Pharmacological interventions for improved colonic anastomotic healing: A meta-analysis

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AIM: To identify pharmaceuticals for the prophylaxis of anastomotic leakage (AL), we systematically reviewed studies on anastomosis repair after colorectal surgery.

METHODS: We searched PubMed and EMBASE for articles published between January 1975 and December 2012. We included studies in English with the primary purpose of promoting healing of anastomoses made in the colon or rectum under uncomplicated conditions. We excluded studies on adverse events from interventions, nutritional interventions or in situ physical supporting biomaterials. The primary outcome was biomechanical strength or AL. We performed meta-analyses on therapeutic agents investigated by three or more independent research groups using the same outcome.

The DerSimonian-Laird method for random effects was applied with $P < 0.05$.

RESULTS: Of the 56 different therapeutic agents assessed, 7 met our inclusion criteria for the meta-analysis. The prostacyclin analog iloprost increased the weighted mean of the early bursting pressure of colonic anastomoses in male rats by 60 mmHg (95%CI: 30-89) vs the controls, and the immunosuppressant tacrolimus increased this value by 29 mmHg (95%CI: 4-53) vs the controls. Erythropoietin showed an enhancement of bursting pressure by 45 mmHg (95%CI: 14-76). The anabolic compound growth hormone augmented the anastomotic strength by 21 mmHg (95%CI: 7-35), possibly via the up-regulation of insulin-like growth factor-1, as this growth factor increased the bursting pressure by 61 mmHg (95%CI: 43-79) via increased collagen deposition. Hyperbaric oxygen therapy increased the bursting pressure by 24 mmHg (95%CI: 13-34). Broad-spectrum matrix metalloproteinase inhibitors increased the bursting pressure by 48 mmHg (95%CI: 31-66) on postoperative days 3-4. In the only human study, the AL incidence was not significantly reduced in the 103 colorectal patients treated with aprotonin (11.7%) compared with the 113 placebo-treated patients (9.7%).

CONCLUSION: This systematic review identified only one randomized clinical trial and seven therapeutic agents from pre-clinical models that could be explored further for the prophylaxis of AL after colorectal surgery.

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Key words: Anastomotic healing; Colorectal surgery; Experimental; Breaking strength; Bursting pressure; Collagen; Meta-analysis

Core tip: Anastomotic leakage after colorectal surgery is an ongoing challenge and results in high morbidity and mortality. Currently, there is no pharmaceutical compound specifically indicated for the improvement
of anastomotic healing. This situation is remarkable considering the many interventions that have been assessed under experimental conditions. This study reviewed 56 therapeutic agents investigated in 75 separate studies. Iloprost, tacrolimus, erythropoietin, growth hormone, insulin-like growth factor-1, hyperbaric oxygen and matrix metalloproteinase inhibitor therapies reproducibly improved anastomosis stability in experimental models. These therapies, alone or in combination, should be explored further.

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INTRODUCTION

Surgical resection with primary anastomosis is a standard treatment for colorectal cancer and benign diseases, such as diverticulitis, ulcerative colitis and ischemia or for the reversal of an ostomy.

Surgical techniques have been optimized to restore intestinal continuity without compromising blood supply. Nevertheless, the incidence of anastomotic leakage (AL) is 3%-6% after colonic resection and 10%-12% after rectal resection[1,2]. AL increases short-term morbidity, permanent stoma rates and mortality. Furthermore, AL contributes to the recurrence of malignant disease[3]. Risk factors for AL include age older than 60 years, male sex, low serum albumin, prolonged surgery, increased intraoperative blood loss and blood transfusions.

Although the negative impact of corticosteroids[4] and non-steroidal anti-inflammatory drugs[5] have been well documented, there have been no pharmaceutical compounds specifically indicated for the improvement of anastomotic healing. This is remarkable considering the many interventions that have been assessed under experimental conditions. It is even more surprising that this extremely valuable source of scientific data has not been subjected to meta-analysis. Pooled estimates were calculated using the DerSimonian-Laird random-effects model. Heterogeneity among the studies was determined using the inverse-variance weighting method with Q tests.

Inclusion and exclusion criteria

We included controlled studies published in English that primarily investigated a therapeutic agent with the purpose of promoting colonic anastomotic healing under uncomplicated conditions, measured as BPR, BST or AL.

We excluded studies on interventions assessed under complicated conditions, such as intestinal ischemia, generalized peritonitis, colitis, large bowel obstruction, jaundice, diabetes, radiation, malnutrition or the presence of an ostomy. Furthermore, studies with the primary aim of investigating the adverse events associated with therapeutic agents were excluded. The influences of nutritional interventions were similarly excluded. Finally, the effects of mechanical enforcement, such as fibrin sealants, omental pedicle grafts or carboxymethylcellulose coatings, of anastomoses were recently reviewed and thus were excluded here[10].

Data extraction

The titles of the articles were retrieved and screened. Subsequently, the abstracts or the full texts of potentially relevant publications were scrutinized for eligibility. At least two authors decided whether a paper qualified. Disagreements were resolved by discussion among the four authors. The abstracted data included the investigated compound, dosage, route of administration, species, sex, sample size, assessment day and primary outcome of the study.

Statistical analysis

Therapeutic agents investigated by at least 3 independent research groups using the same primary outcome were subjected to meta-analysis. Pooled estimates were calculated using the inverse-variance weighting method with the DerSimonian-Laird random-effects model. Heterogeneity among the studies was determined using F tests. Analyses were conducted using Review Manager, version...
5.1 (The Cochrane Collaboration). The level of statistical significance was $P < 0.05$.

RESULTS

We included 75 studies (Figure 1) that were performed in rats ($n = 68$), rabbits ($n = 4$), guinea pigs ($n = 1$), dogs ($n = 1$) and humans ($n = 1$). The most frequently reported outcome was BPR ($n = 62$), followed by BST ($n = 19$), whereas only one study used AL.

We identified 56 different compounds that were subgrouped into immunomodulators ($n = 20$), hormones and growth factors ($n = 14$), miscellaneous ($n = 15$) and proteinase inhibitors ($n = 7$).

### Immunomodulators

Of the 20 different immunomodulating compounds identified in the search, data for iloprost$^{[12-14]}$ and tacrolimus$^{[15-17]}$ were subjected to meta-analysis (Table 1). This analysis demonstrated that iloprost increased the weighted mean of the early bursting pressure by 60 mmHg (95%CI: 30-89, $P < 0.0001$) vs controls (Figure 2A). The corresponding figure for tacrolimus was 29 mmHg (95%CI: 4-53, $P = 0.02$) (Figure 2B).

Recombinant human granulocyte macrophage-colony stimulating factor increased BPR on days 3 and 7 in one study$^{[18]}$, but this treatment was found to be ineffective in two other studies$^{[19,20]}$. Interleukin-2 decreased both BPR and BST in male rats$^{[21]}$. Both parthenolide and resve-
### A

| Study or subgroup | Iloprost | Control | Mean difference | Mean difference |
|------------------|----------|---------|-----------------|----------------|
|                    | Mean     | SD      | Total (95% CI)  | IV, random, 95% CI |
| Bostanoglu et al., 1998 | 64 39 10 | 35 29 10 | 31.6% | 29.00 [-1.12, 59.12] |
| Galanopoulos et al., 2011 | 162 27 10 | 117 27 10 | 36.3% | 65.00 [41.33, 88.67] |
| Vasiliadis et al., 2007 | 191 44 10 | 107 18 10 | 32.1% | 84.00 [54.54, 113.46] |

**Total (95% CI):** 30

**Heterogeneity:** \( \tau^2 = 475.6; \chi^2 = 6.78, df = 2 \ (p = 0.03); \ I^2 = 71\% \\
**Test for overall effect:** \( Z = 3.98 \ (p < 0.0001) \)

### B

| Study or subgroup | Tacrolimus | Control | Mean difference | Mean difference |
|------------------|------------|---------|-----------------|----------------|
|                    | Mean      | SD      | Total (95% CI)  | IV, random, 95% CI |
| Kiyama et al., 2002 | 146 29 10 | 119 23 11 | 17.3% | 27.00 [4.47, 49.53] |
| Rapits et al., 2012 | 158 33 11 | 119 23 11 | 17.0% | 39.00 [15.23, 62.77] |
| Schäffer et al., 2005 | 151 19 10 | 119 23 11 | 18.3% | 32.00 [14.02, 49.98] |
| Rapis et al., 2012 | 217 35 10 | 136 21 10 | 16.7% | 81.00 [55.70, 106.30] |
| Schäffer et al., 2005 | 82 24 11 | 102 38 10 | 14.6% | 8.00 [-26.2, 42.20] |

**Total (95% CI):** 61

**Heterogeneity:** \( \tau^2 = 777.57; \chi^2 = 30.49, df = 5 \ (p < 0.0001); \ I^2 = 84\% \\
**Test for overall effect:** \( Z = 2.28 \ (p = 0.02) \)

### C

| Study or subgroup | EPO | Control | Mean difference | Mean difference |
|------------------|-----|---------|-----------------|----------------|
|                    | Mean | SD      | Total (95% CI)  | IV, random, 95% CI |
| Kaimpf et al., 2010 | 154 5 5 | 74 17 5 | 36.3% | 60.00 [44.47, 75.53] |
| Moran et al., 2012 | 200 16 9 | 205 48 8 | 26.7% | 5.00 [-39.87, 50.87] |
| Ozel Turkcu et al., 2012 | 150 20 8 | 94 14 8 | 36.3% | 66.00 [49.08, 82.92] |

**Total (95% CI):** 22

**Heterogeneity:** \( \tau^2 = 602.19; \chi^2 = 13.36, df = 2 \ (p = 0.001); \ I^2 = 85\% \\
**Test for overall effect:** \( Z = 2.86 \ (p = 0.004) \)

### D

| Study or subgroup | GH | Control | Mean difference | Mean difference |
|------------------|----|---------|-----------------|----------------|
|                    | Mean | SD      | Total (95% CI)  | IV, random, 95% CI |
| Adas et al., 2013 | 95 21 10 | 81 20 10 | 21.3% | 14.00 [-3.97, 31.97] |
| Christensen et al., 1990 | 92 73 10 | 77 25 10 | 6.9% | 65.00 [17.18, 112.82] |
| Christensen et al., 1991 | 38 24 11 | 22 13 12 | 23.2% | 16.00 [0.02, 51.98] |
| Christensen et al., 1992 | 56 24 9 | 20 10 11 | 22.5% | 36.00 [19.24, 52.76] |
| Karahasanoglu et al., 1998 | 51 13 10 | 45 16 10 | 25.9% | 6.00 [-6.78, 18.78] |

**Total (95% CI):** 50

**Heterogeneity:** \( \tau^2 = 158.61; \chi^2 = 11.76, df = 4 \ (p = 0.02); \ I^2 = 66\% \\
**Test for overall effect:** \( Z = 2.89 \ (p = 0.004) \)

### E

| Study or subgroup | IGF-1 | Control | Mean difference | Mean difference |
|------------------|-------|---------|-----------------|----------------|
|                    | Mean  | SD      | Total (95% CI)  | IV, random, 95% CI |
| Eggert et al., 2001 | 139 14 6 | 85 24 6 | 67.1% | 54.00 [31.77, 76.23] |
| Mantzoros et al., 2006 | 339 44 20 | 272 183 20 | 4.9% | 67.00 [-15.49, 149.49] |
| Zacharakis et al., 2007 | 346 55 20 | 269 56 20 | 28.0% | 77.00 [42.60, 111.40] |

**Total (95% CI):** 46

**Heterogeneity:** \( \tau^2 = 0.00; \chi^2 = 1.23, df = 2 \ (p = 0.54); \ I^2 = 0\% \\
**Test for overall effect:** \( Z = 6.57 \ (p < 0.00001) \)

### F

| Study or subgroup | HBO | NO HBO | Mean difference | Mean difference |
|------------------|-----|--------|-----------------|----------------|
|                    | Mean | SD      | Total (95% CI)  | IV, random, 95% CI |
| Erenoglu et al., 2003 | 221 6 10 | 190 18 10 | 49.4% | 31.00 [19.29, 42.76] |
| Hamzaoglu et al., 1998 | 123 18 10 | 104 19 10 | 32.2% | 19.00 [7.78, 35.52] |
| Yagci et al., 2005 | 119 17 10 | 107 33 10 | 18.6% | 12.00 [11.01, 35.01] |

**Total (95% CI):** 30

**Heterogeneity:** \( \tau^2 = 26.01; \chi^2 = 2.75, df = 2 \ (p = 0.25); \ I^2 = 27\% \\
**Test for overall effect:** \( Z = 4.27 \ (p < 0.0001) \)
Hormones and growth factors

In this category, sufficient data for the meta-analysis were available for erythropoetin (EPO)\textsuperscript{[19,34-37]} growth hormone (GH)\textsuperscript{[38-43]} and insulin-like growth factor (IGF)-1\textsuperscript{[44-47]} (Table 2). The meta-analyses showed that EPO increased BPR by 45 mmHg (95%CI: 14-76, \(P < 0.00001\)) (Figure 2C), \(GH\) increased BPR by 21 mmHg (95%CI: 7-35, \(P = 0.004\)) (Figure 2D) and IGF-1 increased BPR by 61 mmHg (95%CI: 43-79, \(P < 0.00001\)) cf. the controls (Figure 2E).

In addition to these substances, full-length or truncated keratinocyte growth factor increased anastomotic strength on postoperative days 2-7\textsuperscript{[44,49]} Compounds investigated in isolated studies reporting enhancement

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**Figure 2** Forest plots of the bursting pressure in mmHg. The results of the meta-analysis for A: Iloprost at days 3-5 (cf. Table 1); B: Tacrolimus (cf. Table 1); C: Erythropoietin (EPO) at days 5-7 (cf. Table 2); D: Growth hormone (GH) at day 4 (cf. Table 2); E: Insulin-like growth factor-1 (IGF-1) at days 4-7 (cf. Table 2); F: Hyperbaric oxygen (HBO) at days 4-7 (cf. Table 3); G: Broad-spectrum matrix metalloproteinase inhibitors (MMPi) at days 3-4 (cf. Table 4).

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**Figure 3** Effects of broad-spectrum matrix metalloproteinase inhibitors on anastomotic breaking strength (A) and morphology (B) on postoperative day 3. A: Forest plot of the results of the meta-analysis in N (cf. Table 4); B: Diminution of the gap between the two large bowel ends (indicated by double-headed arrows) after treatment with the broad-spectrum matrix metalloproteinase inhibitor (MMPi) GM6001 (left image) compared with the vehicle treatment (right image). The photomicrographs are representative of the overall significant (\(P < 0.05\)) effect of GM6001 (median: 320 μm) compared with the vehicle (median: 900 μm).
Table 2  Studies on the hormones erythropoietin and growth hormone, and the growth factor insulin-like growth factor-1 in colonic anastomotic healing

| Study                     | Compound | Species          | Sex | Sample size | Dosage | Route | Test | Test day | Effects |
|---------------------------|----------|------------------|-----|-------------|--------|-------|------|----------|---------|
| Faruquzeaman et al[26]    | EPO      | Guinea pig       | Male| 20          | 500    | SC    | BPR  | 7        | NS      |
| Fatouros et al[27]        | EPO      | Rat              | Male| 30          | 500    | SC    | BPR  | 7        | 137     |
| Kaemmerer et al[28]       | EPO      | Rat              | Male| 20          | 5000   | SC    | BPR  | 3/5      | NS/182  |
| Moran et al[29]           | EPO      | Rat              | Male| 20          | 500    | SC    | BPR  | 7        | NS      |
| Ozel Turkcu et al[30]     | EPO      | Rat              | Male| 16          | 500    | IM    | BPR  | 7        | 193     |
| Adas et al[31]            | GH       | Rat              | Male| 20          | 2.0    | SC    | BPR  | 4        | 116     |
| Christiansen et al[32]    | GH       | Rat              | Male| 20          | 2.0    | SC    | BPR  | 4/6      | 104/1232/NS |
| Christiansen et al[33]    | GH       | Rat              | Female| 72         | 2.0    | SC    | BPR  | 4/6      | 155/NS/NS |
| Christiansen et al[34]    | GH       | Rat              | Female| 72         | 2.0    | SC    | BPR  | 4/6      | 104/1232/NS |
| Christiansen et al[35]    | GH       | Rat              | Female| 50         | 0.125/0.5/2.0/8.0 | SC    | BPR  | 4        | NS/NS/1270/1430 |
| Christiansen et al[36]    | GH       | Rat              | Female| 36         | 2.0↑/4↑ | SC    | BST  | 4        | NS/NS/134/159 |
| Karahasanoglu et al[37]   | GH       | Rat              | Male| 20          | 2.0    | SC    | BPR  | 4        | 111     |
| Egger et al[38]           | IGF-1    | Rat              | Male| 76          | 1.0    | IP    | BPR  | 4/6      | 162/167/161 |
| Mantzoros et al[39]       | IGF-1    | Rat              | Female| 40         | 2.0    | IP    | BPR  | 7        | 125     |
| Petersen et al[40]        | IGF-1    | Rat              | Female| 26         | about 2.5 | SC    | BST  | 3        | NS      |
| Zacharakis et al[41]      | IGF-1    | Rat              | Male | 40          | 2.0    | IP    | BPR  | 7        | 129     |

1Total number of animals; 2EPO: IU/kg; GH: mg/kg; IGF-1: mg/kg; ↑% increase (P < 0.05) or ↓% decrease (P > 0.05) vs controls; 3Preoperative; 4Postoperative; 5Preoperative and postoperative. BPR: Bursting pressure; BST: Breaking strength; EPO: Erythropoietin; GH: Growth hormone; IGF-1: Insulin-like growth factor-1; IM: Intramuscular; IP: Intraperitoneal; SC: Subcutaneous; NS: Not statistically significant.

Table 3  Studies on exogenous oxygen in colonic anastomotic healing

| Study                     | Species | Sex | Sample size | Dosage | Test | Test day | Effects |
|---------------------------|---------|-----|-------------|--------|------|----------|---------|
| Erenoğlu et al[42]        | Rat     | Male| 20          | Hyperbaric | BPR  | 7        | NS      |
| Hamzaoglu et al[43]       | Rat     | Male| 20          | Hyperbaric | BPR  | 4        | 118     |
| Kirk et al[44]            | Rat     | Male| 36          | 50%     | BWT  | 7        | NS      |
| Yagci et al[45]           | Rat     | Male| 40          | Hyperbaric 3/4/5 | BPR  | 5        | NS/NS/NS |

1Total number of animals; 2↑% increase (P < 0.05) or ↓% decrease (P > 0.05) vs controls; 3Preoperative; 4Postoperative; 5Preoperative and postoperative. BPR: Bursting pressure; BWT: Bursting wall tension; NS: Not statistically significant.

Miscellaneous

Although the results from animal studies on oxygen therapy were inconsistent,[61-63] (Table 3), hyperbaric oxygen significantly increased BPR by 24 mmHg (95%CI: 13.34, P < 0.0001) in the meta-analysis (Figure 2F). However, the sole human study on oxygen therapy that we retrieved was recently retracted by the journal that published it[64].

Gentamicin, administered systemically and locally, increased BPR on day 5 but not on day 3 in two separate studies.[65,66] Kanamycin in combination with erythromycin was more effective than kanamycin alone in increasing BS of colonic anastomoses on day 7 in dogs.[64] The calcium channel blocker nifedipine increased BPR on days 3 and 7 in one study.[67] Phenytion increased BPR on days 3 and 7, either by oral or rectal administration at clinically relevant dosages.[68] Male rats that received pharmacological doses of vitamin A for five days preoperatively and for six days postoperatively showed increased BPR.[69] Simvastatin orally administered to rats resulted in increased BPR on days 3 and 7.[70] Zinc intraperitoneally administered to rabbits increased BPR on day 7[71] but showed no significant effect on BST on days 3 or 7 in male rats given an equivalent zinc dosage[72]. Heparin had no effect on BST, but low-molecular-weight heparin increased BST at day 7[73]. A dextran derivative with heparin-like properties increased BPR by more than two-fold on day 2 but not at later time points in two separate studies.[74,75] Albumin,[67] colloid[76] and hydroxyethyl starch[76,77] had no significant impacts on anastomotic strength.

Proteinase inhibitors

Matrix metalloproteinase (MMP) inhibitors, including AG3340,[78] BB-94,[79] BB-1101,[80] BE166227B[81], doxycline[82,83] and GM6001[83], consistently resulted in improved anastomotic strength on postoperative days 3-4 but not later (Table 4). The meta-analysis of the three studies using the outcome of BPR showed a weighted mean increase in BPR of 48 mmHg (95%CI: 31-66 mmHg, P < 0.00001) vs the vehicle. The studies that used BST as an outcome measurement demonstrated a significant increase in the weighted mean of BST of 0.45 N (95%CI: 0.27-0.63, P < 0.00001) (Figure 3A). In a study on GM6001, light microscopic examination
Table 4  Studies on matrix metalloproteinase inhibitors in colonic anastomotic healing

| Study           | Compound     | Species | Sex | Sample size | Dosage (mg/kg) | Route | Test  | Test day | Effect     |
|-----------------|--------------|---------|-----|-------------|----------------|-------|-------|----------|------------|
| de Hingh et al  | BB-94        | Rat     | Male| 60          | 30             | IP    | BPR/BST| 1        | NS/NS      |
|                 |              |         |     |             |                |       | BPR/BST| 3        | ↑34/↑27    |
|                 |              |         |     |             |                |       | BPR/BST| 7        | NS/NS      |
| Kiyama et al    | BE16627B     | Rat     | Male| 21          | About 10       | SC    | BPR   | 4        | ↑28        |
| Pasternak et al | Doxycycline  | Rat     | Male| 40          | NA             | LO    | BST   | 3        | ↑17        |
| Siemonsma et al | Doxycycline  | Rat     | Male| 80          | About 40       | SC    | BST   | 5        | NS/NS      |
|                 |              |         |     |             |                |       | BPR/BST| 1        | NS/NS      |
|                 |              |         |     |             |                |       | BST   | 3        | ↑90/↑127   |
|                 |              |         |     |             |                |       | BST   | 3        | ↑36/↑NS    |
|                 |              |         |     |             |                |       | SC    | 3        | ↑79/↑88    |
|                 | BS-300M      |         |     |             |                |       | SC    | 1        | ↑28        |
| Syk et al       | BB-1101      | Rat     | Male| 48          | 30             | SC    | BPR   | 1/3/7    | NS/148/NS  |
| Ågren et al     | AG3340       | Rat     | Male| 120         | 10             | SC    | BST   | 3        | ↑17        |
|                 | GM6001       | Rat     | Male| 10/100      | SC             | BST   | 3        | 179/188   |

*Total number of animals; ↑% increase (P < 0.05) or ↓% decrease (P < 0.05) vs controls; BPR: Bursting pressure; BST: Breaking strength; IP: Intraperitoneal; LO: Local; NA: Not applicable; SC: Subcutaneous; NS: Not statistically significant.

DISCUSSION

Summary of results

We reviewed the data on 56 different therapeutic agents intended to promote anastomotic wound healing with the purpose of identifying interventions with prophylactic potential in colorectal AL. Approximately half of these agents were assessed in one study only. To obtain more robust conclusions, we performed a meta-analysis of the products that were tested by three or more independent research groups. Meta-analyses were undertaken for iloprost, tacrolimus, EPO, GH, IGF-1, hyperbaric oxygen and MMP inhibitors. These therapies reproducibly improved anastomotic stability in uncomplicated pre-clinical models. Thus, exploration of these agents, alone or in combination, would be the next step in the search for effective interventions for AL prophylaxis.

Anastomotic wound healing

Anastomotic healing follows the chronological phases of tissue repair, which are largely regulated by cytokines and growth factors. The initial hemostatic response results in a fibrin/fibronectin matrix that temporarily seals and connects the two bowel ends. Subsequently, inflammatory cell infiltration contributes to loss of the existing collagen of the adjacent submucosa by tissue-destructive proteinases, notably MMPs. From postoperative day 3, the provisional matrix is gradually converted into granulation tissue, which contains many new blood vessels, macrophages and fibroblasts. The collagen synthesis rate then increases dramatically and peaks on days 6-7 in BST, but not BPR, was correlated with the increase in collagen deposited in the anastomosis during the first week.

Anastomotic repair can be improved via different non-overlapping mechanisms, including inhibition of the degradation of submucosal collagen, promotion of angiogenesis and acceleration of granulation tissue deposition and epithelialization.

Discussion of the seven candidate pharmaceuticals

The prostacyclin analog iloprost enhanced anastomotic strength, possibly through increased neoangiogenesis and intestinal blood perfusion. Tacrolimus also improved anastomotic healing, and light microscopy revealed reduced inflammatory cell infiltration and preserved morphology of the two colonic ends in the tacrolimus group. However, tacrolimus is an immunosuppressive drug that targets T-cell activation and interleukin-2 transcription. Based on these results, iloprost is a potential candidate for further exploration, while tacrolimus is not due to its general immunosuppressive effects.

Although the main indication for EPO is anemia, its non-hematopoietic properties could have positive effects on anastomotic healing. EPO treatment also enhanced anastomotic strength under normal situations. Interestingly, improved BPR coincided with reduced MMP-8 expression in anastomotic wounds. A more obvious place for EPO therapy would perhaps be under complicated situations, such as ischemia.

The beneficial effect of GH on anastomotic strength was reproduced on postoperative days 2-4 when administered at a dosage of 2.0 mg/kg per day or more. The positive effects could be ascribed to earlier deposition and reorganization of neocollagen in anastomotic wound.
gaps\textsuperscript{[41,42]}. Similarly, overexpression of GH profoundly stimulated early granulation tissue formation in wounds\textsuperscript{[45]}. One caveat is that most of the studies were performed in female animals. GH treatment was seemingly less effective in male rats, possibly due to the negative influence of testosterone on wound healing\textsuperscript{[43]}. GH stimulated hepatic synthesis of IGF-1, which is believed to mediate the local effects of GH\textsuperscript{[44]}. Exogenous IGF-1 also raised anastomotic collagen levels and anastomotic strength when administered intraperitoneally\textsuperscript{[44,47]} However, the systemic adverse effects of GH and IGF-1 and the possible danger of using mitogenic substances in colorectal cancer patients might disqualify these agents from further exploration in clinical trials. To circumvent the risk of harm, IGF-1 could be delivered locally\textsuperscript{[49]}.

Oxygen therapy for anastomotic wound healing is theoretically attractive. In a series of elegant experiments, Shandall et al\textsuperscript{[50]} demonstrated a strong positive correlation between tissue oxygen tension and the breaking strength of colon anastomoses, largely due to increased hydroxyproline. The results here were inconsistent, although there was a statistically significant, but mediocre, increase in BPR with hyperbaric oxygen therapy\textsuperscript{[59-61]}. On the other hand, hyperbaric oxygen therapy increased intra-abdominal adhesions\textsuperscript{[90]}. Interestingly, a more clinically useful regimen, with 50\% oxygen at atmospheric pressure, was ineffective\textsuperscript{[81]}. This outcome agrees with the findings of the PROXI trial, in which no significant effect of supplemental oxygen on anastomotic dehiscence was observed\textsuperscript{[93]}. The PROXI trial was excluded here because AL was not the primary outcome, and patients undergoing emergency surgery were also enrolled. MMPs comprise a 23-member family of human zinc-dependent endopeptidases\textsuperscript{[88]}. While extracellular matrix remodeling by MMPs is part of the normal physiological response to injury\textsuperscript{[88]}, increased activity of MMPs could be deleterious to anastomotic strength due to excessive collagen degradation\textsuperscript{[89]}. Synthetic MMP inhibitors unequivocally improved anastomotic integrity. Kiyama et al\textsuperscript{[86]} attributed the increased mechanical strength to more collagen fibers in the wound gap connecting the large bowel ends. Morphologically, the smaller wound gap observed after administration of GM6001 strongly suggested that MMP inhibitors protected the existent submucosal collagen network from degradation\textsuperscript{[88]}. Intuitively, this observation also indicated a decreased risk of leakage of the intraluminal content into the peritoneal cavity\textsuperscript{[88]}. Interestingly, GM6001 treatment did not increase the formation of intra-abdominal adhesions\textsuperscript{[99]}.

**Limitations**

The pathogenesis of AL is multifactorial. The studies in our review used surrogate outcomes that could not be directly translated into clinical AL. Furthermore, there was only one study conducted in patients who were subjected to colorectal surgery. Neither the quality nor publication bias of the included studies was evaluated here because the studies were too small in sample size and too few in number\textsuperscript{[100]}.

The efficacy of a therapeutic agent depends on the conditions in which the anastomoses are constructed. We chose to focus on studies investigating anastomotic healing under uncomplicated conditions because these cases are representative of the majority of patients with colorectal cancer\textsuperscript{[101-103]}.

To conclude, despite these limitations, our review indicated several promising therapeutic agents for the prevention of AL.

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