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Blood Transfusion–induced impairment of Colonic Anastomotic Healing in Rats is Associated with Decreased TGFβ1 Expression

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INTRODUCTION-AIM

Dehiscence of colonic anastomoses is a significant cause of perioperative morbidity and mortality in patients undergoing colonic surgery (1). Various experimental models are being employed in order to elucidate the factors affecting colonic anastomotic healing and improve its efficacy (2). Whole blood transfusions have been recently shown to compromise colonic anastomotic healing in rats, an effect attributed to the leukocyte fraction (3). Postoperative administration of the H_2-receptor antagonist ranitidine, known to elicit upregulation of interleukin-2 receptors in surgical patients (4), has resulted in a decreased number of absesses but has not affected anastomotic strength significantly. Other investigators have shown that expression of transforming growth factor beta-one ($TGF\beta_1$) in the perianastomotic tissue increases gradually, reaching a maximum on the 7th postoperative day (5). In this report, we examine the effect of whole blood transfusion, with, and without ranitidine administration, in the expression of $TGF\beta_1$ in rat perianastomotic tissue, on the 7th postoperative day following experimental colonic anastomosis.

METHODS

Details of the animals used, the operative technique and blood transfusions have been published elsewhere (3). Ranitidine was administered twice daily, at 7 mg/kg, i.v., from the 1st to the 7th postoperative day, at which point the animals were sacrificed. Total RNA was isolated, using an RNA extraction kit, from segments of large intestine, 2 cm long, containing the anastomotic sites, previously removed from the experimental animals and maintained at -70 °C. The isolated RNA was reversed transcribed to cDNA with MMLV reverse transcriptase and random hexamer primers, in a final volume of 100 μl, using standard procedures. Following completion of the reverse transcription, 10 μl of each reaction mixture were used for PCR amplification of either the $TGF\beta_1$ (forward primer: TATAGCAACAATTCCTGGCG, reverse primer: CAGAAGTTGGCATGGTAGCC) or the hypoxanthine-guanine ribosyltransferase (HPRT, forward primer: GTCAAGCAGTACAGCCCCAAAATGG, reverse primer: TAGTGCAAATCAAAAGGGACGCAGC) transcript, in a total volume of 25 μl. The PCR amplification was conducted with 35 cycles of 94 °C for 30 sec, 62 °C for 45 sec and 72 °C for 1 min. Under these conditions, the $TGF\beta_1$ transcript produces a 446 bp, and the HPRT, a 385 bp product. Separation and identification of the amplified DNA was performed with 2% gel electrophoresis. The ratio of $TGF\beta_1$ over HPRT band intensities was taken as a measure of relative $TGF\beta_1$ expression in each tissue segment from which RNA was derived. Statistical comparison of the results obtained from different study groups was done with Student's t-test.

RESULTS

$TGF\beta_1$ expression in the perianastomotic tissue of the rats which had been infused with whole blood (n=6) was decreased approximately by half, compared to the rats infused with normal saline following colonic anastomosis (n=7, $p<0.001$). Ranitidine administration to rats infused with whole blood (n=5) appeared to suppress $TGF\beta_1$ expression even further.

CONCLUSIONS

The observed decrease in $TGF\beta_1$ expression in the animals infused with whole blood, compared to the ones receiving normal saline, is well in accord with the previously reported reduction of anastomotic strength in these animals (3) since $TGF\beta_1$ has been suggested to play a significant role in promoting collagen synthesis in healing anastomoses (5). The fact that the relative expression of $TGF\beta_1$ remained low in the group of animals treated with ranitidine after whole blood transfusion, is a reflection of the apparent inability of this drug to prevent the impairment of colonic anastomoses in rats, in the doses employed in this study.

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