A neurokinin-1 receptor antagonist that reduces intra-abdominal adhesion formation decreases oxidative stress in the peritoneum

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INTRA-ABDOMINAL ADHESIONS LEAD TO SIGNIFICANT POSTOPERATIVE MORBIDITY, INCLUDING SMALL-BOWEL OBSTRUCTION, INFERTILITY, AND CHRONIC PELVIC PAIN (33). ADHESIONS OCCUR IN UP TO 94% PATIENTS THAT HAVE UNDERGONE ABDOMINAL OPERATIONS (6) AND INCUR A SIGNIFICANT FINANCIAL BURDEN ON THE HEALTHCARE SYSTEM (39). ADHESIONS HAVE BEEN RECOGNIZED FOR MORE THAN 250 YEARS (33). ADHESIONS OCCUR IN UP TO 94% PATIENTS THAT HAVE UNDERGONE ABDOMINAL OPERATIONS (6) AND INCUR A SIGNIFICANT FINANCIAL BURDEN ON THE HEALTHCARE SYSTEM (39).

Adhesiogenesis is a complex interaction of cellular components involved in inflammation and wound repair. Abdominal operations initiate an acute inflammatory response in the peritoneum, and this postoperative inflammation, although part of the normal healing response, leads to deposition of a fibrin-rich matrix capable of forming permanent attachments to adjacent viscera (33). Peritoneal mesothelial cells and newly recruited neutrophils, mast cells, and macrophages secrete proinflammatory mediators, including cytokines, growth factors, nitric oxide, and reactive oxygen species (ROS), that may contribute to adhesion formation (13). Pursuant to the latter, studies suggest that acute oxidative stress in the peritoneum subsequently induces mesothelial cell loss or dysfunction, peritoneal fibrosis, and intra-abdominal adhesion formation (23). In support of this, agents that reduce oxidative stress such as vitamin E, allopurinol, superoxide dismutase, and catalase have been shown to limit adhesion formation in animal models (16, 42, 51).

In mice, it was shown that oral administration of allopurinol, a xanthine oxidase inhibitor, reduced the severity of peritoneal adhesions, purportedly by limiting the production of the superoxide radical (42). In other studies, reduction of oxidative stress by intraperitoneal administration of NO donors (NOnOates) or the NO precursor L-arginine was shown to reduce adhesion formation in rats (10, 35). Furthermore, ten Raa et al. (51) demonstrated that the antioxidant enzymes superoxide dismutase and catalase reduce adhesion formation in a rat model. In addition, intraperitoneal methylene blue and melatonin, possibly via their antioxidant properties, have been shown to reduce adhesions in a rat model (4, 19, 24). Studies in rabbits (17) have shown that pneumoperitoneum alone increases peritoneal oxidative stress, and a recent study in rats (23) showed that acute oxidative stress induced by intraperitoneal injection of the oxidant agent deoxycholate resulted in extensive fibrosis, mesothelial cell loss, and adhesion formation.

Recently, we showed that the intraperitoneal administration of a nonpeptide neurokinin-1 receptor (NK-1R) antagonist inhibits adhesion formation in a rat model (15, 41). Because the proinflammatory peptide substance P, the best-characterized NK-1R ligand, can induce leukocyte adhesion and ROS generation (14), it follows that the NK-1R antagonist would also reduce ROS formation. In support of this, rat peritoneal mast cells have been shown to generate intracellular ROS after stimulation with nanomolar concentrations of substance P (9), and Substance P has been shown to increase leukocyte ROS production in a dose-dependent manner (12). Substance P has also been shown to induce rapid mobilization of adhesion molecules (i.e., VCAM-1 and ICAM-1) to the cell surface at sites of tissue injury, leading to the homing and extravasation of leukocytes and neutrophils, primary sources of ROS (38).

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Chien et al. (14) showed that exogenous substance P increases ROS in the bladder and whole blood via enhanced ICAM expression and that this response is blocked by treatment with a NK-1R antagonist. Furthermore, pretreatment of rats with the specific NK-1R antagonist SR-140333 was shown to reduce recruitment of leukocytes to the peritoneum within 4 h of formalin injection (44), and administration of another NK-1R antagonist, CP-96345, was demonstrated to decrease inflammation and oxidative stress in a rat colitis model (48). This antagonist has also been shown to suppress mRNA expression of ICAM-1 in mouse lung during acute pancreatitis (30). A potential role for activation of the NK-1R in adhesion formation is further supported by the fact that substance P is present in peritoneal fluid (43), substance P and NK-1R mRNA levels increase in peritoneal tissue following abdominal operation (40), and substance P-containing sensory neurons have been demonstrated in peritoneal adhesions (50).

The aim of this study was to examine the possibility that the NK-1R antagonist CJ-12255 (Pfizer, Groton, CT) inhibits adhesion formation, in part, by reducing oxidative stress in the postoperative peritoneum. The effect of NK-1R antagonist administration on key markers of oxidative stress and inflammation, including 8-isoprostane (8-IP), protein carbonyl, NADPH oxidase, and myeloperoxidase (MPO), was determined in rat peritoneal tissue following a surgical procedure to induce adhesions. Protein carbonyl content is a commonly used marker of protein oxidation (46), and tissue 8-IP, a lipid-peroxidation byproduct, is also a reliable biomarker of oxidative stress in vivo (31). Elevated levels of 8-IP and protein carbonyl have been documented in tissues from animals and humans with conditions associated with oxidative stress (18). NADPH oxidase and MPO are enzyme markers of leukocyte infiltration and are also considered reliable indicators of oxidative stress in inflamed tissue. The production of superoxide by activated neutrophils and other phagocytic cells is catalyzed by NADPH oxidase, whereas MPO, predominantly synthesized in neutrophils, leads to formation of hypochlorous acid (45). Superoxide and hypochlorous acid are major components of the phagocytic cells’ bactericidal mechanisms that can lead to oxidative tissue damage.

The effect of a specific NK-1R antagonist on peritoneal oxidative stress has not, to our knowledge, been examined. The results indicate that the NK-1R may be involved in postoperative oxidative stress and adhesion formation following abdominal surgery. The NK-1R antagonist may reduce intra-abdominal adhesion formation, in part, by decreasing the level of damaging ROS in the postoperative peritoneal environment through an inhibition of neutrophil recruitment to the peritoneal cavity and an increase in peritoneal fluid antioxidant capacity.

**MATERIALS AND METHODS**

**Materials.** All chemicals were obtained from Sigma (St. Louis, MO) unless otherwise noted. The nonpeptide NK-1R antagonist (3R,4S,5S,6S)-6-diphenylmethyl-1-(5-isopropyl-2-methoxybenzylamino)-1-azabicyclo[2.2.2]octane-3-carboxylic acid (CJ-12255) was a gift from Pfizer (Groton, CT). CJ-12255 is a structurally related analog of the parent compound CJ-11974 (Ezlopitant; Pfizer) that is highly specific for the NK-1R with no affinity for the NK-2 or NK-3 receptors. CJ-12255, when administered intraperitoneally at the dose used in this study (25 mg/kg), has been shown to completely block a substance P-induced salivary response in rats (47).

**Animals.** Male Wistar rats weighing between 200 and 250 g (Charles River Labs, Wilmington, MA) were used for all experiments. The rats were housed at a constant room temperature, with 12-h light and dark cycles, and were provided standard rodent chow and water ad libitum. The Institutional Animal Care and Use Committee at Boston University School of Medicine approved these studies, and all procedures and animal care were performed in accordance with recommendations outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

**Induction of intra-abdominal adhesions and experimental design.** Intra-abdominal adhesions were induced in rats as we have previously described (40). Briefly, laparotomies via a midline incision were performed under 3–4% isoflurane anesthesia, and four ischemic buttons, spaced 1 cm apart, were created on both sides of the parietal peritoneum. Adhesion formation was quantifiable 7 days after surgery. Sham-operated animals could not be used in this study because the laparotomy itself induces adhesion formation to the midline incision as well as biochemical changes to the peritoneal wall in uninjured areas (40).

To assess the effect of the NK-1R antagonist on biomarkers of oxidative stress, adhesions were surgically induced in 32 rats randomized to experimental groups that were lavaged, at the time of surgery, with 1 ml of the NK-1R antagonist CJ-12255 (25 mg/kg) or sterile saline (vehicle). Eight animals per time point (4 vehicle and 4 antagonist) were killed at 2, 4, 6, and 24 h after surgery, and peritoneal tissue was collected from within a 0.5-cm radius of the ischemic buttons as previously described (40). Peritoneal fluid, collected in 5 mM citrate and 0.1 M acetate (final concentration), was also taken for analysis of antioxidant capacity. For comparison, peritoneal tissue and fluid samples were also collected from nonoperated animals (n = 4). The 6 h (n = 4/group) and 24 h (n = 3/group) time points, as well as three nonoperated controls, were repeated in a second experiment. All samples were immediately frozen in liquid nitrogen and were stored at −80°C until use. Protein levels were determined for each sample (tissue homogenate or peritoneal fluid) by using the method of Bradford (7). Adhesion formation was assessed in another group of animals that were killed at 7 days (n = 6 for NK-1R antagonist and n = 11 for vehicle). Adhesion formation was quantified in a blinded fashion, with each animal receiving a percent adhesion score based on the number of ischemic buttons with attached adhesions.

**NADPH oxidase assay.** The NADPH oxidase capacity of peritoneal tissue samples was determined by using a lucigenin-amplified chemiluminescence assay, the most sensitive method of superoxide detection, which measures the NADPH oxidase-mediated ROS-generating system (2, 20). Peritoneal tissue samples were homogenized via mortar and pestle, under liquid nitrogen, in 3 volumes of phosphate-buffered saline containing 10 mM Tris, pH 7.4, and 1 mM EDTA. The homogenates were allowed to thaw on ice, and NADPH oxidase capacity, an indicator of oxidative stress, was measured immediately by mixing 50 μl of homogenate with 950 μl of reaction mix (phosphate-buffered saline containing 5 μM lucigenin, 2 mM CaCl₂, and 100 μM NADPH) in the dark. Chemiluminescence counts were integrated over a 2- to 4-min period from the time of the addition of 100 μM NADPH to plateau with a TD 20e luminometer (Turner Biosystems, Sunnyvale, CA). Light emission was measured as mean arbitrary light units per minute and was expressed as the percent of nonoperated control levels.

**MPO assay.** Tissue MPO activity, an enzyme marker of inflammatory and neutrophil infiltration, can be used as a marker for neutrophil content (8). Peritoneal tissue samples were assayed for MPO, as described by Barone et al. (5), with the following modifications. Approximately 20 mg of tissue was homogenized on ice with a Polytron tissue homogenizer in 4 ml ice-cold 5 mM phosphate buffer, pH 6.0. The homogenate was centrifuged at 30,000 g for 30 min at 4°C. The supernatant was discarded, the pellet was resus-
pendent in phosphate buffer, and the homogenization procedure was then repeated. The second pellet was washed three times with ice-cold 5 mM phosphate buffer, pH 6.0, and then resolubilized in 10 volumes of ice-cold 0.5% hexadecyltrimethylammonium bromide in 50 mM phosphate buffer by sonication on ice at full power for 10–15 s. Sonication was repeated four times with 30-s cooling on ice between bursts. The extract was then incubated at 4°C for 20 min and was centrifuged at 12,500 × g for 15 min at 4°C. MPO activity in the supernatant was assayed, in a 96-well microtiter plate, by mixing 20 μl of the supernatant (either undiluted or diluted 1:2, 1:5, and 1:10 with 50 mM phosphate, pH 6.0) with 80 μl of assay buffer (80 mM phosphate buffer, pH 5.4, 0.48 mg/ml tetramethylbenzidine, 0.001% hydrogen peroxide). The change in absorbance was measured with a Spectra Max 250 spectrophotometer (Molecular Devices, Sunnyvale, CA) over 3 min, and one unit of MPO activity was defined as that degrading 1 μmol of hydrogen peroxide per minute.

Measurement of 8-IP and protein carbonyl groups. Protein carbonyl content and 8-IP, well-established biomarkers of oxidative damage to protein and lipid, respectively, were measured as follows (32). Peritoneal tissue samples were homogenized under liquid nitrogen via mortar and pestle in 3 volumes of phosphate-buffered saline containing 10 mM Tris, pH 7.4, 1 mM EDTA, and 10 μM butylated hydroxytoluene. The homogenates were then centrifuged at 10,000 × g for 10 min, and the supernatants were taken. Concentrations of 8-IP, a marker of lipid peroxidation, were determined by mixing 50 μl of the supernatant with an equal volume of 5.35 M KOH and were neutralized with HCl, and 15 μl of sodium acetate, pH 3.5, was added before extraction with 0.5 ml ethyl acetate. The extracts were dried under nitrogen and were resuspended in 100 μl enzyme immunoassay (ELIA) buffer and then were assayed by using a commercially available 8-IP ELIA kit (Cayman Chemical, Ann Arbor, MI) according to the manufacturer’s instructions. The protein carbonyl content was determined in 500 μl of the supernatant by using a protein carbonyl assay kit (Cayman Chemical) according to the manufacturer’s instructions.

RNA isolation and relative RT-PCR. Total RNA was isolated from 20–50 mg of peritoneal tissue by using the SY Total RNA Isolation System (Promega, Madison, WI) according to the manufacturer’s instructions. Reverse transcription of total RNA samples and PCR were accomplished by using the GeneAmp RNA PCR kit (Applied Biosystems, Foster City, CA), and PCR was carried out in a reaction volume of 20 μl by using bulk master mixes, except template, prepared for multiple reactions as previously described (40). The PCR cycle number was determined empirically for each primer set such that amplification of products was linear with respect to the amount of input RNA. The primer sequences and cycling conditions for ICAM-1 and VCAM-1 are described elsewhere (40). PCR products (15 μl) were subjected to electrophoresis on 2% agarose gels containing 0.03 μg/ml ethidium bromide, and quantitation of transcript level was carried out by analysis of scanned photographs of gels by using Scion Imaging software (Scion, Frederick, MD). Levels of mRNA expression were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a constitutively expressed gene that did not significantly vary among treatment groups.

Total antioxidant status. The antioxidant capacity of peritoneal fluid was determined by using the Total Antioxidant Status kit (Randox, Oceanside, CA) according to the manufacturer’s instructions. The principle of this assay is based on the generation of a free radical produced by the incubation of 2,2′-azino-di-[3-ethylbenzthiazoline sulphonate] (ABTS) with a peroxidase (methmyoglobin) and hydrogen peroxide to produce the radical cation ABTS+. The presence of antioxidants in the sample quenches ABTS+ in proportion to their concentration. For relative comparison, the water-soluble vitamin E analog Trolox (0, 31.2, 62.5, 125, 250, and 500 μM) was also assayed, thereby allowing the data to be presented as Trolox equivalents.

Statistical analysis. Log-transformed data were analyzed by one-way ANOVA using SIGMASTAT software (SPSS, Chicago, IL). When significant effects (P < 0.05) were detected, the differences between specific means were determined with the Student-Newman-Keuls test. Groups were deemed to be significantly different from one another when P < 0.05. All data are expressed as means ± SE.

RESULTS

The NK-1R antagonist reduces adhesion formation. The ischemic button model of adhesion formation is a well-validated and reproducible surgical model developed in this laboratory (40), whose major advantage is that adhesion formation can be objectively quantified. Typically, >70% of the buttons form adhesions to various structures within the peritoneal cavity, such as the small intestine and liver. In the current study, a single bolus dose of the NK-1R antagonist (25 mg/kg) was administered at the time of surgery, a dosing regime we previously demonstrated as effective in reducing adhesion formation at 7 days (15). The animals administered the antagonist (n = 6) had 33.3 ± 8.6% adhesions, a 57% reduction in adhesion formation (P < 0.05), compared with the vehicle-control animals (n = 11), which had 77.1 ± 9.2% of their ischemic buttons associated with adhesions. These results confirm our previously published data (15).

The NK-1R antagonist delays neutrophil recruitment to peritoneal tissue following surgery. Abdominal surgery increases peritoneal oxidative stress. Neutrophil infiltration was evident by 2 h following surgery, and by 6 h significant numbers of neutrophils had infiltrated the adhesion site (Fig. 1A, vehicle) and were associated with a significant respiratory burst (Fig. 1B, vehicle). MPO levels in vehicle-administered animals were significantly (P < 0.05) increased by 84-fold and 125-fold at 6 and 24 h after surgery, respectively, compared with nonoperated levels. This increase was delayed in animals administered the NK-1R antagonist. At the 4- and 6-h time points, the tissue MPO levels for NK-1R antagonist-administered compared with vehicle-administered animals were 12.5 vs. 19.8 U/g protein (P = 0.079) and 13.25 vs. 35.9 U/g protein (P < .001), respectively. However, by 24 h there was no difference in tissue MPO levels for NK-1R antagonist- and vehicle-administered animals (53.7 vs. 52.7 U/g protein; Fig. 1A). NADPH oxidase capacity, measured in vitro, was significantly (P < 0.05) increased at 6 h by 115% and at 24 h by 205% compared with samples from nonoperated animals (arbitrary units set at 100%). Administration of the NK-1R antagonist did not affect these increases (Fig. 1B).

The NK-1R antagonist reduces postoperative levels of adhesion molecule mRNA in peritoneal tissue. The effect of NK-1R antagonist administration on ICAM-1 (Fig. 2A) and VCAM-1 (Fig. 2B) mRNA expression in postoperative peritoneal tissue was assessed. ICAM-1 mRNA levels were significantly (P < 0.05) increased at 2, 4, and 6 h following surgery by 696, 545, and 376%, respectively, compared with levels in nonoperated tissues (arbitrary units set at 100%). Administration of the NK-1R antagonist led to a significant (P < 0.05) reduction (~50%) in ICAM-1 mRNA levels at 2 h. At 4 and 6 h, ICAM-1 mRNA levels were lower in the NK-1R antagonist-administered animals compared with the vehicle controls; however, the differences were not significant. By 24 h after surgery, ICAM-1 mRNA levels in both groups were the same as that in nonoperated animals. Although there was not a

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postoperative increase, the mRNA levels for VCAM-1 were significantly lower (P < 0.05) in NK-1R antagonist-administered animals than in vehicle-administered animals at 2 and 4 h (74 vs. 109% and 56 vs. 87%, respectively). By 24 h following surgery, VCAM-1 mRNA levels in both NK-1R antagonist- and vehicle-administered animals were significantly decreased by ~50% (P < 0.05) compared with levels in nonoperated tissues.

**The NK-1R antagonist reduces oxidative stress in peritoneal tissue following surgery.** Because oxidative stress is known to increase in the peritoneum following abdominal surgical procedures, the effect of NK-1R antagonist administration on markers of oxidative stress in the postoperative peritoneum was examined at 2, 4, 6, and 24 h following surgery. Levels of 8-IP in peritoneal tissue from vehicle-administered animals were significantly lower (P < 0.05) in NK-1R antagonist-administered animals at 6 and 24 h were not different from nonoperated levels. However, due to variability at the 6-h time point, the difference in 8-IP levels between vehicle- and NK-1R antagonist-administered animals was only significant (P < 0.05) at the 24-h time point.

Administration of the NK-1R antagonist also led to a reduction in peritoneal tissue protein carbonyl content, an index of the degree of oxidative damage to tissue protein (Fig. 3B). At the 6-h time point, protein carbonyl content in both the vehicle- and NK-1R antagonist-administered animals was significantly elevated (P < 0.05) over nonoperated levels. However, at 2, 4, and 6 h following surgery the protein carbonyl content was significantly lower (P < 0.05) in the NK-1R antagonist compared with the vehicle-administered animals. By 24 h, the protein carbonyl contents did not differ between the two operated groups and were similar to the levels observed in nonoperated controls.
The NK-1R antagonist increases total antioxidant capacity of peritoneal fluid. The total antioxidant capacity of peritoneal fluid collected 24 h following surgery from animals that received vehicle or NK-1R antagonist was also determined (Fig. 4). This assay tests the ability of antioxidants in the peritoneal fluid to inhibit the oxidation of the chromophore ABTS in an oxidizing environment. For comparison, a dose response to the water-soluble vitamin E analog Trolox was also determined, and the data are presented as Trolox equivalents. Peritoneal fluid collected from animals that were administered the NK-1R antagonist had significantly greater ($P < 0.05$) antioxidant capacity at all time points compared with vehicle-administered animals at same time point.

**DISCUSSION**

We have recently shown that a NK-1R antagonist reduces adhesion formation in a rat model (15, 41); however, the mechanism(s) remains unknown. Because activation of the NK-1R by its primary ligand, substance P, can lead to increased oxidative stress, and because a number of antioxidant treatments have been shown to inhibit intra-abdominal adhesion formation, these studies were undertaken to determine whether administration of a specific NK-1R antagonist, CJ-12255, reduces adhesion formation via an attenuation of postoperative oxidative stress. The major finding of this study was that NK-1R antagonist administration reduces oxidative stress in the postoperative peritoneum, and these data suggest that this may be via inhibition of neutrophil recruitment to peritoneal adhesion tissue and an increase in peritoneal fluid antioxidant capacity.

A significant rise in peritoneal tissue MPO levels, observed 6 h following surgery in vehicle-control animals, was not evident in animals administered the NK-1R antagonist. However, by 24 h both the vehicle- and antagonist-administered animals had similarly elevated levels of MPO. These data suggest that the antagonist delays recruitment of polymorphonuclear cells (PMNs) or neutrophils to the postoperative peritoneum until after the 6-h time point. This may be a consequence of a significant decrease in peritoneal tissue ICAM-1 and VCAM-1 levels measured in the NK-1R antagonist-administered rats as early as 2 h following surgery. ICAM-1 and VCAM-1, expressed on endothelium, enable leukocytes to migrate through blood vessel walls at sites of tissue injury (27) and initiate a potent local inflammatory response, a critical first step in adhesion formation. The NK-1R antagonist may delay this inflammatory response and reduce adhesion formation by creating an environment conducive to normal restitution of peritoneal injury. Indeed, previous results from this laboratory demonstrate that the first few hours after surgery are critical to adhesion outcome in this rat adhesion model (15). We have shown that administration of the NK-1R antagonist must occur within 5 h of surgery to decrease adhesion formation (15). Interestingly, although the NADPH oxidase levels also increased postoperatively, we did not observe a significant effect of NK-1R antagonist administration on its levels. This may be
because NADPH oxidase exists in both leukocyte-specific and ubiquitous isoforms. The failure to observe a change in this enzyme may, in part, relate to the relative activities of these isoforms in our peritoneal tissue samples.

Animals administered the NK-1R antagonist also had reduced postoperative oxidative stress in peritoneal tissue, as measured by 8-IP and protein carbonyl. Protein carbonyl content and 8-IP are well-established biomarkers of oxidative damage to protein and lipid, respectively (32). Tissue 8-IP, produced primarily via a noncyclooxygenase, free radical-induced lipid peroxidation of membrane-bound arachidonic acid, is a measure of lipid peroxidation, whereas protein carbonyl content is a measure of protein oxidation (46). In vehicle controls, the 8-IP and protein carbonyl levels were significantly increased over nonoperated levels by 6 h after surgery. Administration of the NK-1R antagonist significantly reduced postoperative lipid peroxidation and protein oxidation, suggesting that a delay in neutrophil recruitment to the peritoneum may protect peritoneal tissue from postoperative oxidative stress. An increase in peritoneal fluid antioxidant capacity may also be a contributing factor.

The NK-1R antagonist-administered animals had significantly increased peritoneal fluid total antioxidant capacity at 2 h. Although the NK-1R antagonist alone does have some, albeit small, antioxidant capacity, it is not nearly as striking as the 128% increase in antioxidant capacity measured in the peritoneal fluid from the NK-1R antagonist-administered animals compared with vehicle-administered animals (Fig. 4). Addition of 900 μM of the NK-1R antagonist, a dose approximately equivalent to the initial concentration administered in the peritoneum, to peritoneal fluid from vehicle-administered animals only increased its antioxidant capacity by 14% (K. L. Reed, unpublished observation). This increase was, however, statistically significant (P < 0.05). The increased antioxidant capacity of peritoneal fluid in the presence of the antagonist may effectively scavenge free radicals produced following surgery and thereby protect peritoneal tissue from oxidative damage. Further investigation is warranted to fully understand how the NK-1R antagonist affects peritoneal fluid antioxidant capacity and subsequent oxidative stress in the peritoneum. This response may be separate from NK-1R antagonist effects on neutrophil recruitment and may involve extravasation of protein into the peritoneal cavity. We have observed a significant increase in peritoneal fluid protein content following surgery that was further enhanced in antagonist-administered animals (K. L. Reed, unpublished observation).

The involvement of oxidative stress in adhesion formation has been well documented (4, 10, 17, 22, 23, 28, 35, 37, 42). In this study, MPO and NADPH oxidase, markers of leukocyte infiltration and activation, were significantly elevated by 24 h in the postoperative tissue samples, providing further evidence that peritoneal oxidative stress is present under conditions that are favorable for adhesion formation. MPO and NADPH oxidase are prooxidant enzymes found primarily in PMNs. They catalyze the production of reactive oxygen metabolites and are hence capable of mediating extensive tissue damage (29). The data presented in this study demonstrate that within hours of peritoneal trauma there is a rapid PMN infiltration, as evidenced by a 70-fold increase in peritoneal tissue MPO activity and a 3-fold increase in NADPH oxidase capacity. The magnitude of this response suggests a pathological component of PMN-derived oxidative stress in postoperative tissue damage and adhesion formation. In support of this conclusion, studies have been conducted that demonstrate that inhibition of adherence and migration of PMNs into the peritoneal cavity lowers the degree of postoperative adhesion formation (3, 51, 52).

Substance P may play a role in both recruitment of PMNs to the peritoneum and in the subsequent induction of oxidative stress. Substance P is known to induce ICAM-1 and VCAM expression in endothelial and epithelial cells, leading to increased extravasation of leukocytes (38), and substance P is also known to increase leukocyte recruitment (12). Therefore, it is not surprising that Santos et al. (44) demonstrated that the NK-1R antagonist SR-140333 inhibited leukocyte recruitment to the peritoneal cavity. Their model, intraperitoneal injection of rats with formalin, induced two peaks of leukocyte recruitment, one within 2–4 h and another at 24 h. The NK-1R antagonist SR-140333 reduced the early, but not the late, leukocyte peak. The authors suggest that substance P release, in part, accounts for the formalin-induced leukocyte migratory activity into the peritoneum (44). Although substance P or perhaps other NK-1R ligands may similarly affect leukocyte recruitment in the postoperative peritoneum, it remains to be determined which NK-1R agonist is most relevant to adhesion formation. The NK-1R, expressed on numerous cell types found in the peritoneum, including fibroblasts, endothelial cells, and macrophages, is activated by several tachykinin ligands, with substance P being the best characterized (25). Other potential NK-1 receptor agonists include the recently identified hemokinin-1 and endokinins A and B. All three functionally bind the NK-1R with affinity that is similar to but less than that of substance P (36).

The mechanism by which oxidative stress promotes adhesion formation is unclear but could include regulation of intraperitoneal fibrinolytic activity. Surgical trauma is known to impair peritoneal fibrinolytic activity, in part, via a reduction in tissue plasminogen activator (tPA) activity (26). There are several studies that demonstrate that increasing postoperative peritoneal tPA levels reduces the incidence of adhesion formation (21, 26, 49), and other studies suggest an association between tPA levels and oxidative stress (1, 11). Using the rat ischemic button model, we have demonstrated that administration of the NK-1R antagonist CJ-12255 leads to an increase in peritoneal fibrinolytic activity via an increase in tPA activity (41). We have also demonstrated that intraperitoneal administration of methylene blue attenuates oxidative stress, increases peritoneal fibrinolytic activity, and inhibits intra-abdominal adhesion formation (24). Furthermore, antioxidants have been shown to increase tPA gene expression in endothelial cell cultures and to increase plasma fibrinolytic activity in humans (11, 34, 54). Thus it is possible that the NK-1R antagonist inhibits adhesion formation via an oxidative stress/tPA mechanism and that these events occur within hours of surgery to affect adhesion outcome. Further studies are necessary to firmly establish a link between the NK-1R, oxidative stress, and peritoneal fibrinolytic activity.

Overall, this study further supports a role for oxidative stress in intra-abdominal adhesion formation and suggests that activation of the NK-1R, by substance P or other tachykinin ligands, contributes in part to the generation of ROS and oxidative stress in the postoperative peritoneum. These data also suggest that the NK-1R antagonist may reduce postoper-
ative adhesion formation, in part, by reducing postoperative oxidative stress through an inhibition of neutrophil recruitment and an increase in peritoneal fluid antioxidant capacity. These data contribute to our understanding of the pathophysiology of adhesion formation and raise the possibility of utilizing NK-1R antagonists clinically in adhesion prevention.

GRANTS

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REFERENCES


