Tissue hydroxyproline measurement

Quantification of collagen in enteric anatomosis is synonymous with quantification of hydroxyproline, an amino acid unique to collagenous proteins in most tissues. Stored tissues were cleared from anastomotic materials. Approximately 50 mg of tissue was taken from each sample and colorimetric assay method was used for determination of hydroxyproline [13]. The principal of the method was the hydrolysis of the tissue specimen with 6N hydrochloric acid with the formation of free amino acids from proteins. The results were calculated as micrograms(μg) of hydroxyproline per milligram(mg) of wet tissue weight.

Histological evaluation

After being stained with haematoxylin and eosin, colonic tissues and anastomosis were examined under light microscopy and were graded in a blind fashion, using a modified 0 to 4 numerical scale by Ehrlich and Hunt (Table 1) [14,15] The evaluated parameters were inflammatory cell infiltration, fibroblast ingrowth, neovascularization and collagen deposition. Each parameter was assessed individually using the numerical scale.

Statistical analysis

Data was stored and evaluated using SPSS 9.0 for Windows. All parameters were expressed as mean ± standard error of the mean (SEM). Statistical comparisons were made by using one way ANOVA and Tukey Post Hoc Tests. A p value of less than 0.05 was considered as significant.

Results

During this experimental study, 7 rats died and new ones were included into the study. There was no spontaneous anastomotic dehiscence, intraabdominal abscesses, or other infection. All data is shown in Table 2.

The comparison of bursting pressures

Bursting pressures was differed significantly between the groups **(p < 0.05)**. When the bursting pressures of the control group and the others were compared, the highest value was observed in Group III (resection+anastomosis+EPO) and the lowest value was observed in Group II (obstruction+resection+anastomosis).

When we compared the results of the groups in which the same surgical intervention was performed (groups I-III, II-IV) but one in the group was given EPO and the other not, there was no statistical difference between groups I and III **(p > 0.05)** but a statistically significant difference was found between groups II and IV **(p = 0.03)**. EPO shoved positive sign of increase in the bursting pressure after obstruction. The mean bursting pressure by groups are shown in table 2.

The comparison of tissue Hydroxyproline levels

In the groups in which EPO was given (Groups III-IV), the tissue HPO levels were higher than in the groups which were not given EPO (Groups I-II) **(p = 0.002)**. The lowest HPO levels were measured in Group II which was an obstruction-resection group. And the highest HPO levels were measured in Group IV which was given EPO after obstruction-resection. In the case where obstruction was performed, EPO increased tissue HPO levels in a more significant way. Although the HPO levels were higher in groups which EPO was given (Group III), when compared to the control group, there was no significant difference between these groups **(p > 0.05)**. The mean tissue Hydroxyproline levels of the groups are shown in table 2.

Histological findings

In all the groups in respect to the control group, inflammatory cell infiltration was observed in a more intense way and the difference between the groups were statistically significant **(p = 0.001)**. In Groups II, III, and IV there was no significant difference in inflammatory cell infiltration **(p > 0.05)**.

There was a significant difference in the neovascularization between the groups which were given and not given EPO. In the resection+anastomosis group in which EPO was given (Group III), neovascularization was observed in an intense way in respect to all the other groups and this difference was significant **(p = 0.001)**. Futhermore, in the obstruction+ resection+anastomosis group in which EPO was given (Group IV), neovascularization was observed more in respect to Group II in which EPO was not given and this was significant **(p = 0.001)**

In Groups I, III and IV, fibroblast proliferation was observed equally and statistically insignificant **(p > 0.05)**. In Group II, in which obstruction+anastomosis was performed, fibroblast proliferation was observed lower than the other groups and the difference was statistically significant **(p = 0.003)**. It has also been observed that the obstruction had a negative effect on tissue fibroblast proliferation and this effect disappeared after EPO injections.

Table 1: Histological Grading Scale*

0 : No evidence
1+ : Occasional evidence
2+ : Light scattering
3+ : Abundant evidence
4+ : Confluent cells or fibers

The following parameters were each assessed individually: inflammatory cell infiltration, blood vessel and fibroblast in growth, and collagen deposition.
*Modified from [14]