Therapeutic improvement of colonic anastomotic healing under complicated conditions: A systematic review

Malene Nerstrøm, Peter-Martin Krarup, Lars Nannestad Jorgensen, Magnus S Ågren

Abstract

AIM: To identify therapeutic agents for the prophylaxis of gastrointestinal anastomotic leakage (AL) under complicated conditions.

METHODS: The PubMed and EMBASE databases were searched for English articles published between January 1975 and September 2014. Studies with the primary purpose of improving anastomotic healing in the colon or rectum under complicated preoperative and/or intraoperative conditions were included. We excluded studies investigating the adverse effects or risk assessment of an active intervention. Furthermore, investigations of biophysical materials, sealants, electrical stimulation and nutrients were excluded. The primary study outcome was biomechanical anastomotic strength or AL. The meta-analysis focused on therapeutic agents that were investigated in one animal model using the same outcome by at least three independent research groups.

RESULTS: The 65 studies included were divided into 7 different complicated animal models: Bowel ischemia, ischemia/reperfusion, bowel obstruction, obstructive jaundice, peritonitis, chemotherapy and radiotherapy. In total, 48 different therapeutic compounds were examined. The majority of investigated agents (65%) were reported as beneficial for anastomotic healing. Twelve of the agents (25%) were tested more than once in the same model, whereas 13 (27%) of the agents were tested in two or more models of complicated healing. Two therapeutic agents met our inclusion criteria for the meta-analysis. Postoperative hyperbaric oxygen therapy significantly increased anastomotic strength.

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Anastomotic healing; Colorectal surgery; May 27, 2016 vs
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INTRODUCTION

Anastomotic leakage is a challenging compli­
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Key words: Anastomotic healing; Colorectal surgery;
Breaking strength; Bursting pressure; Anastomotic
leakage; Ischemia; Chemotherapy; Reperfusion; Bowel
obstruction; Peritonitis; Radiotherapy

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INTRODUCTION

Colorectal surgery with construction of a primary an­
stomosis is performed for conditions such as cancer, diverticulitis, ulcerative colitis, ischemia or stoma reversal.

Despite improvements in preoperative management
and surgical techniques, anastomotic leakage (AL)
remains a major complication. The incidences of AL
after colonic resection and rectal resection are 3%­7%
and 10%­20%[1­3], respectively. AL is associated with
increased risk of morbidity, short­term mortality, permanent
ostomy, tumor recurrence and a diminished
overall long­term survival[2,4­8].

In animal models of anastomotic healing, anasto­
ometic bursting pressure (BPR) and anastomotic break­
ing strength (BST) are the most common surrogate
outcomes of anastomotic healing. BPR reflects the resis­
tance to increased intraluminal pressure, whereas BST
reflects the increased longitudinal load. The collagen
concentration is important for anastomotic integrity and
depends to a minimum 3 d after the construction of
colonic anastomoses under normal healing conditions[9,10].

Previous studies have identified several local and
systemic factors with deleterious effects on anastomotic
wound healing, including ischemia[11,12], reperfusion[13­15],
bowel obstruction[16], obstructive jaundice[17], peritoni­
tis[18,19], chemotherapy[20] and radiotherapy[12­20].
Reperfusion after intestinal ischemia provokes local and
systemic inflammatory responses[13­15], and ischemia
ultimately leads to tissue necrosis and bowel perfora­
tion[21,22]. Acute bowel obstruction is associated with
ischemia, inflammation and loss of collagen in the
colic wall[9,12,24]. Obstructive jaundice compromises
systemic immune functions[17]. Impaired collagen
thesis is observed in peritonitis[29] and with the use of
chemotherapeutic agents[26]. Preoperative radiotherapy,
which is used to downsize rectal tumors, induces inflam­ma­tion[1,27]. Despite the well­known risk of compromised
anastomotic healing under these conditions, surgical
resection and construction of a primary anastomosis are
pivotal in the treatment algorithm.

A recent meta­analysis identified seven compounds,
including iloprost, tacrolimus, erythropoietin (EPO),
growth hormone (GH), insulin­like growth factor­1 (IGF­
1), hyperbaric oxygen therapy (HBOT) and synthetic
inhibitors of matrix metalloproteinases (MMPs), all
of which have the potential to improve anastomotic
healing under non­complicated conditions[29]. Several
compounds have also been tested in different experimen­
tal models of complicating conditions[29].

The aim of the present systematic review was to
identify therapeutic agents that are potentially capable
of abolishing or reducing the deleterious effects on
anastomotic healing caused by ischemia, ischemia/reper­
fusion (I/R), obstructive bowel, obstructive jaundice,
peritonitis, chemotherapy or radiotherapy.

MATERIALS AND METHODS

Methods

This systematic review was conducted according to the
Preferred Reporting Items for Systematic Reviews and
Meta­Analyses guidelines[30].

Search strategy

The PubMed and EMBASE databases were searched for
articles published between January 1975 and September
2014 using the following syntax: (((Surgical anastomosis*)
OR (intestinal anastomos*) ) OR (anastomos* AND leak*)
OR (anastomos* AND dehiscence) OR (surgical wound
dehiscence) OR (anastomos* AND failure) OR (anastomo­
* AND rupture)) AND (((colorectal surgery) OR
(surgical anastomos*) OR (colo* AND surgery) OR
(rect* AND surgery) OR (large intestine and surgery)
OR (colorectal resection) AND ((burst* pressure) OR (breaking strength) OR (anastomo* AND strength) OR (wound rupture) OR (biomechanical strength) OR (mechanical strength) OR (wound healing) OR (autopsy) OR (anastomo* AND leak*)) AND (((peritonitis) OR (infection) OR (sepsis)) OR ((ischemia) OR (hypoperfusion)) OR ((ileus) OR (bowel obstruction) OR (large bowel obstruction) OR (intestinal obstruction)) OR ((radiation) OR (radiotherapy) OR (radiochemotherapy) OR (chemotherapy))).

Cross-references from the included studies were manually reviewed.

Data extraction and outcomes
Titles of the articles identified in the search were reviewed, and potentially relevant abstracts or full-text articles, if necessary, were assessed for eligibility. Two or more authors decided whether a study qualified for inclusion, and disagreements were solved by discussion among the four authors.

The abstracted data included the complicated animal model used, the investigated compound, the time of administration, the species, the gender, the sample size, the dosage, the route, the day of anastomotic testing, the primary outcome and the effects on BPR, BST or AL of the compound investigated.

Missing data were gathered by contacting the authors.

Inclusion and exclusion criteria
English publications with the primary aim of investigating the potential beneficial properties of a pharmacological agent to improve anastomotic healing during complicated conditions were included. Studies on animals or humans with colo-colonic or colorectal anastomoses without a protecting ostomy reporting BPR, BST and AL relative to a proper control group were included.

Studies with the aim of clarifying adverse effects or risks of a therapeutic agent on anastomotic healing in complicated conditions were excluded. Likewise, studies on the effects of electrical stimulation, mechanical enforcement, such as biofragmentable anastomotic rings, endoluminal prosthesis/tube or amniotic membranes, together with sealants, such as fibrin glue, cyanoacrylates or collagen matrix bound coagulation factor sealants, and nutrients were also excluded.

Statistical analysis
Compounds investigated in one complicated animal model by at least three independent research groups using the same primary outcome were subjected to a meta-analysis. For these analyses, Review Manager (RevMan, Version 5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014) was used. Pooled estimates were calculated using the inverse-variance weighting method with the DerSimonian-Laird random-effects model. Heterogeneity among the studies was determined using I² tests. The level of statistical significance was 0.05.

RESULTS
A total of 65 studies were included in the study (Figure 1). These studies were divided into 7 different animal models (Figure 2): Bowel ischemia models (n = 21) in rats (n = 20) and dogs (n = 1), I/R injury models (n = 8) in rats, models of colonic obstruction (n = 5) in rats (n = 4) and guinea pigs (n = 1), an obstructive jaundice model in the rat (n = 1), models of peritonitis (n = 16) in rats (n = 15) and mice (n = 1), chemotherapeutic models (n = 8) in rats and irradiation models (n = 6) in rats (n = 5) and pigs (n = 1). The reported outcomes were BPR (n = 62), BST (n = 4) and AL (n = 5). More than one outcome was applied in 6 studies. No human studies were retrieved by our search criteria.

Forty-eight different compounds were identified; 12 (25%) compounds were tested more than once in the same model, and 13 (27%) were tested in more than one complicated model. Enhancement of anastomotic healing was reported for 31 (65%) of the compounds; a non-significant effect was reported for 7 (15%) of the compounds, inconsistent results were reported for 9 (18%) different compounds and 1 (2%) compound was found to be detrimental to anastomotic healing.

Bowel ischemia
Twenty-two different compounds were tested in models of intestinal ischemia (Table 1). Experimentally, ischemia in the anastomotic segment was induced by ligation[31,33,35] or coagulation[34] of vessels in the mesocolon. The anastomosis was then constructed in the ischemic segment during the same surgical procedure.

Four studies tested the effect of postoperative HBOT in rats[31,33,35,36]. The meta-analysis demonstrated that HBOT significantly increased anastomotic BPR by a mean 28 mmHg (95%CI: 17 to 39 mmHg, P < 0.00001) compared with controls (Figure 3A). The inconsistency between studies was moderately large (I² = 40%). HBOT increases tissue oxygenation[31,33,35,36], which may explain the elevated hydroxyproline concentration in the anastomosis[33,35]. HBOT was ineffective when only administered preoperatively[33]. The possible adverse effects of HBOT are oxygen toxicity, air embolization and pneumothorax[31,33,35].

Guzel et al[35] found that rats receiving a post-operative intraperitoneal injection of β-1,3-glucan alone also significantly improved BPR by 67% compared with 50% for HBOT alone. β-1,3-glucan alone also significantly improved HBOT by 86%. Supplementing postoperative HBOT with low molecular weight heparin (enoxaparin) had no further effect on BPR despite increasing neovascularization in the anastomotic area[36]. Enoxaparin did not significantly improve BPR[36].

Growth factors and hormones are pivotal in wound healing[37]. Vascular endothelial growth factor (VEGF)-A and fibroblast growth factor (FGF)-2 plasmids were injected directly into the anastomotic tissue intraoperatively. The gene therapy increased VEGF and FGF-2
indicated a direct mitogenic effect of leptin on colonic epithelial cells [39]. Intraperitoneal leptin also increased the anastomotic strength of right-sided colon anastomoses in rats [39].

Pentoxifylline enhanced anastomotic BPR on day 8 [34], but not on day 5 [40].

The vasoactive adrenomedullin increased BPR and hydroxyproline levels on postoperative days 3 and 7 [41]. Furthermore, adrenomedullin treatment decreased anastomotic tissue concentrations of tumor necrosis factor-α and interleukin-6 [41]. Increased vascularization and less oxidative damage of the anastomoses were observed with adrenomedullin [41]. Adrenomedullin causes significant hypotension that may impair the colonic blood flow [41]. Another caveat is that adrenomedullin may induce neoplasia [41,42].

The beneficial effects of the endothelin receptor antagonist, bosentan, on anastomotic healing were possibly due to the increased blood flow and increased hydroxyproline level in the anastomotic area [43]. Bosentan significantly reduced adhesion formation [43].

Allopurinol reduced the induced superoxide anion production in ischemic anastomoses and increased the hydroxyproline levels [22].

Allogeneic mesenchymal stem cells (MSCs) derived from bone marrow of rats were cryopreserved. The cells were thawed and injected (1 × 10⁶ viable MSCs) into newly constructed anastomoses in ischemic rat colon. This cell therapy resulted in enhanced BPR on both day 4 and day 7 [44], whereas systemically applied MSCs resulted in a significant effect on day 4 only [45].

Locally applied granulocyte macrophage-colony stimulating factor (GM-CSF) enhanced anastomotic BPR on days 3 and 7 [46].

### Figure 1
Flow diagram of the identified and selected studies. BPR: Bursting pressure; BST: Breaking strength; AL: Anastomotic leakage.

### Figure 2
Number of studies included, divided into the 7 models of complicated anastomotic wound healing.
A study on the effect of a prostacyclin analogue (OP-41483) on AL was undertaken in dogs with colonic ischemia of variable severity. Colonic ischemia was induced by devascularization of marginal vessels resulting in slight (40%–60% decrease in colonic blood flow), moderate (60%–80%) or severe (80%–100%) ischemia measured by a hydrogen gas clearance method. All eight agents tested in rat I/R injury models were evaluated in single studies (Table 2). Before, more commonly, after construction of an anastomosis, I/R injury is induced by occluding mesenteric vessels of a 2–3 cm segment of the left colon or by occluding the superior mesenteric artery with microvascular clamps for 30–60 min before reperfusion. Notably, antithrombin III (ATIII) and ethyl pyruvate treatment increased anastomotic BPR in the I/R injured rats by more than 60%, possibly because of an enhanced anastomotic BPR. The mechanism of action remains elusive because amelogenin had no effect on hydroxyproline levels.

**Table 1 Studies on therapeutic compounds in ischemic models**

| Ref. | Compound | Time of administration | Species | Sex | Sample size | Dosage | Route | Test | Test day | Effect |
|------|----------|------------------------|---------|-----|-------------|--------|-------|------|----------|--------|
| Yagci et al[43] | HBOT | Preoperative | Rat | M | 20 | | | BPR | 5 | NS |
| Hamzaoğlu et al[44] | HBOT | Postoperative | Rat | M | 16 | 20 | | BPR | 4 | ↑ 32 |
| Guezl et al[45] | HBOT | Postoperative | Rat | F | 20 | | | BPR | 4 | ↑ 50 |
| Kernik et al[46] | HBOT | Postoperative | Rat | F | 20 | | | BPR | 4 | ↑ 80 |
| Adas et al[47] | VEGF-A plasmid | Intraoperative | Rat | M | 40 | 0.001 | LO | BPR | 4 | ↑ 16 |
| Sarıbeyoğlu et al[48] | PDGF-BB | Intraoperative | Rat | M | 20 | 125 | LO | BPR | 4 | ↑ 8 |
| Yarımçay et al[49] | GH | Preoperative and postoperative | Rat | M | 28 | 1 | SC | BPR | 3/7 | 87/32 |
| Tasdelen et al[50] | Nandrolone | Preoperative | N/A | | | | | IM | | 55/NS |
| Parra-Memberies et al[51] | Leptin | Postoperative | Rat | M, F | 38 | 50 | IP | BPR/BST | 8 | 74/1 |
| Stein et al[52] | Pentoxifylline | Postoperative | Rat | M, F | 20 | 50 | IP | BPR | 5 | NS |
| | Pentoxifylline | Postoperative | Rat | M, F | 20 | 50 | IP | BPR | 5 | NS |
| Vinpocetine | | Postoperative | Rat | F | 32 | 0.002 | SC | BPR | 3/7 | 5/20 |
| Karatepe et al[53] | Adrenomedulin | Postoperative | Rat | F | 20 | 3.5 | IP | BPR | 6 | ↑ 46 |
| Cetinkaya et al[54] | Bostantan | Postoperative | Rat | F | 20 | 50 | IP | BPR | 4 | ↑ 74 |
| Garcia et al[55] | Allopurinol | Preoperative and postoperative | Rat | M | 20 | 50 | PO | BPR | 4 | ↑ 74 |
| Adas et al[56] | MScs | Intraoperative | Rat | M | 40 | 0.5 | LO | BPR | 4/7 | 110/1.86 |
| Adas et al[57] | MScs | Postoperative | Rat | M | 40 | 0.5 | iv | BPR | 4/7 | 142/8 |
| Dinc et al[58] | GM-CSF | Intraoperative | Rat | M | 72 | 0.050 | LO | BPR | 3/7 | 30/26 |
| Ikeda et al[59] | Prostacyclin analogue (OP-41483) | Intraoperative and postoperative | Dog, M, F | | 10² | 0.00004 | iv | AL | 3 | NS |
| Cohen et al[60] | Neomycin + erythromycin | Preoperative | N/A | | 12 | 20 | PO | AL | 7 | 83 |
| Karatas et al[61] | Amelogenin | Intraoperative | Rat | M | 16 | 0.14 | LO | BPR | 4 | ↑ 25 |
| Ikrorcu et al[62] | Sildenafil | Intraoperative | Rat | M | 27 | 10 | PO | BPR | 4 | NS/NS |
| Connely et al[63] | Compound 48/80 | Preoperative | Rat | M | 20 | 1 | iv | BPR | 4 | NS |

†Total number of animals; ↑% increase (P < 0.05) or ↓% decrease (P < 0.05) vs controls; †mg; †mg/kg; †IU/kg; †mL; †Slight ischemia; †Moderate ischemia; †Severe ischemia. AL: Anastomotic leakage; BPR: Bursting pressure; BST: Breaking strength; F: Female; FGF-2: Fibroblast growth factor-2; GH: Growth hormone; GM-CSF: Granulocyte macrophage-colony stimulating factor; HBOT: Hyperbaric oxygen therapy; IP: Intraportaneous; iv: Intravenous; LMWH: Low molecular weight heparin; LO: Local; M: Male; MScs: Bone marrow derived mesenchymal stem cells; N/A: Data not available; NS: Not statistically significant; PDGF-BB: Platelet-derived growth factor-BB; PO: Per os; SC: Subcutaneous; VEGF-A: Vascular endothelial growth factor-A.
increase in hydroxyproline concentrations.

The antioxidant N-acetyl-cysteine (NAC) significantly increased the hydroxyproline level; histological evaluation also revealed increased collagen deposition compared with the I/R injured control group, independent of the administration route[53].

Other compounds that prevented I/R-induced reductions in anastomotic patency included tempol[55], the immunomodulating compounds activated protein C[58], caffeic acid phenethyl ester[59] and pyrrolidine dithiocarbamate[54].

One study reported significantly increased anastomotic BPR and hydroxyproline levels after treatment with montelukast administered intraperitoneally[52].

Colonic obstruction

Four different agents were investigated in models of colonic obstruction (Table 3). In these models, typically the left-sided colon was obstructed by suture ligation for 24 h. Re-laparotomy was then performed, the obstructed segment was excised, and an end-to-end anastomosis was constructed[23,60-63].

In rats with an obstructed colon, intraoperative lavage with povidone iodine (PI) increased anastomotic BPR significantly on day 6 compared with untreated controls in two independent studies[23,60]. Because the BPR was similar to lavage with saline alone, the additional value of PI remains questionable[23,60]. NG-nitro-L-arginine methyl ester, an inhibitor of nitric oxide synthase, was found to be detrimental to anastomotic healing in rats with an obstructed colon[23]. Intra-abdominal irrigation is time consuming, costly, cumbersome and possibly increases the risk of spillage[64,65]. These circumstances should be taken into account when investigating new lavage agents.

EPO administered after construction of the anastomosis of the obstructed colon significantly increased BPR on day 7 in rats and guinea pigs[64,65]. EPO possibly enhanced anastomotic healing through increased neovascularization and fibroblast proliferation leading to more collagen in the anastomotic wound[65,63]. Therefore, further exploration of EPO to improve anastomotic

Table 2  Studies on therapeutic compounds in ischemia/reperfusion injury models

| Ref.       | Compound       | Time of administration | Species | Sex | Sample size | Dosage | Route | Test | Test day | Effect |
|------------|----------------|------------------------|---------|-----|-------------|--------|-------|------|----------|--------|
| Tekin et al[54] | AT III        | Preoperative and postoperative | Rat | M | 16 | 250 µg | iv | BPR | 6 | | |
| Unal et al[57] | Ethyl pyruvate | Preoperative and postoperative | Rat | M | 24 | 50 mg | IP | BPR | 5 | | |
| Kabali et al[58] | NAC          | Preoperative | Rat | F | 30 | 300 mg | PO/IP | BPR | 7 | | |
| Aydin et al[59] | Tempol       | Preoperative and postoperative | Rat | M | 20 | 30 µg | iv | BPR | 5 | | |
| Teke et al[52] | Activated protein C | Preoperative and postoperative | Rat | M | 24 | 0.1 µg | iv | BPR | 7 | | |
| Teke et al[52] | Caffeic acid phenethyl ester | Preoperative and postoperative | Rat | M | 24 | 0.028 µg | iv + IP | BPR | 7 | | |
| Teke et al[52] | Pyrrolidine dithiocarbamate | Preoperative and postoperative | Rat | M | 20 | 100 µg | iv | BPR | 6 | | |
| Celik et al[52] | Montelukast   | Preoperative and postoperative | Rat | M | 24 | 10 mg | IP | BPR | 5 | | |

| Sample size | Dosage | Route | Test | Test day | Effect |
|-------------|--------|-------|------|----------|--------|

Heterogeneity: Tau = 49.94, χ² = 5.02, df = 3 (P = 0.17); I² = 40%
Test for overall effect: Z = 5.03 (P < 0.00001)

Heterogeneity: Tau = 265.25, χ² = 26.60, df = 2 (P < 0.00001); I² = 92%
Test for overall effect: Z = 0.03 (P = 0.97)

**Figure 3** Forest plots of the bursting pressure in mmHg. The results of the meta-analysis for A: Hyperbaric oxygen therapy (HBOT) at days 4-5 in ischemic models (confer, Table 1); B: Granulocyte macrophage-colony stimulating factor (GM-CSF) at days 3 and 7 in chemotherapeutic models (confer, Table 5).
wound healing under these conditions seems justified.

Iloprost increased BPR on days 4 and 8 in rats, possibly by stimulating angiogenesis and fibroblast activity[62]. Moreover, iloprost reduced the levels of immunodetectable MMP-13 in the anastomotic tissue, which may explain the increased collagen deposition with iloprost. Significantly more intra-abdominal adhesions formed, which were assessed according to the scale of van der Ham et al[66], in the rats with obstruction compared with the animals without obstruction[62]. The prostacycline analog iloprost did not reduce adhesion formation in the obstructed animals[63]. Although iloprost seemed to reduce AL (10% vs 30% for saline controls), this difference did not reach statistical significance[63].

**Obstructive jaundice**

Obstructive jaundice was modeled by ligation of the common bile duct. The anastomosis was constructed 7 d later[72]. GM-CSF, the only agent investigated in this clinical condition, increased BPR and the hydroxyproline level[73] (Table 3). Increased mononuclear infiltration of the anastomoses was suggested to be the mechanism for the improved anastomotic wound healing with GM-CSF[17].

**Peritonitis**

Sixteen different compounds were identified, none of which qualified for the meta-analysis (Table 4). Experimental peritonitis is commonly established by puncture of the colon[67], ligated cecum[66-71], or intraperitoneal administration of fecal suspension[72]. *Escherichia coli* suspension[73] or the Gram-negative wall component lipopolysaccharide (endotoxin)[74]. Anastomoses were then performed 5-14 h later. In one study, the cecum was ligated and punctured after the anastomosis was performed[75].

In one study, the vasomodulating agent sildenafil was administered intraperitoneally after the anastomoses were constructed in female rats with peritonitis. Sildenafil decreased intra-abdominal adhesions and increased BPR by 43% on day 7 compared with the controls that received saline alone[67]. Furthermore, sildenafil stimulated new vessel formation in the anastomoses[67].

In another rodent peritonitis model, intravenous administration of the thrombin inhibitor ATIII increased anastomotic BPR by 34% on day 2 and by 38% on day 7 compared with the peritonitis control[69]. Diller et al[45] attributed the improved anastomotic healing with ATIII to increased numbers of perfused capillaries and reduced clot formation, although these improvements failed to reach the levels of the non-infected controls.

Unfractionated heparin (UFH) and low molecular weight heparin (LMWH) prevented the reduction in BPR in animals with peritonitis[72]. Histopathological examinations revealed increased vascularization and collagen formation of the anastomotic wounds treated with UFH and LMWH[72]. Notably, UFH and LMWH facilitate intraperitoneal bacterial clearance by preventing formation of fibrin that may act as a reservoir for bacteria[72].

Ethyl pyruvate increased BPR by 51%, possibly due to its anti-inflammatory effects[76].

Reactive oxygen species (ROS) are thought to delay wound healing under septic conditions. Tempol is a stable piperidine nitroxide that may dampen the negative impact of ROS through its intracellular scavenging capacity. Tempol also restored glutathione levels in the anastomoses failed to reach the levels of the non-infected controls.

The anti-inflammatory immunomodulating granulocyte-CSF (G-CSF)[75], activated protein C[69], caffeic acid phenethyl ester[70] and pyrrolidine dithiocarbamate[71] are attractive for prevention of the deleterious effects of peritonitis and also increase anastomotic BPR. Although G-CSF increased hydroxyproline levels, they were still lower than in the normal control group without peritonitis[72]. Activated protein C has been shown to reduce 28-d all-cause mortality in sepsis patients and is now approved for the treatment of patients with severe sepsis[69,71]. The usefulness of activated protein C in colonic anastomotic wound healing in the presence of peritonitis will require more study because of the increased risk of bleeding[77].

Abdominal lavage with the taurine derivative tauro-
Table 4  Studies on therapeutic compounds in models of peritonitis

| Ref.                  | Compound          | Time of administration | Species | Sex     | Sample size | Dosage | Route | Test | Test day | Effect |
|-----------------------|-------------------|------------------------|---------|---------|-------------|--------|-------|------|----------|--------|
| Ayten et al [81]      | Sildenafil         | Postoperative          | Rat     | F       | 14          | 8³     | IP    | BPR  | 7        | ↑ 43   |
| Diller et al [84]     | AT III            | Intraoperative         | Mouse   | M       | 60          | 250⁴   | iv    | BPR  | 2        | ↑ 34   |
| Guerhan et al [79]    | UFH               | Postoperative          | Rat     | M       | 45          | 50³    | SC    | BPR  | 7        | ↑ 12   |
| Oner et al [84]       | Ethyl pyruvate    | Postoperative          | Tempol  | N/A     | 20          | 50³    | IP    | BPR  | 7        | ↑ 51   |
| Aytekin et al [80]    | Tempol            | Preoperative and postoperative | M | 20 | 50³ | iv | BPR | 6 | ↑ 10 |
| Ergin et al [77]      | G-CSF             | Preoperative and postoperative | M | 20 | 0.50³ | SC | BPR | 4 | ↑ 26 |
| Teke et al [85]       | Levamisole        | Preoperative and postoperative | M | 24 | 0.1³ | PO | BPR | 7 | ↑ 9 |
| Teke et al [79]       | Calcium pantothenate | Preoperative and postoperative | M | 24 | 0.0028³ | IP | BPR | 7 | ↑ 9 |
| Teke et al [79]       | Pirrolidine       | Preoperative and postoperative | M | 20 | 0.1³ | iv | BPR | 9 | ↑ 9 |
| Akkus et al [79]      | Tauroline         | Intraoperative         | Rat     | 40     | 0.5³ | Lavage | BPR | 3/7 | ↑ 26/↑ 12 |
| Bicalho et al [79]    | Chlorhexidine     | Intraoperative         | Rat     | 16     | 0.05³ | Lavage | BPR | 7 | NS |
| Wang et al [84]       | Hydroxyethyl starch | Preoperative and postoperative | M | 32 | 7.3³ | iv | BPR | 5 | NS |
| Wang et al [84]       | Hydroxyethyl starch | Preoperative and postoperative | M | 20 | 15³ | iv | BPR | 9 | ↑ 9 |
| Sucullu et al [89]    | HBOT              | Postoperative          | Rat     | M,F    | 32          |         |       | BPR  | 3/7 | ↑ 186/↑ 74 |
| Rocha et al [89]      | HBOT              | Postoperative          | Rat     | M       | 30          |         |       | BST  | 5 | NS |
| Vaneerdegew et al [89]| Gentamicin        | Intraoperative         | Rat     | M       | 30          | 2.6µ/12³ | LO/IM | BPR | 4 | NS/NS |

¹Total number of animals; ²% increase (P < 0.05) or % decrease (P < 0.05) vs controls; ³µg/kg; ⁴IU/kg; ⁵%‐solution; ⁶mL/kg; ⁷mg. AT III: Antithrombin 3; BPR: Bursting pressure; BST: Breaking strength; F: Female; G‐CSF: Granulocyte‐colony stimulating factor; HBOT: Hyperbaric oxygen therapy; IM: Intramuscular; IP: Intrapерitoneal; iv: Intravenous; LMWH: Low molecular weight heparin; LO: Local; M: Male; N/A: Data not available; NS: Not statistically significant; PO: Per os; SC: Subcutaneous; UFH: Unfractionated heparin.

lidine increased BPR on days 3 and 7 [73]. Chlorhexidine lavage had no significant effect on BPR compared with 4‐time lavage with 5 mL of sterile saline prior to construction of the anastomosis [78].

Intravenous administration of hydroxyethyl starch (HES) at 15 mL/kg increased BPR on postoperative day 5 in two studies carried out by the same research group [79,80], whereas 30 mL/kg was detrimental to anastomotic healing [80]. These findings may be explained by the anti‐inflammatory effects of HES at 15 mL/kg [79,80] whereas at higher doses, HES reduces platelet aggregation to the injured endothelium [80]. These facts make HES less practical for use in a clinical setting.

Postoperative HBOT in rats increased BPR by 186% on day 3 and by 74% on day 7 [81]. In another study, HBOT had no effect on BST on day 5 [82].

Local or systemic application of gentamicin [83], as well as levamisole [84], had no effect on BPR in male rats with peritonitis.

Chemotherapy

Five different compounds were tested in rats treated with different chemotherapeutic agents (Table 5). In these studies, 5‐fluorouracil was given preoperatively [84,85] or on postoperative days 3–5 [86–90]. Mitomycin‐C was given as a single intraoperative dose [91].

Three studies investigated the effect of GM‐CSF [86,87,91] and were subjected to meta‐analysis. The combined estimate demonstrated that GM‐CSF failed to increase anastomotic BPR (95%CI: -20 to 21 mmHg, P = 0.97) compared with controls (Figure 3B). The inconsistency between studies was large (I² = 92%). The two studies demonstrating improved anastomotic healing also reported a significantly increased hydroxyproline concentration in the anastomoses, as well as distinct histological changes, including increased mononuclear infiltration compared with chemotherapy alone (fluorouracil or mitomycin‐C) [86,91]. de Waard et al [87] found that GM‐CSF increased BPR, but not BST, in fluorouracil‐treated rats. They also applied a considerably lower dose of GM‐CSF (5 µg) [87] than the other two research groups (50 µg) [86,91]. In addition, GM‐CSF was administered intraperitoneally and not locally. Taken together, these data indicate that the GM‐CSF dose used by de Waard et al [87] was too low. In contrast, a single local application of GM‐CSF in expanded polytetrafluoroethylene tubes implanted subcutaneously in humans inhibited collagen deposition dose‐dependently and resulted in systemic effects on wound healing at doses of 4 µg or more [82].

Iloprost enhanced BPR anastomotic healing on post‐operative day 3 [89] and day 5 [89] compared with both the chemotherapeutic group and the non‐chemotherapeutic
Table 5 Studies on therapeutic compounds in chemotherapeutic model

| Ref. | Compound | Time of administration | Species | Sex | Sample size | Dosage | Route | Test | Test day | Effect |
|------|----------|------------------------|---------|-----|-------------|--------|-------|------|----------|--------|
| Cetinkaya et al[80] | GM-CSF | Postoperative | Rat | N/A | 54 | 0.05 | LO | BPR | 3 | ↑ 26 |
| Erdem et al[80] | GM-CSF | Postoperative | Rat | N/A | 30 | 0.05 | LO | BPR/BST | 3 | ↑ 98 |
| de Waard et al[80] | GM-CSF | Postoperative | Rat | M | 31 | 0.005 | IP | BPR/BST | 7 | ↓ 35/NS |
| Interleukin-2 | | | | | | | | | | |
| Bostanoglu et al[80] | Iloprost | Postoperative | Rat | M | 38 | 0.002 | N/A | BPR | 3/7 | ↑ 63/NS |
| Vasilidas et al[80] | Iloprost | Postoperative | Rat | F | 34 | 0.002 | IP | BPR | 5/8 | ↓ 44/NS |
| Zacharakis et al[80] | IGF-1 | Postoperative | Rat | M | 32 | 2 | IP | BPR/AL | 7 | 30/30 |
| Erenoglu et al[80] | HBOT | Postoperative | Rat | M | 20 | | | BPR | 7 | ↑ 26 |
| Yildiz et al[80] | HBOT | Postoperative | Rat | F | 24 | | | BPR | 5 | NS |

1Total number of animals; ↑% increase (P < 0.05) or ↓% decrease (P < 0.05) vs controls; ↑mg/kg; ↑IU/kg; ↑Chemoradiotherapy model. AL: Anastomotic leakage; BPR: Bursting pressure; BST: Breaking strength; F: Female; GM-CSF: Granulocyte macrophage-colony stimulating factor; HBOT: Hyperbaric oxygen therapy; IGF-1: Insulin-like growth factor-I; IP: Intraperitoneal; LO: Local; M: Male; N/A: Data not available; NS: Not statistically significant; SC: Subcutaneous.

Table 6 Studies on therapeutic compounds in models of radiotherapy

| Ref. | Compound | Time of administration | Species | Sex | Sample size | Dosage | Route | Test | Test day | Effect |
|------|----------|------------------------|---------|-----|-------------|--------|-------|------|----------|--------|
| Demir et al[80] | NAC | Preoperative and postoperative | Rat | M | 24 | 300 | PO/IP | BPR | 4 | ↑ 126/182 |
| Odedemir et al[80] | Amifostine | Preoperative and postoperative | Rat | F | 20 | 200 | IP | BPR | 5 | ↑ 16 |
| Carroll et al[80] | Ribose-cysteine | Preoperative | Rat | M | 72 | 200 | IP | BPR | 7 | ↑ 50 |
| Ribose-cysteine | Preoperative | Rat | M | 72 | 200 | IP | BPR | 7 | ↑ 50 |
| Ribose-cysteine + glutamine | Preoperative and postoperative | Pig | 12 | 100 | IP | BPR | 9/11 | NS |
| Amifostine | Preoperative | Rat | M | 250 | IP | | | | |
| Amifostine | Preoperative | Rat | M | 250 | IP | | | | |
| MgCl2 + ATP | Preoperative | Pig | 10 | 50 | 70 GY | | | | |
| Rowe et al[80] | Ribose-cysteine | Preoperative | Pig | 12 | 100 | PO | BPR | 3/7 | ↑ 19/38 |
| Değer et al[80] | Pycnogenol | Preoperative and postoperative | Rat | M | 40 | 200 | PO | BPR | 18 | 18 |
| Ozel Turkcu et al[80] | EPO | Preoperative | Rat | M | 16 | 500 | IM | BPR | N/A | NS |

1Total number of animals; ↑% increase (P < 0.05) vs controls; ↑40 GY; ↑70 GY; ↑mg/kg; ↑% solution; ↑g/kg; ↑IU/kg. ATP: Adenosine triphosphate; BPR: Bursting pressure; EPO: Erythropoietin; F: Female; IM: Intramuscular; IP: Intraperitoneal; iv: Intravenous; M: Male; NAC: N-acetyl cysteine; N/A: Data not available; NS: Not statistically significant; PO: Per os.

group, but not at later time points[88,89]. Interestingly, iloprost significantly decreased the rate of AL from 30% to 0% in rats receiving chemotherapy[90]. The positive effects on anastomotic strength in models of chemotherapy were possibly due to increased angiogenesis[90] and collagen deposition with iloprost[88,90]. Furthermore, iloprost significantly reduced the severity of intra-abdominal adhesions compared with the chemotherapeutic control group[90].

A single study on IGF-1 treatment reported normalization of anastomotic BPR and hydroxyproline levels on day 7 in fluorouracil-treated rats[89]. IGF-1 had no significant effects on AL[89].

Postoperative HBOT increased BPR on day 7 in one study[84] but had no significant effect on day 5 in another study, in which the rats received combined chemo- and radiotherapy before surgery[85].

Interleukin-2 administered postoperatively had no effect on either BPR or BST[87].

Radiotherapy

None of the eight agents identified and investigated in radiotherapy models qualified for meta-analysis (Table 6). Animals were irradiated with half-body radiation[22,93] or abdomino-pelvic radiation[94-96] in a single dose and as rectosigmoid radiation[97] 5 d a week for 40-45 d.

NAC is theoretically attractive in preventing oxidative damage after radiotherapy. NAC administered before and after construction of the anastomosis also increased BPR[90]. In one study, NAC treatment increased the levels of superoxide dismutase and glutathione, but decreased malondialdehyde[90]. Superoxide dismutase and glutathione are known to neutralize toxic substances in the cell, whereas malondialdehyde is a marker for oxidative stress[90].

Other compounds found beneficial for the prevention of the deleterious effects of preoperative radiation include amifostine[93,94] and magnesium chloride in combination with adenosine triphosphate[93].

Ribose-cysteine[93] administered before surgery improved anastomotic healing day 7 in rats receiving abdominal radiation with 40 GY, but not in irradiated pig colon days 9-11[97]. No beneficial effects were reported by Carroll et al[93], who investigated the effects of ribose-
cysteine combined with glutamine. Pycnogenol administered preoperatively increased BPR on postoperative days 3 and 7 in male rats[27]. EPO had no significant effect on anastomotic strength[95].

DISCUSSION

We have previously reviewed therapeutics intended to enhance normal anastomotic repair in the colorectal region[28]. In this paper we systematically retrieved publications on therapeutic agents intended to promote anastomotic wound healing under the influence of complicating factors, including ischemia, I/R injury, colonic obstruction, obstructive jaundice, peritonitis, chemotherapy and radiotherapy. The majority of the 48 different therapeutic compounds identified were only assessed in one study and/or in one complicated model. Meta-analysis was performed for HBOT and GM-CSF. Postoperative HBOT significantly improved wound healing in a rat model complicated by ischemia in the anastomosis. GM-CSF failed to show a beneficial effect on anastomotic healing in conjunction with chemotherapy. On the other hand, positive effects of GM-CSF were found in models of segmental ischemia[46] and obstructive jaundice[17]; these findings make this agent interesting for further investigation. Iloprost was found to be beneficial for early healing of anastomotic wounds in rats with colonic obstruction[62] and in rats exposed to chemotherapy[88,90], calling for further studies with this agent. The positive actions of NAC after I/R injury[93] and radiotherapy[98] justify further investigations on this antioxidant, as well.

Limitations

Because the 65 pre-clinical studies examined in our review used surrogate outcomes in models mimicking clinical phenomena, the results do not directly translate into clinical AL. Only 12 (25%) of the agents were investigated more than once in the same model. Furthermore, 13 (27%) therapeutic compounds were tested in two or more models of complicated anastomotic wound healing. We were unable to assess publication bias, for example, with Funnel plots, due to the small sample sizes of the included studies[96].

Further exploration of the therapeutic agents identified in this review may be the next step to reach more robust conclusions regarding whether the agents could be effective in preventing AL in high-risk patients.

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