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. 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.

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 malondialdehyde (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. 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. 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. 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.

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 and 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 administration.

| 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                          |
| Wound-breaking strength (mm Hg) | 99.37 ± 4.13                     | 66.12 ± 9.4                      | 105.3 ± 9.04                     | 107.2 ± 9.77                     |
| OHP (μg/mg tissue)              | 0.62 ± 0.03                      | 0.5 ± 0.06                       | 1.23 ± 0.2                       | 1.08 ± 0.8                       |

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, *p < 0.0001, †p < 0.001, ‡p < 0.0001, ††p < 0.001, †‡p < 0.0001, †††p < 0.0001.

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The results were expressed as mmol/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. 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. 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.