V.DISCUSSION

The exposure of mammals to ionizing radiation leads to the development of a complex dose-dependent series of physiological and pathological changes [97]. Most of the deleterious effects are the result of hydroxyl and superoxide radicals as well as hydrogen peroxide produced via the radiolysis of water, imparting severe oxidative stress to all of the cell's components [98]. Increasing evidence also supports the role of chronic oxidative stress in the progression of radiation-induced late tissue injury [16]. A founding concept of radiobiology that deals with x-rays is that this type of radiation indiscriminately damages cellular macromolecules. This concept has been challenged. The lethal effects of radiation appear to be governed by oxidative protein damage, which inactivates enzymes including those needed to repair and replicate DNA[99]. Experimental evidence, led to the conclusion that proteins are more probable initial targets of cellular radiation damage than DNA. The ability of cells to protect their proteins from oxidation by scavenging ionizing radiation-induced ROS has been proposed as the key mechanism for survival of ionizing radiation-resistant microorganisms [98]. This concept led to considering the possible protective effect of a compatible solute like ectoine in this respect since it is known to protect both hydration and folding of proteins[90]. So far the compatible solute ectoine has been shown to prevent signaling events triggered by UVA irradiation in skin epithelial cells. This kind of environmental stress initiates proinflammatory signaling via the induction of ROS in keratinocytes [100]. Therefore, a better understanding of the mechanism by which ectoine confers radioprotection will enable its use more effectively for protection in patients after radiation therapy or in those suffering from overdose or accidental irradiation.

Opposing hypotheses on the potential risks of low-dose radiations have been advanced. One hypothesis proposes that there is no dose of radiation that can be considered completely safe and that the use of radiation must always be determined on the basis of risk and benefit. Another hypothesis suggests that the health risks of diagnostic doses less than 10Gy are not measurable [101], and radiation doses less than 2Gy were reported not to cause significant damage[102]. However at 2-8 Gy, radiation syndrome develops proportional to radiation dose. This was the basis for the choice of the relatively low radiation doses of 2 and 6 Gy used in the present work.

The organs most sensitive to radiation are the hematopoietic, lymphoid, gastrointestinal, reproductive, vascular, and cutaneous systems [103]. The liver has been reported to be a highly radiosensitive hematopoietic organ. It is the primary organ responsible for drug metabolism, detoxifying damaging electrophiles generated during oxidative stress[104,105]. When compared with other organs in the body, the CNS is susceptible to ROS-mediated damage due to its biochemical, physiological and anatomic characteristics. The severity of the effects depends on the radiation dose, frequency and duration of the exposure as well as the size of the exposed area [106]. The most serious complication of radiation exposure to the brain is tissue damage that develops in the form of edema and necrosis, which have been reported to be mediated by free radicals[107].

The normal adult testis is known to be extremely sensitive to the effects of radiation[108]. The relationship between radiation dosage and length of time to recovery has been well-established after single dose irradiation in adult men. Renal involvement [chronic renal failure] may be responsible for marked Leydig cell dysfunction1[109]. In a series of experiments using a rat model Delic and co-workers [110-112] provided evidence

that the pubertal status modified the testicular response to radiation injury. The threshold dose for induction of Leydig cell dysfunction in prepubertal, pubertal and adult rat was about 5 Gy; however, the younger animals appeared to be more vulnerable to persistent Leydig cell damage[113].

Accordingly, these three organs; liver, brain and testicles, were chosen in the present study for their greater risk; although other organs like skin, heart, kidney, intestine and lung are just as important and are at similar risk.

The early biochemical modifications may continue to rise for days and months after the initial exposure to irradiation presumably because of continuous production of ROS[8]. These toxic products initiate a cascade of events on the molecular level, which alter the cytokine content of the microenvironment and affect the balance of antioxidant systems such as glutathione and enzymatic antioxidant defense systems[114-116]. In the present study, this phenomenon was observed in most of the biochemical changes in the tested organs following irradiation by either of the two low x-ray doses. Continued increases in the values of all determined parameters were observed with both doses used between day one and day 7 of irradiation with the exception of MDA. Whether the decrease in MDA observed in day seven may be taken as an early sign of recovery or it may be due to excessive damage of cellular membranes causing depletion of fatty acids, particularly arachidonic acid, because of extensive production of interleukins and PGE_2 or due to decreased activity of the oxidative enzymes, needs to be clarified.

The reported effects of cytokines in case of irradiation are conflicting. Several possible mechanisms of radioprotection have emerged including reduction of oxidation damage through induction of such enzymes as manganese superoxide dismutase (MnSOD) and other scavenging proteins, as well as reduction of apoptosis. It has been suggested that natural levels of IL-1 and TNF-α contribute to radio-resistance of normal mice, and their cooperative interaction is necessary to achieve successful radioprotection [117] and to enhance the ability of the host to recover from lethal radiation[118]. In contrast, sensitizing mechanisms may include increased oxidative damage, which may occur in absence of scavenger induction, as well as enhanced apoptosis and arrest of cells in the G1 phase of the cell cycle at the time of exposure to radiation, which may promote apoptosis [102].

Cytokine measurements in tissue or in the peripheral circulation have been an important part of the process of defining the role various cytokines play in health and disease. It has been suggested that local cytokine levels and activity are of considerably greater value for monitoring of pathological events in a target tissue than are systemic serum cytokine levels [117]. In the present study, the x-irradiated mice showed elevated interleukins in all tested tissues. The magnitude of elevation was dependent on the radiation dose and the time after irradiation. Some of the biochemical changes associated with signal transduction is likely to be cell-specific. This could be seen in the present work in the differences in the magnitude of the changes in the levels of the different interleukins in the tissues tested. The levels in the liver were much higher than in brain or testicles.

In the present study, increases in the concentration of IL-1β were accompanied by concomitant increases in IL-6 in the three organs tested. IL-1β has been reported to induce IL-6 [119], which is considered to be an essential contributor to natural resistance to lethal irradiation. However, by itself IL-6 was shown not afford any protection, but obligatory interaction of IL-1 or TNF-α with IL-6 may be a prerequisite for some of the biological

effects of these inflammatory cytokines . Induction of IL-6 and/or its receptors has been reported to vary in different tissues and many of the actions of IL-1β can be mimicked by IL-6 [120].

It is noteworthy that the increases of the pro-inflammatory cytokines, in the present study, were also accompanied by significant increases in the anti-inflammatory IL-10, which followed the same pattern. High expression of IL-10 has been reported in the thymus on day 5 following irradiation [121,122]. Probably such increase represents a defense mechanism against the high levels of IL-1β and other pro-inflammatory mediators caused by irradiation. It is generally recognized that counteraction of the inflammatory response to radiation is important to attenuate acute radiation effects and prevent consequences [123]. It has been proposed that the biological activities of IL-10 in modulating inflammation in this case may be caused, in part, by down-regulation of pro-inflammatory cytokines and the expression of their receptors and up-regulation of cytokine inhibitors [124]. The down-regulation of IL-6 by IL-10 has been shown and it has been proposed that limiting one step of the inflammatory process cascade might control the progression of the inflammatory reactions. This can be of benefit since it has been widely shown that the inflammatory reaction is intrinsically destructive for surrounding tissues. In addition an early release of cytokines could be responsible for the damage leading to the hypothesis of perpetual cascade of cytokines initiating radiation-induced late effects [125].

The intracellular increase in the level of IL-1 is followed rapidly by induction of several biochemical events[60], some of which are associated with signal transduction and are likely to be cell-specific and some are thought to be initiated by the release of lipid mediators. IL-1 preferentially stimulates new transcripts for the inducible type II form of PLA_2, which cleaves the fatty acid in the number 2 position of cell membrane phospholipids, resulting in most cases in the release of arachidonic acid, which is the rate limiting step in the synthesis of PGs and leukotrienes. Besides, IL-1 induces the transcription of COX-2. Once triggered, COX-2 production is elevated and large amounts of PGE_2 are produced in cells stimulated with IL-1. Therefore, many of the biological activities of IL-1 are proposed to be due to increased PGE_2 production [126].

Prostaglandin E_2, in the present study, increased in all three organs tested proportional to the radiation dose and the time after irradiation. Increased prostaglandin-like activity in most tissues of mice exposed to whole body irradiation was previously reported[127]. The underlying mechanisms through which prostaglandins may be cytoprotective are unknown. It was reported that too little PGE_2 in the early period post-irradiation reduced positive anti-apoptotic and self-renewal effects, while too much PGE_2 signaling at later time points inhibits hepatopoietic progenitor cells expansion and reduces hematopoietic recovery[101]. A broad spectrum of mediators regulates the expression of COX-2. Whereas proinflammatory cytokines such as IL-1β and IL-6 among other factors induce COX-2, the anti-inflammatory cytokine IL-10 inhibits the expression of this enzyme[42,128]. Therefore it seems that the balance between the effects of proinflammatory and anti-inflammatory cytokines may determine the short-term or long-term outcome of irradiation effects.

In the present study indicators of oxidative stress and damage due to increased production of free radicals were highest after one day of exposure to low levels of x-irradiation followed by attenuation on day seven in all tested organs. After gamma

radiation exposure, levels of the lipid peroxidation indicator MDA have been shown to increase in brain [107, 129], liver [130-134], lens[135], serum[136], and skeletal muscle [137] of rats. Ionizing radiation-induced lipid peroxidation reactions can occur at both the cell membrane and the mitochondria membranes, and either can subsequently trigger cell death through apoptosis and/or autophagy [138]. Autophagy is a catabolic process involving the bulk degradation of cellular constituents in lysosomes[139]. Therefore, blockade of lipid peroxidation could be a useful approach to prevent radiation injury.

The data obtained in the present work point out to that the low dose x-irradiation has stronger effect on interleukins and prostaglandin E_2 than on oxidative stress as measured by changes in different forms of glutathione and MDA. The magnitude of effect appears to be radiation dose and organ specific. The effect of low-dose x-irradiation on glutathione was not as strong as may be expected. There were no statistically significant differences in the results of total glutathione among all the groups of mice as compared to control values. However, there were significant differences among the values of the reduced and oxidized forms. The effect was relatively higher after one day of irradiation with a tendency toward recovery and going back to near control. Because of the relatively small initial reduced and oxidized levels in the control group, the percentage changes in the irradiated groups were apparently large. Current concepts of the mechanism of action of ROS include alteration in intracellular redox state and oxidative modification of proteins. Cellular cytosol is normally maintained under strong reducing conditions, which is accomplished by the action of intracellular redox-buffering systems[11]. This could be reflected in the calculated values of redox potential, which showed some variations in the different groups, but they were all within the values indicating that the cells are in the proliferative phase and far from the apoptotic phase [29].

A number of natural and synthetic compounds of diverse structures have displayed significant protection against radiation[140,141]. All of them are presumably working through different mechanisms of action. However, if the main effect of radiation is on protein[99] and the key to cell survival is through conserving the integrity of the antioxidant enzymes [98,142], then compounds known to possess such properties would be good candidates for the protective action. Compatible solutes, including ectoine, are characterized by being effective stabilizers of biomolecules including proteins and nucleic acids as well as biomembranes[91]. These properties make them potential candidates for cellular protection. It was shown that ectoine increases the hydration of a model biological membrane resulting in higher membrane fluidity[90]. The increased hydration and fluidization of the cell membrane may help to withstand membrane damaging stressors and might also accelerate repair mechanisms. Ectoine was also found to block nuclear translocation of NF-κB to down-regulate the expression of the proinflammatory cytokines IL-1, IL-6, IL-8 and TNF-α[91]. These effects might be partially mediated by ectoine's impact on membrane fluidity leading to interference with membrane-coupled proinflammatory signaling [143]. From the results of the present work it could be seen that the effect of ectoine on day seven in all three examined organs; i.e., liver, brain and testicles, was much more prominent than on day one. This probably implies that ectoine gives stronger effects after it accumulates in these organs following multiple dosing.

Results of the present study demonstrated that the 2Gy and 6Gy whole-body irradiation caused a significant increase in the MDA level, whereas the antioxidant levels of GSH were markedly decreased in the livers, brains and testes of irradiated mice.

Administration of ectoine effectively decreased MDA levels in the livers, brains and testes of all irradiated animals. GSH, as an antioxidant, has been considered as the most accurate single indicator of cell health, as GSH depletion represents vulnerability to oxidant attack[144]. Significantly elevated levels of GSH were observed in the livers, brains and testes of mice treated with ectoine, which may be a factor responsible for the inhibition of MDA generated from lipid peroxidation. In addition, the significant increase in GSH protects cellular proteins against oxidative damage through the glutathione redox cycle and also directly detoxifies ROS induced by irradiation[145]. Therefore, ectoine administration can effectively mitigate oxidative stress in the liver, brain and testis. Whether the protective mechanism of ectoine may be due to its ROS scavenging activity or to regulating the activity of antioxidant enzymes needs further investigation.

The ability of ectoine to evoke a proinflammatory process was previously investigated [91]. When keratinocytes were pretreated with ectoine before stimulation with a well-characterized proinflammatory stimulus lipopolysaccharide (LPS), the up regulation of the proinflammatory cytokines IL-1 β, IL-6, IL-8, and TNF-α was not observed as occurred in the LPS-treated cells. However, the sole ectoine treatment did not modify the level of expression for the determined cytokines. It was concluded that ectoine does not have the ability to induce a proinflammatory process; moreover, this compatible solute probably acts as an anti-inflammatory molecule.

In conclusion, protection of biological systems from ionizing radiation is of paramount importance in planned as well as unplanned accidental exposures to radiation. Development of novel and effective agents to combat radiation damages using nontoxic radioprotectors is of considerable interest in health care, particularly in radiodiagnostics and therapy. Despite the lack of clinical studies, results of the present study suggest that ectoine has the potential to protect tissues from radiation injury and is a candidate for further development as a radiation countermeasure. Further experiments and clinical trials are necessary to validate this.

VI.SUMMARY

X-rays represent a form of electromagnetic irradiation that may affect most exposed cells depending on the dose. This type of radiation may cause increased levels of reactive oxygen species (ROS), either directly or through radiolysis of water. Reactive oxygen species can damage electron transport chain proteins that in turn can create more radicals, which cause increased oxidative stress. Whenever the endogenous antioxidant mechanisms are not fully functional, it is expected that the increase in oxidative stress might cause cellular injury by inducing oxidative damage to various molecules, including increased lipid and protein oxidation. Free radicals can interfere with signaling cascades through regulation of protein activities. On the other hand, free radical signaling could be mediated by cytokines, the participation of which in cellular pathways is modulated by the redox status. The cytokine mediators of oxidative stress can alter redox equilibrium by affecting reduced/oxidized glutathione shuttling and recycling. This, by necessity, indicates alterations in the activity of the participating enzymes.

Our strategy of protection against cellular damage by deep x-irradiation was built on restoring homeostasis of the disturbed biochemical pathways by affording protection to and preventing damage of essential macromolecules. The use of agents known to preserve membrane integrity and protect the folding and hydration of proteins would, by necessity, preserve the function of receptors and the activities of the different enzymes including those with antioxidant or DNA repair properties. Compatible solutes, including ectoine, are known to possess such properties and therefore are worth trying to protect cellular elements against the damaging effect of x-irradiation.

The present study was undertaken to investigate the effect of acute exposure to low level of whole body deep x-irradiation on cellular oxidative stress in the mouse. Production of free radicals and the efficacy of antioxidant defense in the liver, brain and testicles were assessed. The relationship between the extent of oxidative stress and the balance between pro-inflammatory and anti-inflammatory cytokines as well as the changes in prostaglandin E_2 were also considered.

male Swiss albino mice, weighing 20-22 g each, were used as the experimental animals and were divided into the following groups:

Group 1: of six (6) animals served as negative controls.

Group 2: of 12 animals were irradiated by a single dose of 2 Gy by exposure for one minute. Six (6) animals were sacrificed after one day and the other six (6) after 7 days.

Group 3: of 12 animals were irradiated by a single dose of 6 Gy by exposure for three minutes. Six (6) animals were sacrificed after one day and the other six (6) after 7 days.

Animals in groups 2 and 3 received intraperitoneal injections of saline.

Group 4: of 12 animals were irradiated by a single dose of 2 Gy. Six (6) animals received a single dose of 200 mg ectoine per kg body weight, and were sacrificed after one day. The other six (6) received the same daily doses of ectoine and sacrificed after one week.

Group 5: of 12 animals were irradiated by a single dose of 6 Gy. Six (6) animals received a single dose of 200 mg ectoine per kg body weight, and were sacrificed after one day. The other six (6) received the same daily doses of ectoine and sacrificed after one week.

At the end of the designated times animals were sacrificed by cervical dislocation. Each sacrificed animal was decapitated and the bones at the top of the skulls were excised to remove the whole brain. The liver and the testicles were also dissected, and the three organs were quickly washed with ice-cold saline. All specimens were properly labeled and kept at -80 □C until assayed for the following parameters:

- Interleukin-1β (IL-1) and interleukin-6, (IL-6) representing pro-inflammatory cytokines
- Interleukin-10 (IL-10), representing anti-inflammatory cytokines
- Malondialdehyde (MDA) as well as total, reduced (GSH) and oxidized glutathione (GSSG) to evaluate oxidative stress
- Prostaglandin E_2 (PGE_2).

The effects of whole body x-irradiation were qualitatively similar on all three organs tested, but quantitatively different depending on the organ and the radiation dose. Both of the pro-inflammatory cytokines, IL-1 and IL-6 increased at one day after irradiation with both x-ray doses (2Gy and 6 Gy) in the liver, brain and testicles with the highest rise seen in the liver.

After one day of irradiation with the 2 Gy dose the level of IL-1in the liver almost doubled (93.1% increase) going up to 3-fold that of control after 7 days. The effect of the 6 Gy dose was more prominent, as the mean values of IL-1 were 3-fold the control after one day and 6-fold after seven days. The changes in the concentration of IL-1in the brain were similar to those in the liver. The 2 Gy dose caused mean increases over control levels of 33.9% after one day and 121.5% after 7 days. Much higher increases were observed with the 6 Gy dose reaching 404.3% after one day and 555.4% after 7 days. The percentage changes were apparently high because the levels in the control group were lower than what was seen in the liver. Such control level was even lower in the testicles. The 2 Gy dose caused increases of 46.0% and 144.4% one and seven days post-irradiation. With the 6 Gy the calculated values were 6.5-fold and 9.3-fold the control in these 2 time points.

The effects of x-rays on IL-6 paralleled those of IL-1. One day after irradiation with 2 Gy the level of IL-6 in the liver was more than 3-fold the control value followed by a substantial increase to more than 10-fold that of control after 7 days. Irradiation with 6Gy gave higher values. The levels of IL-6 in the liver tissue, after one day and 7 days, were more than 8-fold and 16-fold that of control respectively. IL-6 in the brain showed a substantial relative increase as a result of x-irradiation. With the 2 Gy such increases were 521.8% after one day and 919.4% after 7 days. With the 6 Gy IL-6 reached levels more than 12-times the control value after one day and more than 17-times after 7 days. In mouse testicles, one day after the 2 Gy dose an increase of 88.2% of IL-6 was detected, that went up to 160.3% above control after 7 days. The effect of the 6 Gy was greater as IL-6 reached levels about 5.4-fold and 9.7-fold the control, one and seven days after irradiation respectively. These results indicated that the effects of radiation were not momentary, but were progressive with time. The reported effects of cytokines in case of irradiation are conflicting. Several possible mechanisms of radioprotection have emerged including reduction of oxidation damage and apoptosis. In contrast, sensitizing mechanisms may include increased oxidative damage, which may occur in absence of scavenger induction, as well as enhanced apoptosis.

The rise in the pro-inflammatory interleukins was couples with concomitant increase in the anti-inflammatory IL-10. The pattern of change in IL-10 was the same as seen with the other determined interleukins. In the liver, exposure to the 2 Gy radiation dose caused increases, above the mean control level, of 151.8% after one day and 581% after 7 days. These values were higher after the 6Gy dose reaching 443.6% and 945.5% after one day and 7 days. Although the level in the brain was much lower than in the liver, it showed a large increase as a result of exposure to x-irradiation. At day one after the 2 Gy dose its level was about 3.4-fold that of control and went up to more than 6-fold after 7 days. The increase was even greater following irradiation with 6 Gy, as the level exceeded 7.6-times and 12.1-times that of control after one day and 7 days respectively. The level of IL-10 in the testes also increased as a result of exposure of the animals to x-irradiation. Exposure to the 2 Gy radiation dose caused increases of 86.8% after one day and 581% after 7 days, above the mean control level. These values were higher after the 6Gy dose reaching 6.6-fold and 18.5-fold the control value after one day and 7 days. Such increased production of this anti-inflammatory cytokine may represent an attempt of the affected cells to balance the actions of the pro-inflammatory cytokines or to modulate their effects on other signaling pathways. It is generally recognized that counteraction of the inflammatory response to radiation is important to attenuate acute radiation effects and prevent consequences. It has been proposed that the biological activities of IL-10 in modulating inflammation in this case may be caused, in part, by down-regulation of pro-inflammatory cytokines and the expression of their receptors and up-regulation of cytokine inhibitors.

The disturbance by low-dose x-irradiation was also seen in the changes in the levels of PGE_2. The behavior of this prostaglandin was again similar to what was seen with the interleukins. Both doses of x-rays caused an increase in its level starting on the first day

after irradiation and continued to rise to higher levels on day seven. It is known that cytokines, like IL-1, can increase the production of prostaglandins and it is postulated that the effects associated with the rise in interleukin- 1 may actually be due to induction of PG synthesis.

X-rays causes increased ROS production that result in increased lipid peroxidation. Malondialdehyde , the principal product in this case is generally used as indication of oxidative stress. After one day of irradiation by a 2Gy dose the mean level of MDA was 3.8-fold that of control. Contrary to what was expected, the level decreased by 7.1% after seven days although it was still 3.6-times the control level. The 6 Gy dose gave qualitatively similar results, but quantitatively higher levels. The level of MDA was 5.3-fold that of control after one day and only 2.9-fold after 7 days. Whether the decrease in MDA observed on day seven may be taken as an early sign of recovery or it may be due to excessive damage of cellular membranes causing depletion of fatty acids, particularly arachidonic acid, because of extensive production of interleukins and PGE_2 or due to decreased activity of the oxidative enzymes, needs to be clarified.

The effect of low-dose x-irradiation on glutathione was not as strong as may be expected. There were no statistically significant differences in the results of total glutathione among all the groups of mice as compared to control values. However, there were differences among the values of the reduced and oxidized forms. The effect was relatively higher after one day of irradiation with a tendency toward recovery and going back to near control. Because of the initial reduced and oxidized levels in the control group, the percentage changes in the irradiated groups were apparently large.

It could be clearly seen from the results obtained in this work that treatment with ectoine modulated the biochemical effects induced by x-irradiation. Such modulating action was dependent on frequency of dosing of this compatible solute. The effect after a single dose was variable, sometimes increasing the parameter tested and some other times causing a decrease, as could be seen after one day of treatment. However, repeated administration gave more uniform results. The seven consecutive doses given over one week caused all the tested biochemical parameters to go back to near normal values. This gave a clear indication that probably ectoine needs to accumulate in the cells before it affords protection. It should be noted that ectoine accumulates to molar levels in radio-resistant microorganisms. Accordingly, it should be recommended that ectoine be administered repeatedly to protect against irradiation effects. Whether pretreatment with ectoine would prevent cellular damaging effect of ionizing radiation is worth investigating. If successful, it would afford protection to radiologists and technicians continuously exposed to ionizing radiation whether from the x-ray imaging machines or from ionizing radiation devises for tumor treatment.

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الملخص العربى

تمثل أشعة أكس نوعا من الاشعات التى تؤثر فى معظم الخلايا التى تتعرض لها مما قد يؤدى الى زيادة فى شقائق الأكسوجين الحرة بطريقة مباشرة أو عن طريق تحلل الماء و فى حالة عدم عمل مضادات الأكسدة داخل الخلايا بكامل طاقتها فمن المتوقع زيادة الاجهاد التأكسدى مما قد يسبب زيادة أكسدة الدهون و جزيئات البروتينات اللازمة لعمل الخلية . و كما يمكن للشقائق الحرة تنظيم نشاط البروتينات فإن عملها يتأثر بواسطة السيتوكينات التى تنظم عملها الحالة التأكسدية للخلية عن طريق التأثير فى الانزيمات المشاركة.

و قد قامت استراجية حماية الخلايا فى هذا البحث على استعادة توازن المسارات الكيميائية فى الخلايا بواسطة تقديم الحماية و منع الاضرار التى قد تقع للجزيئات اللازمة لحماية الخلية و أن استخدام المركبات التى تحافظ على الأغشية و تحمى شكل جزئ البروتين فى الفراغ يؤدى الى الحفاظ على عمل المستقبلات و نشاط الانزيمات بما فى ذلك الانزيمات التى لها نشاط مضاد للاكسدة أو اصلاح الاحماض النووية . و هذه الخواص موجودة فى المواد الذاتية المتوافقة بما فى ذلك مركب أكتوين.

و قد أجرى هذا البحث لدراسة تأثير التعرض الحاد للمستويات الضعيفة من أشعة أكس العميقة على الاجهاد التأكسدى فى خلايا الفئران . و قد تم تعيين انتاج الشقائق الحرة و مدى فاعلية مضادات الاكسدة فى الكبد و المخ و الخصية كما تم تقييم العلاقة بين السيتوكينات المسببة للالتهابات و تلك المضادة للالتهابات E_2 و كذلك انتاج بروستا جلاندين

و قد استخدم فى هذا البحث ذكور الفئران البيضاء بتراوح وزنها بين ٢٠ و ٢٢ جم و تم تقسيمها الى المجموعات التالية:

المجموعة ١- من ٦ فئران تم استخدامهم كمجموعة حاكمة سالبة

المجموعة ٢- من ١٢ فأرا تم استخدامهم كمجموعة حاكمة موجبة تم تعريضهم لجرعة أشعة أكس ٢ جراى و تم التضحية بعدد ٦ فئران بعد يوم واحد و ٦ فئران بعد ٧ أيام من التعرض للاشعاع و تم استخدامهم كمجموعتين قابضتين موجبتين لمعرفة تأثير جرعة أشعة اكس الأقل.

المجموعة ٣- مكونة من ١٢ فار كمجموعة حاكمة موجبة تم تعريضهم لمستوى ٦ جراى من أشعة اكس و قد تم التضحية بعدد ٦ فئران بعد يوم واحد و ٦ فئران بعد ٧ أيام

المجموعة ٤- من ١٢ فارا تم تعريضهم لمستوى ٢ جراى من الأشعة تمت معالجة ٦ فئران بجرعات أكتوين ٢٠٠ مجم/كجم و تم التضحية بهم بعد يوم واحد كما تمت معالجة ٦ فئران بنفس الجرعة لمدة ٧ أيام و تمت التضحية بهم فى اليوم السابع.

المجموعة ٥- من ١٢ فارا تم تعريضهم لمستوى ٦ جراى من الأشعة و قد تمت معالجة عدد ٦ فئران بجرعة اكتوين ٢٠٠ مجم/كجم و تمت التضحية بهم بعد يوم واحد كما تمت معالجة ٦ فئران بنفس الجرعة لمدة ٧ أيام و تمت التضحية بهم فى اليوم السابع.

و فى الأوقات المحددة تمت التضحية بالفئران و تشريحهم بسرعة و استخراج الكبد و المخ و الخصيتين و تم حفظهم عند درجة حرارة - ٨٠°م حتى تعيين الاتى:

- انترلوكين ١ و انترلوكين ٦ كمثلين للسيتوكينات المسببة للالتهابات-
- انترلوكين-١٠ كممثل للسيتوكينات المضادة للالتهاب
- مالوندای الدهيد و أشكال جلوتاثيون (المختزلة و المؤكسدة و المجموع)
- E_2- بروستاجلاندين

و قد تبين من النتائج ان تأثيرات تشعيع جسم الفئران بالكامل كانت متشابهه من ناحية الكيف و لكنها مختلفة من ناحية الكم معتمدة فى ذلك على عضو الجسم المعنى و جرعة الاشعاع. حيث وجد أكبر تأثير فى الكبد و بمستوى اقل فى المخ و الخصيتين و كما هو متوقع كان تأثير الجرعة العالية ٦ جراى أكبر من ٢ جراى.

بعد يوم واحد من التعرض لجرعة ٢ جراى من اشعة أكس فقد تبين ان مستوى انترلوكين-١ فى الكبد قد بلغ الضعف تقريبا متزايد الى ثلاثة اضعاف مستوى المجموعة الحاكمة بعد ٧ أيام و كان تأثير جرعة ٦ جراى أكبر حيث تضاعف مستوى انترلوكين- ١ثلاث مرات بعد يوم واحد و تزايد بعد ذلك الى ٦ أضعاف بعد ٧ أيام.

و قد كانت التغيرات فى انترلوكين-١ فى المخ متشابهة مع الكبد حيث تسببت جرعة الاشعاع ٢ جراى فى زيادة حوالى ٣٣.٩% بعد يوم واحد و ٢١% بعد ٧ أيام و قد لوحظ ارتفاعات أعلى بكثير بعد جرعة ٦ جراى بلغت ٤٠٤.٣% بعد يوم واحد و ٥٥٥.٤% بعد ٧ أيام و قد كانت التغيرات ظاهريا كبيرة حيث أن المستوى فى المجموعة الحاكمة أقل مما هو موجود بالكبد و كان هذا المستوى فى المجموعة الحاكمة أقل فى الخصيتين حيث تسببت جرعة الاشعاع ٢ جراى فى زيادة ٤٦% و ١٤٤.٤% بعد يوم واحد و سبعة أيام. و قد تسببت جرعة ٦ جراى فى وصول لمستوى انترلوكين-١ إلى٦.٥ و ٩.٣ ضعفا من مستوى المجموعة الحاكمة فى هذين الوقتين.

و قد كان تأثيرالتشعيع بأشعة اكس على انترلوكين-٦ موازيا لما لوحظ فى انترلوكين-١ حيث ارتفع مستوى انترلوكين-٦ فى الكبد الى اكثر من ٣ أضعاف مستوى المجموعة الحاكمة بعد يوم واحد متبوعا بزيادة كبيرة الى أكثر من ١٠ أضعاف بعد ٧ أيام . وبعد جرعة الاشعاع ٦ جراى كان مستوى انترلوكين -٦ فى الكبد بعد يوم واحد و ٧ أيام اكثر من ٨ أضعاف و ١٦ ضعف مستوى المجموعة الحاكمة على التوالى. أما فى المخ فقد ارتفع تركيز انترلوكين -٦ الى أكثر من ٦ أضعاف و ١٠ أضعاف مستوى المجموعة الحاكمة بعد يوم واحد و سبعة أيام من جرعة الاشعاع . و كما هو متوقع فقد كان تأثير الجرعة الاشعاعية ٦ جراى أكبر بكثير حيث بلغ مستوى انترلوكين-٦ اثنى عشر ضعف مستوى المجموعة الحاكمة بعد يوم واحد و ١٧ ضعفا بعد ٧ أيام. أما فى خصية الفأر فقد كانت الزيادة فى مستوى انترلوكين-٦ من جرعة اشعاع ٢ جراى حوالى ٨٨% و قد ازدادت بعد ٧ أيام الى ١٦٠.٣% فوق المستوى الاصلى. و قد كان تأثير ٦ جراى أكبر حيث بلغ المستوى ٥.٤ و ٩.٤ أضعاف المستوى الاصلى بعد يوم واحد و ٧ أيام من التشعيع.

و قد دلت هذه النتائج على أن تأثير اشعة اكس ليس تأثيرا مؤقتا بل متزايد بمرور الوقت.

وقد تزامن ارتفاع مستوى السيتوكينات المسببة للالتهاب مع زيادة فى انترلوكين-١٠ المضاد للالتهاب. ففى الكبد تسبب تعرض الفئران إلى جرعة إشعاع ٢ جراى فى زيادة انترلوكين-١٠ فوق مستوى المجموعة الحاكمة السلبية بمقدار ١٥١.٨% بعد يوم واحد و٥٨١% بعد ٧ أيام وقد كانت هذه الزيادات أعلى بعد التعرض لجرعة إشعاع ٦ جراى حيث بلغت ٤١٣.٦% و ٩٤٥.٥% بعد يوم واحد و٧ أيام على التوالى.

وعلى الرغم من أن المستوى فى المخ كان أقل بكثير من الكبد فقد تسبب التعرض لأشعة اكس فى زيادة ملحوظة فبعد يوم واحد من التعرض لجرعة إشعاع ٢ جراى بلغ مستوى (انترلوكين- ١٠) ٣.٤ أضعاف مستوى المجموعة الحاكمة وتعاقبت الزيادة إلى أكثر من ٦ أضعاف بعد ٧ أيام. وقد كانت الزيادة أكبر بعد جرعة الإشعاع ٦ جراى حيث تخطى المستوى ٧.٦ أضعاف بعد يوم واحد و ١٢.١ ضعفا بعد ٧ أيام كما ارتفع مستوى انترلوكين- ١٠ فى الخصية بعد التعرض لجرعة ٢ جراى من أشعة اكس بمقدار ٨٦.٨% بعد يوم واحد و٥٨١% بعد ٧ أيام. وقد كانت هذه المستويات أعلى بعد التشعيع بجرعة ٦ جراى بالغين ٤٤٣.٦% و ٩٤٥.٥% بعد يوم واحد و٧ أيام على التوالى.

قد تمثل محاولة من الخلايا المصابة لمعادلة تأثير السيتوكينات المسببة للالتهابات. حيث أن من المعروف ان رد الفعل المضاد للالتهابات الناتجة عن الإشعاع مهم لتثبيط تأثيرها الحاد ولمنع المضاعفات.

وقد تم اقتراح أن تأثير انترلوكين-١٠ البيولوجى فى تغير مستوى الالتهاب الناتج عن الإشعاع فى هذه الحالة قد ينتج جزئيا عن تثبيط تركيزات السيتوكينات المسببة للالتهابات وتكوين مستقبلاتهم وزيادة مثبطات السيتوكينات.

وقد لوحظ كذلك أن الاضطرابات الناتجة عن الجرعات المتدنية من أشعة اكس قد تسببت فى تغيرات فى مستوى بروستاجلاندين E2. وقد كانت هذه التغيرات مشابهة لما تمت ملاحظته مع مركبات انترلوكين حيث تسببت جرعة أشعة أكس فى ارتفاع مستوياتها بدءا من اليوم الأول بعد التشعيع مع استمرار الارتفاع لمستويات أعلى فى اليوم السابع.

ومن المعروف أن السيتوكينات مثل انترلوكين-١ تسبب زيادة فى إنتاج البروستاجلاندينات ومن المفترض أن التأثيرات المصاحبة لارتفاع مستوى انترلوكين-١ قد تكون فى الواقع ناتجة عن زيادة تصنيع البروستاجلاندينات.

وتتسبب أشعة اكس فى زيادة إنتاج شقائق الأكسجين الحرة مما يؤدى إلى ارتفاع أكسدة الدهون ويستخدم مركب مالون داى ألدهيد المنتج الرئيسى فى هذه الحالة كمؤشر للإجهاد التأكسدى. وقد كان مستوى مالون داى ألدهيد حوالى ٣.٨ أضعاف المجموعة الحاكمة بعد يوم واحد من التشعيع بجرعة ٢ جراى. وعلى عكس ما كان متوقعا فقد انخفض المستوى بحوالى ٧.١% بعد ٧ أيام بالرغم من أنه مازال ٣.٦ أضعاف مستوى المجموعة الحاكمة. وقد أعطت جرعة ٦ جراى نتائج مشابهة من ناحية الكيف ولكن أعلى من ناحية الكم حيث بلغ مستوى مالون داى الدهيد ٥.٣ أضعاف المجموعة الحاكمة بعد يوم واحد و٢.٩ أضعاف بعد ٧ أيام. ولعل هذا الانخفاض الملحوظ فى اليوم السابع قد يكون علامة مبدئية للتعافى أو ربما يكون ناتجا عن هدم متزايد لأغشية الخلايا متسببا فى نضوب الأحماض الدهنية خاصة حمض أراكيدونيك وذلك بسبب الإنتاج المتزايد للانترلوكينات والبروستاجلاندينات أو بسبب انخفاض نشاط الإنزيمات المؤكسدة وهذا يحتاج إلى دراسة مستقبلية.

لم يكن تأثير الجرعات المنخفضة من أشعة أكس على الجلوتاثيون بالقوة المنتظرة حيث لم يكن هناك فروق معنوية فى نتائج الجلوتاثيون الكلية فى كل المجموعات مقارنة بالمجموعة الحاكمة. ولكن كانت هناك فروق فى مستويات الجلوتاثيون المختزلة وكذلك المؤكسدة. وكان التأثير أكبر بعد يوم واحد مع الميل للعودة نحو مستويات المجموعة الحاكمة بعد ٧ أيام.

وتدل نتائج هذا البحث على أن العلاج بمادة اكتوين كان ذو تأثير واضح على التغيرات التى أحدثها التشعيع بواسطة أشعة اكس وهذا التأثير كان معتمدا على مدى تكرار العلاج بهذه المادة. حيث كان التأثير متباينا بعد جرعة واحدة ولكن تكرار الجرعات أعطى نتائج متشابهة. وقد تسبب إعطاء ٧ جرعات يومية فى خلال أسبوع فى عودة المتغيرات التى تم تعيينها إلى مستويات قريبة من مستويات المجموعة الحاكمة. وقد أعطت هذه النتائج انطباعا واضحا أن مادة اكتوين تحتاج إلى الوصول إلى تركيزات عالية فى الخلايا لتتسبب فى حمايتها.

وفى هذا الصدد يجب ملاحظة أن مادة اكتوين تتجمع إلى تركيزات عالية فى الأحياء الدقيقة المقاومة للإشعاع. وعلى ذلك يمكن التوصية بالتناول المتكرر لمادة اكتوين حتى نحصل على تأثيرها الواقى من الإشعاع. وكذلك التوصية بإجراء بحوث مستقبلية لمعرفة هل العلاج المسبق باكتوين يحمى من تأثير الإشعاع المؤين. وفى حالة نجاح ذلك يمكن استخدامه لحماية الأطباء ومعاونيهم الذين يتعرضون باستمرار للإشعاع المؤين سواء من التصوير بأشعة أكس أو الإشعاع المستخدم فى علاج السرطان.

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معهد البحوث الطبية
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رسالة علمية

مقدمة إلى معهد البحوث الطبية– جامعة الإسكندرية
ايفاءا جزئيا لشروط الحصول على درجة

الماجستير

فى

الاقربازين والعلاج التجريبي

مقدمة من

داليا عادل على محمود سرحان

بكالوريوس العلوم الصيدلية
جامعة الإسكندرية، ٢٠٠٢

معهد البحوث الطبية
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للحصول على درجة
الماجستير

فى

الاقربازين والعلاج التجريبي

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