.2 ± 0.8	7.1 ± 1.8	8.0 ± 1.0
Spleen	1.2 ± 0.2	0.8 ± 0.2	0.8 ± 0.3	0.5 ± --	0.7 ± 0.2
Lung	1.97± 0.5	1.3 ± 0.2	1.1± 0.3	2.4 ± 0.4	0.8 ± 0.1
Heart	0.7 ± 0.6	0.6 ± 0.1	0.3 ± --	0.3 ± --	0.3 ± --
Brain	0.9 ± 0.2	1.0 ± 0.2	1.4 ± 0.2	1.2 ± 0.2	1.4 ± 0.2
Thyroid	2.2 ± 0.3	3.5 ± 0.5	3.6 ± 0.3	3.5 ± 0.3	3.2 ± 0.2
B/Bl., g	0.15	0.23	0.44	0.5	0.77

B: Brain Bl: Blood

Table (8): Biological distribution of ^{99m}Tc (V) DMSA complex in normal animal organs

% Injected dose / organ at different times post injection					
Organs &body fluids	**15**	**30**	**60**	**120**	**240**
Blood	25.9 ± 2.8	15.7 ± 1.5	11.9 ± 1.1	12.2 ± 1.3	11.3 ± 2.5
Muscle	21.3 ± 2.1	17.5 ± 1.4	12.3 ± 1.5	10.9 ± 1.5	11.9 ± 2.2
Bone	23.7 ± 2.1	25.8 ± 1.3	26.9 ± 4.8	21.4 ± 1.2	19.2 ± 0.9
Kidney	1.9 ± 0.4	3.3 ± 0.2	4.4 ± 0.3	5.6 ± 0.5	6.7 ± 1.8
Urine	12.6 ± 1.8	18.9 ± 1.2	19.5 ± 0.6	20.8 ± 1.0	22.5 ± 4.4
Liver	3.4 ± 0.3	5.9 ± 0.5	8.1 ± 1.5	9.6 ± 1.2	10.8 ± 1.4
Intestine	3.1 ± 0.2	6.6 ± 0.9	9.2 ± 1.7	8.0 ± 0.8	11.2 ± 1.2
Stomach	1.1 ± 0.2	1.7 ± 0.3	2.5 ± 0.2	3.8 ± 1.1	2.5 ± 0.2
Spleen	0.4 ± --	0.6 ± 0.2	0.4 ± ---	0.6 ± 0.1	0.7 ± 0.1
Lung	0.8 ± 0.1	0.7 ± 0.2	0.8 ± 0.1	1.0 ± 0.1	1.3 ± 0.5
Heart	0.8 ± --	0.5 ± ---	0.3 ± ---	0.4 ± ---	0.3 ± 0.1
Brain	0.2 ± 0.1	0.5 ± 0.1	0.7 ± 0.2	1.0 ± 0.2	1.1 ± 0.2
Thyroid	0.3 ± 0.1	0.4 ± ---	0.5 ± 0.1	1.9 ± 0.2	1.3 ± 0.3
B/Bl., g	0.05	0.2	0.37	0.5	0.6

B: Brain Bl: Blood

Fig:1

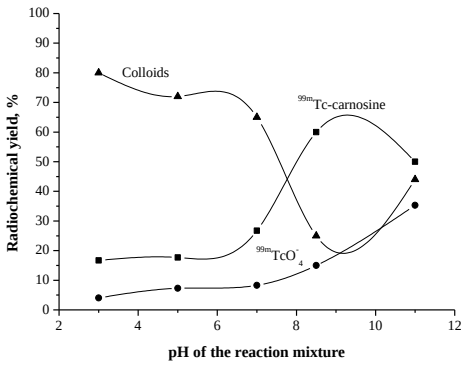


Fig:2

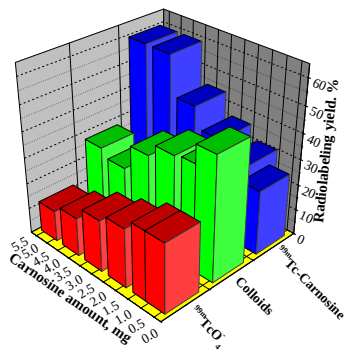


Fig:3

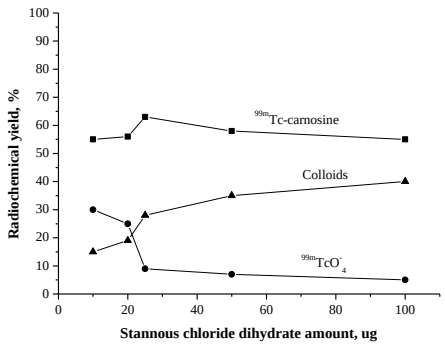
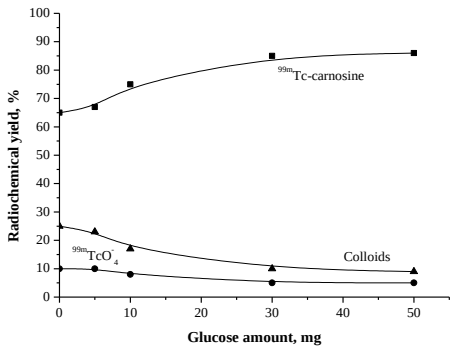


Fig:4



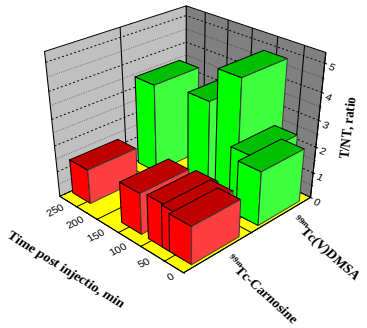


Fig:5

Fig:6a

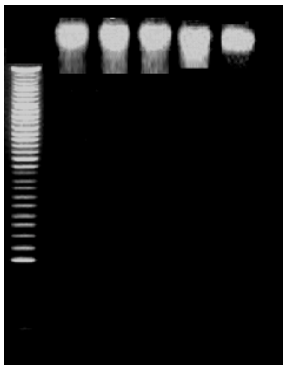


Fig:6b

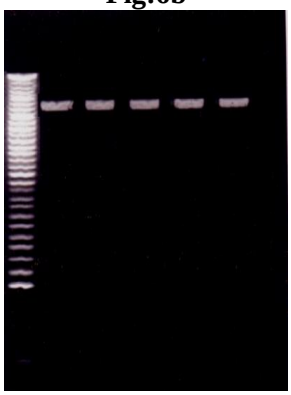


Fig:7a

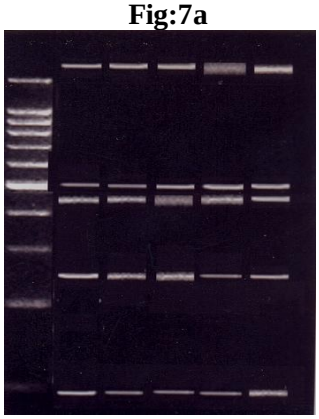


Fig:7b

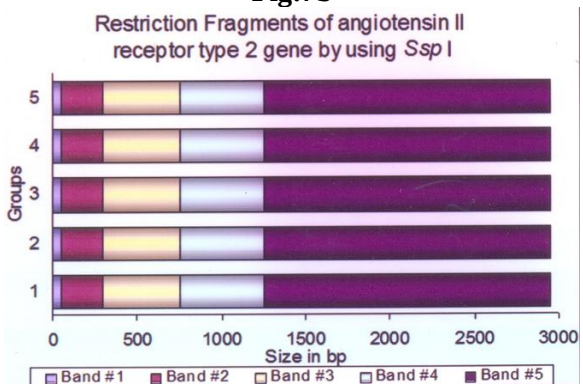
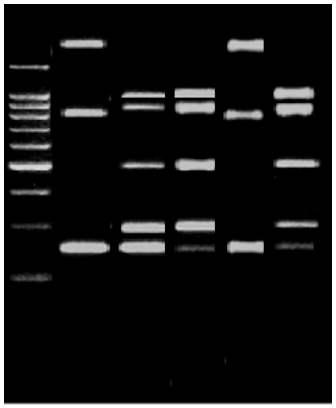


Fig:8a



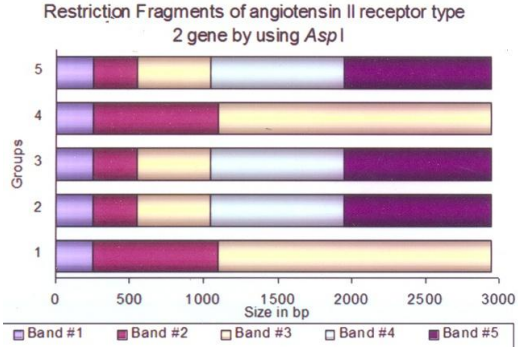


Fig:8b

Fig:9a

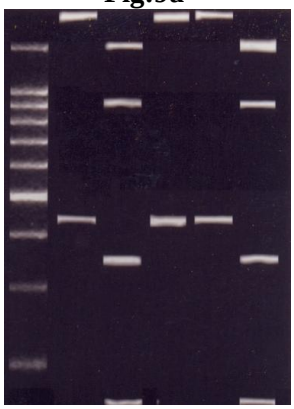


Fig:9b

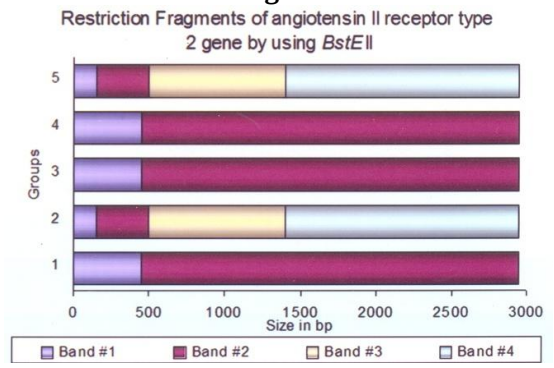


Fig:10a

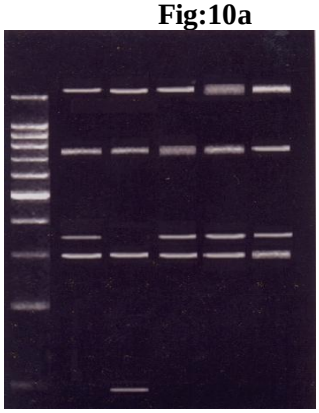


Fig:10b

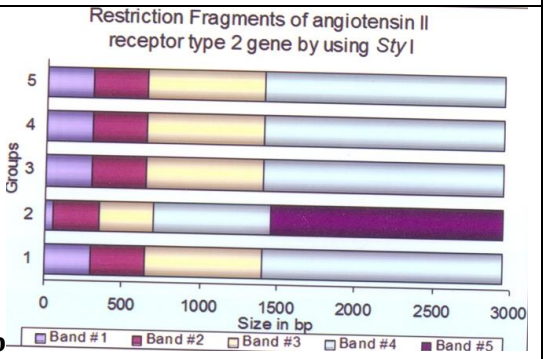


Figure (1): Radiochemical yield of ^{99m}Tc-Carnosine as a function of pH of the reaction mixture
Figure (2): Radiochemical yield of ^{99m}Tc-Carnosine as a function of carnosine amount
Figure (3): Radiochemical yield of ^{99m}Tc-Carnosine as a function of stannous chloride dihydrate amount.
Figure (4): Radiochemical yield of ^{99m}Tc-Carnosine as a function of glucose amount Radiolabeling of DMSA with Technetium-99m
Figure (5): Tumor to non-tumor taken up ratio of both ^{99m}Tc-Carnosine and ^{99m}Tc (V) DMSA in rat muscle
Figure (6): (a) Complete genome and (b) AT_2R Gene.
Figure (7): AT_2R Gene restricted with the endonuclease *SspI*, (a) 1kb ladder lanes 1 to represent the length of the AT_2RG *(*b) Restriction fragments size in bp
Figure (8): AT_2R Gene restricted with the endonuclease *AspI*, (a) 1kb ladder lanes 1 to represent the length of the AT_2RG *(*b) Restriction fragments size in bp
Figure (9): AT_2R Gene restricted with the endonuclease *BstEII*, (a) 1kb ladder lanes 1 to represent the length of the AT_2RG *(*b) Restriction fragments size in bp
Figure (10): AT_2R Gene restricted with the endonuclease *StyI*, (a) 1kb ladder lanes 1 to represent the length of the AT_2RG *(*b) Restriction fragments size in bp

Discussion

The labeling of carnosine with technetium-99m was done via direct labeling. The reducing agent was added to the solution of carnosine followed by pertechnetate. Due to the presence of many donating groups in the structure of carnosine (hydroxyl and amid groups), in addition to the electron enriched atoms

(oxygen and nitrogen), technetium is able to make a coordination bonds with carnosine. Many factors can affect the coordination of technetium-99m with carnosine. The most important one is the pH of the reaction mixture. To investigate the influence of pH on the percent labeling yield of carnosine with ^{99m}Tc, the test was carried out by the addition of pertechnetate to the stannous complex of the ligands at different pH values ranged from 3 up to 11. The data presented in figure 1 clearly showed that, the labeling yield is dependant on the pH of the reaction.

The labelling of many radiopharmaceuticals found to produce high labelling yield at pH8-9 (El-Ghany., *et al* 2000).the labelling of carnosine with technetium reaches the maximum at pH8.5 by using 0.5 M sodium bicarbonate. As the pH decreased towards the acidic one, the ^{99m}Tc-carnosien was decreased also but at higher pH, little decrease in the labelling yield were found.

The reason for such phenomena can be explained as the following; at acidic pH, protons compete with technetium for nitrogen atoms in amine groups of carnosine leading to labeling yield decrease. The higher activity counted in colloid zone may be due to unknown mechanism or formation of secondary complex. But at higher ph than 8.5, it leads to formation of carboxylate anions that are negatively charged entering the resonance stabilization of with electron withdrawing effect on lone pair of electrons that are available for coordination with technetium. So at extreme alkaline pH, a gradual decrease in labeling yield were found and percentage of colloid formation.

Carnosine amounts play an important role in the labeling process. Maximum radiochemical yield of 99mTc-carnosine was obtained at concentration of 4 mg. At low amounts of carnosine, 0.5 and 1.0 mg the radiochemical yield were very low with high percent of free technetium. Increasing the amount of carnosine gradually, the radiochemical yield began to increase. this pattern was due to the low affinity of carnosine to coordinate with technetium-99m especially a maximum yield was obtained after long incubation of carnosine with technetium-99m at room temperature (60 min.) in alkaline pH.

By using stannous chloride dihydrate as reducing agent, the most labeling yield was approximately equal to 25µg of tin for carnosine labelling. Also the results cleared that when the amounts of stannous exceed the values of 25µg/ml, decrease in complexation yield and increase of the colloid formation. Because the labeling of carnosine with technetium was done at alkaline pH 8.5 , an increase in the amount of stannous chloride dehydrate causes the formation of colloids (reduced hydrolyzed technetium and stannous hydroxide).below this value of tin, stannous chloride is not sufficient for complete reduction of pertechnetate that causes increase in free pertechnetate with concomitant decrease in colloid formation . Similar radiolabelling profiles were found in many radiopharmaceuticals (*Ali, and Abdul-megeed, 2002)*.

The effect of addition of varying amounts of dextrose was studied. By addition of moderate amounts of glucose , an increase in the labelling yield were observed with gradual increase in colloid formation as shown in Figure 4 .this may be due to prevention of fast labeled compound degradation with slight increase in reducing power of the solution . Excessive amounts of glucose causes an increase in colloid formation, which may be due to formation of insoluble stannous hydroxide through common ion effect.

Stannous hydroxide reacts with reduced form of technetium-99m to form Tc-tin-colloid excessive amount of dextrose decrease labelling yield which may be due to inhibition of carnosine ionization that decrease the availability of nitrogen and oxygen lone pair of electrons for coordination with technetium.

A huge amount of studies were carried out to prepare pentavalant technetium-99m- DMSA complex (^{99m}TcO (V)-DMSA). This complex has a property differ from that of ^{99m}Tc (III)-DMSA (renal complex) as, it is able to localized in different types of tumor. All these methods were designed to completely control two parameters which have a great influence on the formation of 99mTc (V)-DMSA. The first one is the pH of the reaction mixture, the pentavalant DMSA complex was formed in a critical pH range lies between 8.2 and 9 that showed moderate formation

of pentavalant technetium-99m- DMSA as shown in tables 3 and 4. This pH range can be achieved by adding sodium bicarbonate solution of specific molarity to the renal DMSA kit before pertechnetate addition. The second factor is the concentration of stannous chloride dihydrate, tinny amount of this reducing agent was required to bring the pertechnetate to the oxidation state (V). To achieve this, the reaction mixture of renal DMSA must be purged with sterile oxygen to oxidize the excess stannous chloride and to bring the pertechnetate from the lower oxidation state (III) to the higher oxidation state (V) (Kumar., 2001).

In respect to the results mentioned above, it was cleared that the decrease of the amount of stannous chloride dihydrate to 0.1 mg reduced pertechnetate to the most suitable oxidation state (V) and yielding the highest radiochemical yield of ^{99m}Tc(V)-DMSA as shown in Table 5. The addition of glucose to the DMSA formula did not increase the radiochemical yield of the pentavalant DMSA-technetium-99m complex, but it support the *invitro* stability of the tracer against the radiolysis effect of γ-rays up to 6 hours as shown in Table 6.

As cleared from table (7), the clearance of the tracer from the blood was low due to its high lipophilicity, the blood uptake at early time post injection (15 minutes) was 36% and decreased to its half value at 60 minutes post injection. At the late time (240 minutes) the blood uptake remains high and equal to 11%. This observation reflected the hepatobiliary excretion mode of the tracer, as the liver and intestine hold a high radioactivity (23%) at 60 minutes post injection and was increased gradually as time passed reached to 26%, taken into consideration the radioactivity passed to the feces (not collected). Other route of excretion can be nominated which was via the kidneys, the radioactivity detected in both kidneys and urine was high (21%) at 15 minutes post injection and increased at late time (34% at 240 minutes). The radioactivity detected in both stomach and thyroid reflected the biological stability of the tracer. In respect to this, the total radioactivity detected in both stomach and thyroid was within the accepted limit of technetium-99m radiopharmaceuticals at 30 minutes post injection. However, at late time (240 minutes) the tracer lost some of its stability and free pertechnetate was increased in the stomach and thyroid reached to 11%. The brain uptake of ^{99m}Tc-Carnosine was in its highest value (1.4%) at 60 minutes post injection and remains unchanged even after 240 minutes post injection, so it is useful to use this tracer as brain imaging agent. In respect to the brain/Blood ratio, a maximum ratio (0.77) was obtained at 240 minutes post injection which due to the clearance of the tracer from the blood pool and the imaging of the brain was suitable at this time. The brain uptake of ^{99m}Tc-Carnosine was attributed to the chemical nature of carnosine, as it is amino acid molecule.

The figures outlined in Table (8) showed the localization of high radioactivity in muscles and bone at 15 minutes post injection and decreased gradually as time passed. The uptake of the kidneys which is the target of ^{99m}TC(III)-DMSA was low and equal to 1.9 % and 6.7 % at 15 and 240 minutes post injection, respectively. The localization of the tracer in brain tissues was calculated and presented in Table (8) as ratio of brain uptake to the radioactivity stayed in the blood pool per gram tissue. It clearly showed that the ability of the tracer to pass through the intact blood brain barrier with a relative ratio to the blood sufficient to image the brain tissue (0.7 and 0.77 at 120 and 240 minutes post injection). The in-vivo stability of ^{99m}Tc(V)-DMSA was higher than that of ^{99m}Tc-Carnosine as the sum of radioactivity detected in stomach and thyroid not exceed 3.8% at late time post injection (240 minutes).

In respect to the biodistribution of the two tracers in tumor induced mice, the data of these experiments are presented in Figure (5) which showed that the tumor/ non-tumor(T/NT) of ^{99m}Tc (V)-DMSA was higher than 99mTc-Carnosine during all selected time periods. In addition, the most proper time that showed the highest T/NT of ^{99m}Tc (V)-DMSA was 60 minutes post injection.

Depending on the above data, it was found that polyphylogenicity between all groups which are diagnostic markers for these groups by DNA analysis. The data obtained revealed that group 1 (normal

control) has a characteristic bands with ***Asp1, BstXII, and StyI*** restriction endonucleases. While group 2 (irradiated with 6 Gy) as a positive control group showed a diagnostic polymorphic markers of approximately about 50 and 300 bp for ***StyI*** restriction enzyme. All γ-irradiated groups treated with AOs have diagnostic cuts of 1000 bp using ***BstXII*** restriction endonucleases. So DNA analysis of the AT_2RG polymorphism for different groups is a diagnostic tool for γ-ray adverse effect and to estimate the anti-oxidant activity of carnosine and DMSA.

From oxidative stress point of view, PCR/RFLPs profile of AT_2RG produced high variations between group 1 (normal control), group 2 γ-irradiated (positive control) and groups 3, 4 and 5 (γ-irradiated and treated groups) according to the difference of the profile obtained with ***StyI and BstEII*** restriction endonucleases. On the other side, results obtained of ***Ssp1*** restriction endonucleases did not clarify the difference between all rat groups of AT_2RG based on the similarity of profiles obtained with this restriction endonuclease.

As the results of the digestion of AT_2RG for all groups by different restriction enzymes, it was concluded that there is a polyphylogenetic relationship between AT_2R structures for all groups as the following:

1- Group 2 which exposed to 6 Gy of γ-ray showed different PCR/RFLPs profile of AT_2R gene from normal control (group 1) in all restriction endonucleases except for ***SspI*** indicates DNA fragmentation induced oxidative stress.

2- Carnosine treated group before γ-irradiation (group 3) showed the same PCR/RFLPs profile for two restriction endonuclease enzymes (***StyI and BstEII***) with the normal control (group 1), and similar restriction cuts profile of ***AspI*** restriction enzyme with group 2. All of these data indicate the strong antioxidant activity of carnosine but not enough to completely block radiation induced oxidative stress which compatible with published data (Dizdaroglu 1994 and Halliwell, 1998)

3- Group 4 (treated with both carnosine and DMSA before γ-irradiation) gave the same restriction cuts for StyI***, AspI, and BstEII*** restriction endonucleases in compared to normal control group. So it can be concluded that maximum protection can be assessed through dual action of both DMSA and carnosine.

4- DMSA treated rats before γ-irradiation (group 5) exhibited similarity with PCR/RFLPs profile with ***StyI*** restriction enzyme only in compared to normal control group. In contrast to the above, group 5 has more similarity as shown in the PCR/RFLPs profile of ***AspI and BstEII*** restriction endonucleases with group 2. In addition there are four different PCR/RFLPs profiles from group 1 but with less number of mutations in relation to group 2 with 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 be gives its antioxidant effect through reduction of formed DNA radical because its content of free thiols (Powell and McMillan, 1990).

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 of both chelates.

Concolusions

Carnosine was labeled with technetium-99m via direct labeling using simple method by using glucose and stannous chloride dihydrate as a reducing agents. Minor modification was done to prepare ^{99m}Tc (V)-DMSA tracer in one step by decreasing the amount of stannous chloride dihydrate to 100 μg with addition of glucose. The biodistribution of the two tracers in normal and tumor-induced mice clarified that ability of both tracers to penetrate the blood brain barrier with

superiority of ^{99m}Tc-carnosine. In addition, the two tracers were able to localized a tumor site but the priority to the ^{99m}Tc (V)-DMSA at all the designed time periods.

The molecular investigation of the anti-oxidant activity of both carnosine and DMSA in 6 Gy γ-irradiated rats using the anti-inflammatory angiotensin II subtype II receptor gene (AT_2RG) as indicator referred to the following: 1- AT_2RG was separated successfully into a purified fragments using PCR technique. 2- The size of AT_2RG was found approximately 2950 bp for rats brain. 3- 6 Gy γ-irradiation showed different PCR/RFLPs profile of AT_2RG from normal control in all restriction endonucleases except for ***SspI***. 4- Strong antioxidant activity of carnosine was not enough to block radiation induced oxidative stress. 5- Moderate antioxidant activity of DMSA through free iron chelation and chelation of iron released through oxidative stress. 6- Maximum protection was achieved through the dual action of both DMSA and carnosine.

References

1. **Nunomura A Castellani J Zhu X Moreira P Perry G and Smith A. (2006):**Involvement of Oxidative Stress in Alzheimer Disease. J. Neuropathol. Exp. Neurol., 65 (7): 631.
2. **Ali S and Abdul-Megeed F (2002):** Synthesis and technetium labeling of pentadentate aminophenol ligand and studies of its biodistribution and biochemical changes in animal organs.Az.J. Pharm. Sci., 29:361.
3. **Ambrosone C B Freudenheim JL and Thompson PA (1999):** Manganese Superoxide Dismutase (*MnSOD*) Genetic Polymorphisms, Dietary Antioxidants, and Risk of Breast Cancer Cancer Res.,59: 602.
4. **Blocher D (1988);** DNA double strand break repair determines the RBE of alpha particles. A new mechanism for DNA alterations induced by alpha particles. Int. J. Radiat. Biol.; 54, 761.
5. **Chaudhry MA and Wienfeld M (1995):** the action of *Escherichia coli* endonuclease III on multiply damaged sites in DNA. J. Mol. Biol., 49: 914.
6. **Chaudry MA and Weinfeld M (1997):** Reactivity of human apurinic/apyrimidinic endonuclease and *Escherichia coli* exonuclease III with bistranded abasic sites in DNA. J. Biol. Chem., 272: 15650.
7. **David-Cordonnier MH Laval J and O'Neill P (2000):** Clustered DNA damage, influence on damage excision by XRS5 nuclear extracts and *Escherichia coli* Nth and Fpg proteins. J. Biol. Chem., 275: 11865.
8. **David-Cordonnier M H Boiteux S and O'Neill P (2001);** Excision of 8-oxoguanine within clustered damage by the yeast OGG1 protein. Nucleic Acids Res.,29: 1107.
9. **David-Cordonnier M H Laval J and O'Neill P (2001):** Recognition and kinetics for excision of a base lesion within clustered DNA damage by the *Escherichia coli* proteins Fpg and Nth. Biochemistry.,40: 5738.
10. **David-Cordonnier MH Boiteux S and O'Neill P (2001):** Efficiency of excision of 8-oxo-guanine within DNA clustered damage by XRS5 nuclear extracts and purified human OGG1 protein. Biochemistry.,40: 11811.
11. **David-Cordonnier MH Cunniffe S M T Hickson I D and O'Neill P (2002):** Efficiency of incision of an AP site within clustered DNA damage by the major human AP endonuclease. Biochemistry., 41: 634.
12. **Dizdaroglu M (1994):** Chemical determination of oxidative DNA damage by gas chromatography-mass spectrometry. Methods Enzymol., 234: 3.
13. **E l-Ghany E Attia F Marzouk F and Kollaly M (2000):**Organic synthesis and technetium labeling of some amino-phenol ligands.J.Rad.Nuc.Chem.245(2)237-245.
14. **Goodhead D T (1994):** Initial events in the cellular effects of ionizing radiations: clustered damage in DNA. Int. J. Radiat. Biol., 65: 7.
15. **Guiotto A Calderan A Ruzza P and Borin G (2005):** Carnosine and carnosine-related antioxidants: a review.Curr Med Chem.; 12(20): 2293.
16. **Gulston M Fulford Jenner T de Lara C and O'Neill P (2002):** Clustered DNA damage induced by gamma radiation. Nucleic Acids Res., 30: 3464.
17. **Halliwell B (1998):** Can oxidative DNA damage be used as a biomarker of cancer risk in humans. Free Radic. Res., 29: 469.
18. **Harrison L Hatahet Z Purmal A A and Wallace S S** (1998): Multiply damaged sites in DNA: interactions with *Escherichia coli* endonucleases III and VIII. Nucleic Acids Res., 26: 932 (2).
19. **Harrison L Hatahet Z and Wallace S S**

(1999):*In vitro* repair of synthetic ionizing radiation-induced multiply damaged DNA sites. J. Mol. Biol., 290: 667.

20. **Hunninghake G W and Crystal R G (1983):** Cigarette smoking and lung destruction. Accumulation of neutrophils in the lungs of cigarette smokers. Am Rev Respir Dis; 128, 833.
21. **Jeggo P (1990);** Studies on mammalian mutants defective in rejoining double strand breaks in DNA. Mutat. Res., 239: 1.
22. **Jenner T de Lara C M O'Neill P and Stevens D L (1993):** Induction and rejoining of DNA double-strand breaks in V79-4 mammalian cells following gamma- and alpha-irradiation. Int. J. Radiat. Biol., 64: 264.
23. **Jenner T J Fulford J and O'Neill P (2001)**: Contribution of base lesions to radiation-induced clustered DNA damage: Implication for models of radiation response. Radiat. Res., 156: 590.
24. **Johnston P J. MacPhail S H. Stamato T D Kirchgessner C U and Olive P (1998):** Higher-Order Chromatin Structure-Dependent Repair of DNA Double-Strand Breaks: Involvement of the V (D) J Recombination Double-Strand Break Repair Pathway. Radiat. Res., 149: 455.
25. **Kumar V:(2001):**Evaluation of stannous oxidation in the preparation of high purity Tc(V)DMSA.Nucl.Med.Comm.,22:1261-6.
26. **Lomax M E Cunniffe S M T and O'Neill, P (2004):** 8-OxoG retards the activity of the ligase III/XRCC1 complex during the repair of a single-strand break, when present within a clustered DNA damage site. DNA Repair., 3:, 289.
27. **Moseley E and Belin B (1984):** "Radiation Damage and its Repair in Non-sporulating Bacteria", In the Revival of Injured Microbes, Radiation Biology" M.H.E. Andrew and Russell eds., Academic press, London., 147: **(3)**
28. **Nikjoo N O'Neill P Goodhead D T and Terrissol M (1997):** X-ray absorption near-edge structure (XANES) spectral changes of 2-deoxy-D-ribose by irradiation within the energy region around the oxygen K-shell absorption edge. Int. J. Radiat. Biol., 71: 467.
29. **Nikjoo H O'Neill P Wilson W E and Goodhead D T (2001):** Computational approach for determining the spectrum of DNA damage by ionizing radiation. Radiat. Res., 156: 577.
30. **O'Neill P and Fielden E M (1993):** Primary free radical processes in DNA. Adv. Radiat. Biol.,; 17:120.
31. **Pfeiffer P (1998):** The mutagenic potential of DNA double-strand break repair Toxicol. Lett., 96: 119.
32. **Powell S and McMillan T J (1990):** DNA damage and repair following treatment with ionizing radiation. Radiother. and Oncol., 19: 95 .
33. **Prise K M Ahnstrom G Belli M Carlsson J Frankenberg D Kiefer J Lobrich M Michael B D Nygren J Simone G and Stenerlow B (1998):** A review of DSB induction data for varying quality radiations. Int. J. Radiat. Biol., 74: 173.
34. **Pryor W A and Stone K (1993):** Oxidants in cigarette smoke. Radicals, hydrogen peroxide, peroxynitrate, and peroxynitrite. Ann NY Acad. Sci.,.686: 12.
35. **Shimoda-Matsubayashi S. Matsumine H and Kobayashi T (1996):** Structural dimorphism in the mitochondrial targeting sequence in the human manganese superoxide dismutase gene. A predictive evidence for conformational change to influence mitochondrial transport and a study of allelic association in Parkinson's disease. Biochem Biophys Res Commun.,226: 561.
36. **Sutherland, B.M. Bennett, P.V. Sidorkina, O. and Laval, J. (2000);** Clustered DNA damages induced in isolated DNA and in human cells by low doses of ionizing radiation. Proc. Natl Acad. Sci. USA., 97: 103.
37. **Sutherland B.M., Bennett, P.V. Sutherland, J.C.and Laval J. (2002);** Clustered DNA damages induced by x-rays in human cells. Radiat. Res.; 157, 611.
38. **von Sonntag; C. (1987);**"The Chemical Basis of Radiation Biology" Taylor & Francis, Basingstoke, UK. ISBN 0-85066: 375.
39. **Wallace, S.S. (2002)**; Engineering Functional Changes in *Escherichia coli* Endonuclease III Based on Phylogenetic and Structural Analyses. Free Radic. Biol. Med., 33: 1.
40. **Wang L.I., Neuberg, D. and Christiani, D.C. (2004):** Asbestos Exposure, Manganese Superoxide Dismutase (MnSOD) Genotype, and Lung Cancer Risk J Occup. Environ. Med., 46: 556.

ترقيم الكارنوزين و ثنائى كبريت حمض السكسنيك بالتكنسيوم-99م و التوزيع البيولوجى لهذه المركبات وتقدير التاثير المضاد للتقطيع الجينى الناتج عن التشعيع الجامى

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إن المركبات المخلبية هى مركبات تحتوى على أكثر من مكان جاهز لتكوين الروابط التنلسقية وهى واسعة الإستخدام فى مجال الطب النووى و الكيمياء الحيوية.أثنين من هذه المركبات تم ترقيمهم بإستخدام عنصر التكنسيوم المشع مع عئد ترقيمى 85% و 97% للكارنوزين و ثنائى كبريت حمض السكسنيك . وتم ترقيم الكارنوزين عند تركيز 4 مجم و25 ميكرو جرام من ثانى اكسيد القصديروز و30 مجم جلوكوز. أما ثنائى كبريت حمض السكسنيك فتم تعلية نسبة ترقيمه بإستخدام تركيز 1 مجم من ثنائى كبريت حمض السكسنيك و تركيز 100 ميكرو جرام من العامل المختزل. وبعمل التوزيع البيولوجى لها داخل الفئران تبين أن هذه المركبات تتركز فى المناطق السرطانية بنسب عالية مما يسمح باستخدامها فى المسح الذرى للاورام السرطانية. وتبين تركيز هذه المعدات داخل العظام بصورة عالية وبالتالى قد تكون صالحة للمسح الذرى فى العظام ولقد تبين وجود تركيز كافى لهذه المركبات فى المخ والغدة الدرقية مما يسمح بإستخامها فى المسح الذرى لهذه الأعضاء. وحيث أن المركبات المخلبية ترتبط بالحديد الحر والذى يسبب ذيادة الأكسدة و الأكسدة المفتعلة إشعاعيا و بالتالى تثبط تفاعل فينتون المحفز بالمعادن. و حيث أن تفاعلات الأكسدة والإختزال هى تفاعلات تستهدف الحمض النووى فكان لابد من إستخدام تقنيات الهندسة الوراثية و البيولوجيا الجزيئية للكشف عن التأثيرات بالكيمياء الحيوية.ولقد تم عمل خمس مجموعات من الجرذان و كانت المجموعة الأولى هى المجموعة الضابطة السلبية و المجموعة الثانية هى مجموعة ضابطة إيجابية حيث تم تشعيعها بجرعة 6 جرامن إشعاع الجاما . بينما المجموعة الثالثة والخامسة تم إعطائهم 200مجم/كج من الكارنوزين و50 مجم/كج من ثنائى كبريت حمض السكسنيك علة التوالى لكل من المجموعتين قبل التشعيع بساعة بينما المجموعة الربعة أعطيت كلا من المادتين قبل التعريض لأشعة الجاما. ولقد تم عزل الجين الخاص بمستقبلات الأنجيوتينسين من النوع الثانى فى المخ بواسطة تفاعل البلمرة المتسلسل ثم تم تقطيع المحددات الجينية باستخدام الانزيمات المقطعة. ولقد تبين فى هذه الدراسة أن الكارنوزين أقوى من ثنائى كبريت حمض السكسنيك فى التأثير المضاد للأكسدو أن اتحادهما معا حققت أقصى درجات الحماية . و تبين امكانية استخدام المركبات المخلبية ضد العوامل المسرطنة و الطفرات الجينية و تبين من استخدام الجين السابق من امكانية عمل هذه المركبات كمضادات للأكسدة.