ORIGINAL ARTICLE

N-acetylcycsteine attenuates the deleterious effects of radiation therapy on incisional wound healing in rats

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Abstract

Background: During preoperative radiotherapy, effective doses of ionizing radiation occasionally cause wound complications after subsequent surgery. This study was designed to determine the effects of intraperitoneally or orally administered N-acetylcysteine (NAC) on anastomotic healing of irradiated rats.

Material & Methods: Forty Wistar albino rats were randomized into four groups containing 10 rats each. A 3 cm long surgical full-thickness midline laparotomy was performed to all groups (Groups 1-4). Group 1 was designed as a control group without radiation therapy and NAC treatment. Groups 2, 3 and 4 received a single abdominal dose of 10 Gy irradiation before laparotomy and groups 3 and 4 received oral and intraperitoneal NAC, respectively.

Results: Group comparisons demonstrated that breaking strength was significantly higher in NAC treated rats. A statistically significant difference was determined in terms of superoxide dismutase (SOD), malondealdehyde (MDA) and glutation (GSH) values between groups ($p<0.001$). Nevertheless, advanced oxidation protein products (AOPP) levels were found to be similar between groups ($p=0.163$). Serum GSH and SOD levels were significantly higher in groups 3 and 4 when compared to group 2 ($p < 0.05$). Similarly, there was a significant increase in serum MDA concentration, predicting lipid peroxidation, in group 2 when compared to groups 1, 3 and 4 ($p < 0.05$). There was not a significant difference between Groups 3 and 4 regarding GSH, MDA, SOD, and AOPP levels. Histopathological analysis revealed that NAC administration, either orally or intraperitoneally, leads to a better incisional healing in terms of inflammation, granulation, collagen deposition, reepithelization and neovascularization.

Conclusion: The present study supports the hypothesis that NAC administration alleviates the negative effects of radiotherapy on incisional wound healing by means of reducing oxidative stress markers and improving histologic parameters independent of the route of administration. Hippokratia 2014; 18 (1): 17-23.

Keywords: N-acetylcysteine, ionizing irradiation, radiotherapy, incisional healing, lipid peroxidation

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Introduction

Currently, radiation therapy is a well-established treatment modality in various malignant diseases as an adjuvant or neoadjuvant setting. Although the main aim of radiation therapy is to kill cancerous cell lines, it also damages healthy cells. Adverse effects of radiation are very well-known depending on the sensitivity of the body sites being treated, the volume of normal tissue irradiated, the rate of dose accumulation and the total dose. The skin is particularly affected by radiation damage. Side effects are more evident in rapidly proliferating tissues, such as skin or mucosa. Radiation-induced epithelial injury causes inflammatory responses in the underlying supportive tissue. Ionizing radiation also leads to the generation of free radicals and cytotoxic peroxides, which damage DNA, proteins and membranes. One of the most dreaded consequences is its deleterious effects on wound healing[1]. Wound healing in irradiated tissue constitutes a great surgical and clinical challenge leading to defects on oncologic treatment protocols[2]. Despite ongoing research, radiation damaged tissue remains an impending catastrophe. Wound healing problems are characterized by decreased wound strength, decreased collagen deposition and decreased angiogenesis. The consequences of these

problems may lead to delayed or non-healing ulcerations (radiation ulcers), superimposed infections, carcinogenesis and cosmetic failure. These complications are due to the combination of radiation-induced DNA mutations, microvascular damage and fibrosis.

N-acetylcysteine (NAC) is a thiol containing small molecule with antioxidant properties and has been in clinical practice primarily as a mucolytic agent. As a consequence of its diverse biological effects, oral, intravenous and intraperitoneal routes of administration have been the focus of experimental and clinical research[3]. Moreover, NAC exerts positive effects on nephrotoxicity, hepatotoxicity, ischemia/reperfusion injury, and radiation damage[4-8]. The literature highlights the positive outcome on wound healing in diabetic patients together with ischemic and irradiated anastomosis[9-11]. Moreover, the effect of NAC on wound healing following abdominal incisional trauma after radiation therapy has not been experimentally verified. Therefore, the present study was designed to evaluate the effect of oral and intraperitoneal administration of NAC on the histological, biochemical and mechanical parameters of abdominal incisional wound healing in an irradiated rat model.

Material and Methods

Animals

The surgical procedure, use of anesthesia, and animal care methods in the experiments were consistent with the guidelines in the National Institute of Health's Guide for the Care and Use of Laboratory Animals (National Institutes of Health publication no. 86–23, revised 1985, Bethesda, MD). The animals were housed in wire bottom cages at room temperature with a 12-hr light/dark cycle. They were fed standard laboratory diet until the night before the operation and had access to water ad libitum. Care was taken to avoid unnecessary stress and discomfort to the rats throughout the experimental period. Forty female adult Wistar albino rats weighing 280-320 g were included in the study and allocated into four groups of 10 each, randomly. A 3 cm long surgical full-thickness midline laparotomy was performed to all groups (Figure1) (Groups 1-4). Group 1 was designed as a control group without radiation therapy and NAC treatment. Groups 2,3 and 4 received irradiation before laparotomy. Oral and intraperitoneal NAC (300mg/kg/d) (Asist, Husnu Arsan Ilac, Istanbul, Turkey) were administered to groups 3 and 4 after irradiation, respectively. The average weight and behavior of the animals did not differ significantly between the beginning and the end of the study. All rats were sacrificed at postoperative day 4 by means of intracardiac puncture.

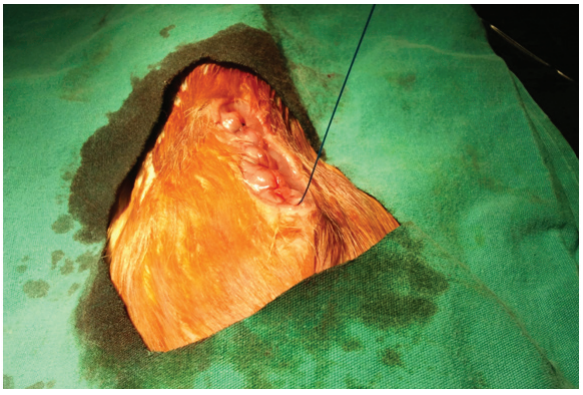

Figure 1: The method of continuous suturing of the abdomen.

Irradiation

Radiotherapy was administered to the groups 2-4, one week before the surgical procedure. The rats were lightly anesthetized before irradiation using ketamine hydrochloride 50 mg/kg (Ketalar; Parke Davis, Eczacibasi, Istanbul, Turkey) and xylazine 5 mg/kg (Rompun; Bayer AG, Leverkusen, Germany). A single abdominal dose of 10 Gy RT was performed using a 6 MV linear accelerator with source axis distance (SAD) technique. The source–axis distance was 100 cm and irradiation target fields were 5 × 5 cm pelvic regions. The other parts of the abdomen were spared by means of lead shields (Figure 2).

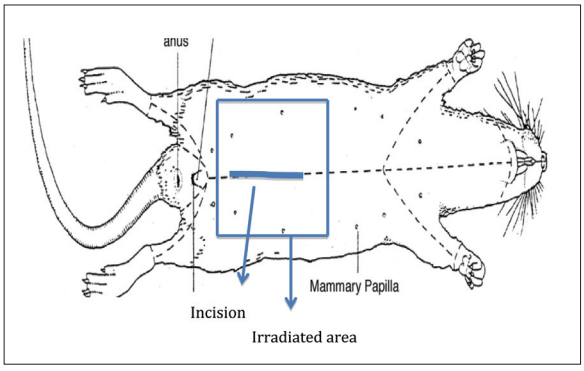

Figure 2: The fields of Radiotherapy on rat's abdomen.

Surgical procedure

All of the surgical procedures were performed under sterile conditions. After an overnight fast, all rats were anesthetized by an intramuscular injection of ketamine hydrochloride 50 mg/kg (Ketalar) and xylazine 5 mg/kg (Rompun). Animals were allowed to breathe spontaneously during the surgery. A heating lamp was used to preserve the body temperature at approximately 37°C. Postoperative dehydration was avoided by injecting 5 ml Ringer's lactate solution subcutaneously. The abdomen was shaved and prepared with povidone-iodine (Betadine®, Kansuk, Istanbul, Turkey) and covered in a sterile fashion. A 3-cm long full thickness incision (including peritoneum), simulating lower midline laparotomy was performed with a scalpel, and was afterwards closed in a continuous fashion with running 3/0 prolene sutures in all rats by the same surgeon. All rats were given water and a regular diet ad libitum on the day of the operation.

Incisional breaking strength measurement

Necropsy was performed on postoperative day 4, by means of intracardiac puncture and blood samples were taken. Three surgeons, who were blinded to the groups, performed tensile strength measurement according to the method described by Atkinson et al[12]. Incisional breaking strength was defined as the pressure (mm Hg) that caused the incision line to separate. Then, the entire incision line together with a 1 cm wide intact abdominal wall layer was excised longitudinally in an en-bloc fashion and divided into two equal pieces. One piece of this sample was fixed in 10% formalin solution for histopathologic examination. The remaining piece, wrapped in aluminum foil, was kept in a biochemistry laboratory for tissue hydroxyproline (OHP) measurement.

Determination of OHP Level

After weighing, tissue samples were frozen (by bedside liquid nitrogen), lyophilized, and pulverized. Twenty-five-microliter samples taken from hydrolyzation were lyophilized and soluted in the 1 ml 50% (v/v) isopropyl alcohol. Chloramine-T was added to these samples 10 min later. Then, they were incubated for 90 min at 50°C after adding 1 ml Erlich's reagent. A color change after the reaction was evaluated under 560-nm wavelength spectrophotometer. Under the same conditions, OHP standards with 0.2, 0.4, 0.6, 0.8, 1.2, and 1.6 mg were also studied. Sample concentrations were calculated with the help of standard curve. Results were calculated as micrograms per milligram of wet tissue[13].

Biochemical Analysis

Blood samples were centrifuged at 3,000 rpm for 10 min and serum aliquots were stored at -80°C for further examination. Oxidative stress and lipid peroxidation were determined by measuring serum malondealdehyde (MDA), glutathion (GSH), advanced oxidation protein products (AOPP), and superoxide dismutase (SOD). GSH, a multifunctional intracellular nonenzymatic antioxidant, is critically important for detoxifying an array of toxic substances, including peroxide compounds, and other free radical generating molecules. The GSH concentrations of the serum were measured using the method described by Ellman[14]. The results were expressed as μmol/l. The serum MDA assay was performed as described by Yoshioka et al[15]. MDA, a product of lipid peroxidation, reacts with thiobarbituric acid under acidic conditions at 95°C to form a pink-colored complex with an absorbance maximum at 532 nm. The results were expressed as nmol/ml. The products of the oxidative modification of proteins are known as AOPP. The serum AOPP assay was performed by modification of Witko-Sarsat's method[16]. The results were expressed as μmol/l of chloramine-T equivalents. SOD is an important enzyme in the neutralization of free oxygen radicals. SOD activity was measured in serum samples as previously described by Sun et al[17]. The principle of the method is based on the inhibition of nitroblue-tetrazolium (NBT) reduction by the xanthine–xanthine oxidase system as a superoxide generator. One unit of SOD was defined as the enzyme amount causing 50% inhibition in the NBT reduction rate. The results were expressed as U/ml.

Histotopathological Analysis

The samples for histology were dehydrated and embedded in paraffin. From all paraffin blocks, 5 μm sections were cut, and staining was performed with hematoxylin and eosin. An expert pathologist blinded to experimental groups sampled the specimens for examination. Five high power fields were evaluated per each region. Histologic grading was performed according to the wound-healing histologic scoring system described by Abramov et al[18].

Statistical analysis

Statistical analyses were performed with SPSS 18.0 software (SPSS Inc., Chicago, IL, USA). Results were expressed as median (minimum-maximum). Differences among the groups were analyzed by the Kruskal-Wallis test. Dual comparisons among groups with significant values were evaluated with the Bonferroni adjusted Mann-Whitney U-test. A p value of less than 0.05 was considered to be statistically significant.

Results

Incisional breaking strength and tissue OHP levels

All animals survived throughout the experimental procedure with no complications. The mean ± SD values of wound breaking strength (WBS) and OHP levels of abdominal wounds with the statistical comparisons of the groups are demonstrated in Table 1. The OHP content of the wounds in NAC treatment groups (Group 3-4) was found to be superior to the groups 1 and 2 with statistical significance ($p < 0.001$). The highest levels of OHP were determined after intraperitoneal NAC administra-

Table 1: Comparison of the study groups in terms of wound-breaking strength and tissue hydroxyproline (OHP) levels. Values are expressed as mean ± standard deviation.

	Group 1	Group 2	Group 3	Group 4
Breaking pressure (mm Hg)	99.37 ± 4.13	66.12 ± 9.4 ^{a}	105.3 ± 9.04	107.2 ± 9.77
OHP (μg/mg tissue)	0.62 ± 0.03	0.5 ± 0.06	1.23 ± 0.2 ^{b}	1.08 ± 0.8 ^{b}

$p < .05$ was considered statistically significant, Group 1: Control group, Group 2: Radiation therapy group, Group 3: Radiation therapy plus oral NAC administration group, Group 4: Radiation therapy plus intraperitoneal NAC administration group, ^{a}: compared with groups 1, 3 and 4 ($p < 0.001$), ^{b}: Compared with groups 1 and 2 ($p < 0.001$).

tion whereas the lowest in group with radiation therapy only (p<0.001). The comparison in terms of OHP values between oral and intraperitoneal NAC administration after irradiation revealed an incline favoring intraperitoneal NAC treatment without statistical significance (p>0.05). In Group 1 (without treatment or RT) OHP values were found to be higher than radiation therapy group (Group 2), but lower than both NAC treatment groups (Groups 3-4). Similarly, a statistically significant difference was determined between groups in terms of wound breaking strength (p<0.001). The mean ± SD values of pressures (mm Hg.) predicting wound breaking strength were significantly higher in NAC treatment groups (Group 3-4) when compared to control (Group 1) and radiation therapy only (Group 2) (p<0.001). In accordance with OHP levels, the highest-pressure values were obtained in intraperitoneal NAC administration whereas the lowest in group with radiation therapy only. The breaking strength for midline incision after radiation therapy were significantly decreased when compared to the control and NAC treatment groups (p<0.001). Moreover, both routes of NAC treatment improved breaking strength after irradiation according to the pressure data.

Histological Analysis

Acute inflammation score for Group 2 was significantly higher than that of the control (Group 1) and NAC treatment groups (p=0.002). A statistically significant decrease in acute inflammation score was detected after NAC administration either orally or intraperitoneally when compared to group 2 (p<0.001). There was also a statistically significant difference among groups in terms of chronic inflammation (p=0.006). Chronic inflammation scores were found to be significantly higher in group 2 when compared to group 4 (p=0.006). The difference among the groups in terms of the amount of granulation tissue on POD 4 was statistically significant (p=0.004). The highest scores were obtained in Group 2, as expected. Granulation tissue fibroblast maturation in the group 4 was higher than that of the other groups, with the lowest scores in group 2 (p= 0.001). A statistically significant difference was determined between groups in terms of collagen deposition (p<0.001). Collagen deposition was significantly decreased in Group 2 and a gradual increase was determined in Groups 3 and 4 (p<0.001). A statistically significant decrease in reepithelialization scores was found in Group 2, when compared to other groups (p=0.001). There was also a significant difference between groups 3 and group 4 (p=0.001). Reepithelialization score was highest in group 4. A significant difference was determined among the four groups regarding neovascularization (p<0.001), with the highest scores in group 3 and the lowest in group 2 (p=0.006). Nevertheless, the difference was not significant between groups 1, 3 and 4, in terms of neovascularization (p>0.05). Comparisons of histological scores of four groups are shown in Table 2. A linear correlation was determined between breaking pressure values and histologic parameters including collagen deposition, fibroblast maturation, reepithelielization and neovascularization. Acute inflammatory scores and gran-

Table 2: Comparison of the study groups in terms of histopathological analysis according to Abramov's histologic scoring system (range 0-3 for each parameter). Values are expressed as mean ± standard error of the mean (SEM).

	Group 1	Group 2	Group 3	Group 4	P value
Acute inflammation	1.87 ± 0.39	3	2 ± 0.25	1.1 ± 0.34	p=0.002
Chronic inflammation	2.12 ± 0.22	2.5 ± 0.18	1.9 ± 0.17	1.4 ± 0.16	p=0.006
Granulation tissue amount	2.25 ± 0.25	3	2.4 ± 0.22	1.9 ± 0.17	p=0.004
Fibroblast maturation	1.25 ± 0.16	0.37 ± 0.18	1.5 ± 0.16	2 ± 0.25	p=0.001
Collagen deposition	1.37 ± 0.18	0.12 ± 0.12	1.5 ± 0.16	2 ± 0.25	p<0.001
Reepithelialization	2 ± 0.26	0.62 ± 0.18	1.7 ± 0.26	2.4 ± 0.22	p=0.001
Neovascularization	1.87 ± 0.12	1 ± 0.18	2.1 ± 0.1	1.9 ± 0.1	p<0.001

p < .05 was considered statistically significant, Group 1: Control group, Group 2: Radiation therapy group, Group 3: Radiation therapy plus oral NAC administration group, Group 4: Radiation therapy plus intraperitoneal NAC administration group.

Table 3: Comparison of the study groups in terms of biochemical parameters. Values are expressed as mean ± standard deviation.

	Group 1	Group 2	Group 3	Group 4
SOD (U/ml)	12.55 ± 2.87	10.6 ± 1.81	21.57 ± 2.66 ^{a}	18.72 ± 3.2 ^{a}
MDA (nmol/ml)	12.5 ± 1.19	26.5 ± 6.25 ^{b}	12.4 ± 2.71	13.6 ± 1.64
GSH (µmol/l)	0.24 ± 0.08	0.16 ± 0.02	0.47 ± 0.07 ^{a}	0.43 ± 0.05 ^{a}
AOPP (µmol/l)	16.75 ± 3.6	15.5 ± 1.06	16.7 ± 2.83	18 ± 3.2

p < .05 was considered statistically significant, Group 1: Control group, Group 2: Radiation therapy group, Group 3: Radiation therapy plus oral NAC administration group, Group 4: Radiation therapy plus intraperitoneal NAC administration group, SOD: superoxide dismutase, MDA: malondealdehyde, GSH: glutation, AOPP: advanced oxidation protein products, ^{a}: compared with groups 1 and 2 (p < 0.001), ^{b}: Compared with groups 1,3,4 (p < 0.001).

ulation tissue amount were found be inversely correlated with breaking pressure values.

Biochemical analysis

A statistically significant difference was determined in terms of SOD, MDA and GSH values between groups ($p<0.001$). Nevertheless, AOPP levels were found to be similar between groups ($p=0.163$). Serum GSH and SOD levels were significantly higher in groups 3 and 4 when compared to group 2 ($p < 0.05$). Similarly, there was a significant increase in serum MDA concentration, predicting lipid peroxidation, in group 2 when compared to groups 1,3 and 4 ($p < 0.05$). There was not a significant difference between Groups 3 and 4 regarding GSH, MDA, SOD, and AOPP levels ($p > 0.05$). The results of biochemical analysis are demonstrated in Table 3.

Discussion

Wound healing problems after neoadjuvant radiation is a matter of fact that is really difficult to deal with[19]. Consequently, agents with the potential to reverse radiation damage to healthy tissue attract great enthusiasm over researchers. The hypothesis of the present experimental study was to evaluate the effects of oral or intraperitoneal NAC administration on abdominal wall wound healing process after irradiation. The wound healing process in tissue samples obtained from irradiated rats with or without NAC treatment and from healthy controls was compared for breaking strength measurements, tissue OHP levels, histological characteristics and biochemical parameters. Administration of NAC either orally or intraperitoneally seemed to improve the healing process of incisional wounds in irradiated animals.

Wound healing is an active harmonic process with multiple phases. Optimal tissue regeneration could be reestablished only if these phases follow a predictable sequence. The impairment of the exact timeline and cell population during healing period may lead to inappropriate consequences. Repetitive radiation injury disrupts this highly organized sequence of events, resulting in repetitive inflammatory responses and ongoing cellular regeneration[20]. The early effects of radiation may disrupt the inflammatory and proliferative phases of wound healing[19]. Accordingly, the presented experimental model was designed to evaluate the early effects of radiation therapy on POD 4 together with NAC administration.

Factors promoting wound healing by an induction of collagen deposition have been shown to be reduced in irradiated wounds of experimental animals[21, 22]. The other proposed mechanism of action of radiation injury is associated with its effects on oxidative stress pathways. Oxidative stress has been demonstrated to be an important factor to impair wound healing process in literature[23]. From this point of view, we aimed to evaluate the potential of NAC treatment to reverse radiation-induced injury on wound healing in terms of tensile strength, histologic analysis and biochemical parameters.

Two parameters are of paramount importance to evaluate the healing process and to examine the tissue repair. One was the tensile strength of the tissues. The other parameter was OHP levels reflecting tissue collagen concentration[24,25]. In the present study, WBS and tissue OHP levels for the laparotomy incision after radiation therapy were significantly higher in NAC treated rats than other groups. In accordance with literature, WBS was found to be significantly decreased after radiation therapy[20]. NAC treatment both orally and intraperitoneally caused an increase in WBS, with statistical significance ($p<0.05$). Similarly, tissue OHP level, predicting collagen amount of the wounds, was determined to be significantly decreased in irradiated wounds and displays significant increase after NAC administration. All these data support the hypothesis that radiation injury plays an important role in early phase of wound healing by means of decreasing collagen deposition with a clinical impact of decreased tensile strength and NAC treatment alleviates this effect of irradiation.

Collagen is major structural protein providing biochemical strength to tissue. During wound healing, fibroblasts deposit increasing amounts of fibrillar collagen, which is an important determinant for the development of the wound's strength and tissue integrity. Collagen deposition by skin fibroblasts has been demonstrated to start within 3-5 days after injury and to continue for several weeks[26]. Abramow et al demonstrated that collagen deposition displays a sharp incline within first 4 days after injury in abdominal wounds[18]. NAC has been reported to increase collagen deposition in normal and diabetic wound healing[10]. In accordance with these data, the current study demonstrated a progressive increase in collagen deposition in standard laparotomy incisions on the fourth postoperative day. The significant decrease after radiation therapy was determined to be reversed by means of NAC treatment.

Oxidative damage has long been investigated as an adjunct in impaired wound healing, particularly under ischemic circumstances. Radiation therapy causes oxidative stress in healthy tissue by means of increasing the amount of free radicals. The antioxidant enzymes such as SOD counteract oxidative damage by means of scavenging free radicals. Doctrow et al reported that SOD mimetics promotes wound healing in irradiated rat skin[27]. The positive effect of NAC on oxidative damage has previously been attributed to its free radical scavenging properties[28,29]. In these studies, NAC exerts antioxidant action after ischemia / reperfusion injury by means of decreasing lipid peroxidation and increasing antioxidant enzymes. Moreover, Gulbahar et al. demonstrated that NAC administration before radiation exposure decreases protein oxidation and lipid peroxidation in the brain tissue[30]. The radio-protective effect of NAC on γ-radiation induced toxicity in hepatic tissue has been reported by Mansour et al. by measuring MDA, as an index of lipid peroxidation, SOD, glutathione peroxidase and reduced glutathione[31]. NAC has been shown to alleviate radiation induced oxidative damage in

liver homogenate and in serum similar to radio-protector agents in clinical practice[32]. In the current study, SOD activities together with MDA, AOPP, and GSH levels, were analyzed to predict the oxidative stress in terms of lipid peroxidation, protein oxidation, and thiol-redox status of the tissue. The data revealed that NAC served as an antioxidant in the experimental laparotomy wound after irradiation and prevents lipid peroxidation by decreasing MDA levels together with potentiating free radical scavenging activity by increasing SOD and GSH levels. Nevertheless, no statistically significant difference was determined between study groups in terms of AOPP levels.

It is a well-known fact that the gold standard method to verify regular wound healing process is histologic assessment. Nevertheless, quantitative evaluation of wound healing is challenging as a result of its complex nature with several physiologic processes taking place within overlapping time frames. Moreover, every phase consists of multiple cellular or humoral determinants that should be in perfect order for proper healing. In order to achieve more realistic results, Abramow's modified histologic scoring system was performed for the presented study [18]. Acute inflammation was reported to peak at post-wounding day 4, while chronic inflammation was determined to peak at days 4-7 in the abdomen[18]. According to the design of the current study that the specimens were obtained on the fourth postoperative day, the results of acute inflammation seemed to be more meaningful, since the preferred time point was earlier than the initiation and completion of chronic inflammation. Acute inflammatory scores were found to be significantly increased after irradiation, as expected ($p<0.05$). The addition of NAC treatment, either orally or intraperitoneally, declined acute inflammatory scores to the level of control group and the data confirmed no statistically significant difference at the laparotomy site in terms of acute inflammation between controls and irradiated rats after NAC treatment. Chronic inflammatory scores were highest in the irradiated rats. This was an expected end point as a result of experimental design that the sacrification time was postoperative day 4, aiming to evaluate early effects of irradiation not the chronic. Granulation tissue amount was abundant in all irradiated rats without NAC treatment. This score was similarly declined in rats treated with NAC after radiation therapy and reached to the scores of control group. Fibroblast maturation scores were significantly decreased in irradiated wounds. NAC administration seemed to improve fibroblast maturation up to the scores of control rats. One of the most significant differences was determined in terms of collagen content of the wounds among the study groups. The collagen deposition was nearly nill in irradiated wounds. The administration of NAC improves collagen deposition scores higher than the control group. This data might suggest that one of the most crucial mechanisms of NAC action over healing process in irradiated abdominal wounds was improving collagen structure. This is in accordance with the biochemical analysis verifying higher OHP levels in wounds of irradiated rats with NAC treatment. Reepithelialization in dermal tissues was shown to occur within 24-48 hours after wounding when spurs of epithelial cells moved from the wound edges along the cut margins of the dermis[33]. Reepithelialization establishes an external barrier to the regenerating wound and serves as a biologic occlusive dressing. In the present study, radiation exposure resulted in significant decrease in reepithelialization scores, whereas NAC treatment seemed to reverse this effect and improve scores up to the control group. The last histologic parameter evaluated in the current study to predict wound healing was neovascularization. Neovascularization is one of the most important components of the healing process that includes branching of adjacent blood vessels as well as recruitment of endothelial progenitor cells[34]. Angiogenesis is a must for proper healing process and any factor compromising neovascularization may lead to impaired wound regeneration. Diabetes is a systemic disease exerting negative impact on healing process with various mechanisms, one of which is inadequate angiogenic response[35]. Recently, Aktunc et al reported that NAC improves wound healing in a diabetic mouse model of incisional wounds by promoting angiogenesis[10]. In the present study, the comparison in terms of neovascularization scores revealed a significant difference between irradiated laparotomy incisions with and without NAC treatment. Independent of the administration route, NAC seemed to improve angiogenesis after radiation therapy.

Similar to any other experimental study, several limitations should be considered with regard to this research. One of the most crucial disadvantages of the experimental design is the difficulty to reflect its results to clinical practice. While wound healing process includes multiple phases and radiation therapy causes early and late side-effects over healing, the presented study aimed to evaluate the effects of NAC administration on radiation induced damage of incisional wound healing in inflammatory and early proliferative phase. Accordingly, the late side effects of radiation therapy together with NAC treatment on incisional wound healing might be the topic of further investigations. Moreover, various radiation doses with different NAC administration routes at multiple time-points during the wound healing process and the underlying mechanisms responsible for impaired wound healing after irradiation with possible cellular or humoral pathways accounting for the positive effects of NAC over irradiation damage were all beyond the scope of this study and should be considered in future research.

In conclusion, the presented study verifies the previous results of the experimental research stating the negative impact of radiation therapy on incisional wound healing process. Moreover, the data determines that NAC, with diverse biological effects, possesses the ability to reverse some of the deleterious effects of irradiation and promotes healing parameters independent of the route of administration at inflammatory and early proliferative phase of healing. Since radiation induced tissue damage is a consequence of multiple mechanisms, NAC seems to act on

oxidative stress markers, generation of collagen synthesis, progression of reepithelialization and stimulation of neovascularization coupled with an inhibition of overinflammation and radiation induced fibrosis. Although the precise cellular mechanisms by which NAC enhances incisional wound healing after radiation therapy is not clear, both antioxidant and anti-inflammatory effects might be responsible for overall favorable clinical outcome with improved WBS verified by tissue collagen amount.

NAC seems to alleviate oxidative stress by means of decreasing lipid peroxidation and increasing free radical scavenging activity, improve collagen synthesis, inhibit radiation induced fibrosis and overinflammation coupled with an upregulation of neovascularization, producing an overall favourable clinical outcome of a higher WBS.

Conflict of interest

None.

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