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Combined intraoperative administration of a histone deacetylase inhibitor and a neurokinin-1 receptor antagonist synergistically reduces intra-abdominal adhesion formation in a rat model

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Abstract

Background—Intra-abdominal adhesions are the most frequent postoperative complication after abdominopelvic surgery. Our laboratory has previously shown that an intraoperative peritoneal lavage containing either the histone deacetylase inhibitor valproic acid (VPA) or a neurokinin-1 receptor antagonist (NK-1RA) reduced adhesions by approximately 50% in a rat model. The objective of this study was to determine whether the combination of these 2 drugs was more effective in reducing adhesions than either alone.

Methods—Rats underwent laparotomy with creation of peritoneal ischemic buttons to induce adhesions. A single dose of VPA (25 mg/kg), NK-1RA (50 mg/kg), a combination of both, or 0.9% saline was lavaged intraperitoneally just before wound closure. On postoperative day 7, adhesions were quantified. To investigate early mechanisms of adhesiogenesis, adhesions were created as described and adhesive button tissue was harvested at 30 minutes and 3 hours postoperatively and fibrinogen and vascular endothelial growth factor (VEGF) protein levels, both indices of peritoneal extravasations, were determined by Western blot analysis. Peritoneal fluid was collected in similar experiments at 30 minutes, and 3 and 6 hours to measure fibrinolytic activity, an index of the ability of the peritoneum to degrade fibrinous adhesions.

Results—The coadministration of VPA plus NK-1RA reduces adhesions by 72.6% relative to saline ($P < .001$); this reduction was greater than either compound alone ($P < .001$). Peritoneal fibrinolytic activity was significantly increased at 3 and 6 hours postoperatively in animals administered the combination therapy versus saline ($P = .01$). VPA plus NK-1RA significantly decreased fibrinogen and VEGF protein levels at 3 and 6 hours compared with saline controls.

Conclusion—These results suggest that a combined pharmacologic approach targeting multiple adhesiogenic pathways provides optimal adhesion prevention.

Intra-abdominal adhesions remain among the most challenging and unresolved problems in surgery today. Despite having been recognized >200 years ago,¹ the incidence of adhesions

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is disturbingly high and unacceptable, developing in nearly 100% of patients who have undergone an open abdominopelvic operation.² The consequences of adhesions can be life threatening and debilitating: Approximately 75% of all small bowel obstructions, nearly one third of secondary female infertility, and the majority of chronic abdominal and pelvic pain syndromes are adhesion related.^{1,3,4} Despite decades of research, the development of a broadly effective and easy-to-use technique to prevent the formation of adhesions remains elusive. The only effective treatment for adhesion-related complications is adhesiolysis; however, the reformation rate is reportedly as high as 97% within months.⁵ Hence, a viable, permanent solution that prioritizes effective prevention is needed.^{3,4,6}

Other than meticulous operative technique, surgeons have very limited options for adhesion prevention. The only prevention strategies approved by the US Food and Drug Administration are bioresorbable barriers, such as Seprafilm (Genzyme Corp, Cambridge, MA) that facilitate adhesion-free healing by preventing the apposition of injured peritoneal tissues, but do not address the underlying pathophysiology.⁷⁻⁹ Despite their localized efficacy, physical barriers are not completely effective in preventing small bowel obstructions,¹⁰⁻¹² can be cumbersome to handle, and are not amenable to laparoscopic applications. Hence, they have not been widely adopted by the surgical community and there remains an unmet need for more effective preventative therapies.

Our laboratory has been studying adhesion biology for over a decade and it has become apparent that the etiology of adhesiogenesis is multifactorial, leading us to conclude that a single approach would not be completely effective. For example, it is well-established that postoperative inflammation induces the extravasation of a fibrinous exudate into the peritoneum and the simultaneous downregulation of the peritoneal fibrinolytic system prevents its degradation.^{13,14} If not either inhibited from forming or rapidly degraded, the fibrin matrix ultimately becomes the scaffolding for permanent adhesion formation. Thus, pathways critical to fibrin formation, extravasation, and fibrinolysis are all potential targets for intervention.

Our group¹⁴⁻¹⁶ and others^{17,18} have demonstrated that early resolution of the fibrinous exudate is a critical event in adhesion prevention. We have also shown that several classes of pharmacologic compounds including a neurokinin-1 receptor antagonist (NK-1RA),¹⁴ statins,¹⁵ methylene blue,¹⁹ and *N*-acetyl-L-cysteine¹⁶ reduce adhesions by ~50% in a rat model. Even though they act on different mechanisms, they do not synergize when administered in combination, presumably because they all eventually increase peritoneal fibrinolytic activity.

We have more recently described the efficacy of histone deacetylation inhibitors in adhesion prevention. A single perioperative dose of valproic acid (VPA), a prototypical class 1 histone deacetylation inhibitor, was shown to reduce postoperative adhesions by approximately 50% in a rat model.²⁰ VPA does not modulate the peritoneal fibrinolytic system, but instead reduces adhesions by decreasing signaling molecules that can mediate fibrin accumulation in the peritoneum. Specifically, VPA significantly reduced adhesion tissue levels of vascular endothelial growth factor (VEGF), a key modulator of vascular permeability as well as tissue factor and fibrinogen, key participants in the formation of the peritoneal fibrin matrix.

Because NK-1RA and VPA seem to target 2 independent pathways central to adhesion formation, namely the peritoneal fibrinolytic system and the peritoneal permeability pathways, the goal of this study was to determine if they acted synergistically if coadministered at the time of operation in a rat model of adhesion formation.

MATERIALS AND METHODS

Materials

Sodium valproate (USP grade) was obtained from Sigma Aldrich (St. Louis, Mo). The NK-1RA (3R,4S,5S,6S)-6-diphenylmethyl-5-(5-iso propyl-2-methoxybenzylamino)-1-azabicyclo[2,2,2] octane-3-carboxylic acid (CJ-12,255) was obtained from Pfizer (Groton, CT). This antagonist is highly specific for the NK-1R with no affinity for the NK-2 or NK-3 receptors.

Animals

Male Wistar rats (range, 200–225 g; Charles River Laboratories, Wilmington, MA) were housed at a constant room temperature and under 12-hour light/12-hour dark cycles and allowed access to food and water ad libitum. The Institutional Animal Care and Use Committee at the Boston University School of Medicine approved this study, and all procedures described were performed in accordance with recommendations outlined in the Guide for the Care and Use of Laboratory Animals: Eighth Edition (NRC 2011), published by the National Academies of Science.

Adhesion operation

Intraperitoneal ischemic buttons were created as described previously.^{14,21} In brief, after a midline laparotomy, 6 ischemic buttons (3 on each side of midline wound) were created by grasping and ligating 5 mm of peritoneum with 4-0 silk suture. The test compounds and vehicle controls were lavaged intraperitoneally just before the wound was closed in 2 layers, as described herein. Rats survived until postoperative day 7, at which time they were humanely killed, their abdomens explored, and adhesion formation was quantified by scoring the percentage of buttons with attached fibrinous adhesions in each rat as previously described.^{14,21} For example, an animal that had adhesions attached to 4 of its 6 ischemic buttons received a score of 66%.

Efficacy studies

To determine the efficacy of the VPA or NK-1RA either alone or in combination, 102 male Wistar rats underwent laparotomy with ischemic button creation as described. Rats received intraperitoneal administration of either 1 mL of 0.9% normal saline (control), 1 mL VPA at 50 mg/kg in normal saline, 1 mL NK-1RA at 25 mg/kg in normal saline, or 1 mL of the combination of VPA plus NK-1RA in normal saline. The peritoneal cavity was lavaged at the time of surgery by pipetting 1 mL of the NK-1RA, VPA, the combination of both, or normal saline directly into the peritoneum via the midline incision. After administration, the abdomen was gently massaged to distribute the solutions in the peritoneal cavity; however, it was not necessary to ensure that all peritoneal surfaces were coated. Animals were humanely killed on postoperative day 7 and adhesions were quantified as described.

Colonic wound healing

To assess whether the combination of VPA plus NK-1RA affects normal wound healing, 15 male Wistar rats underwent laparotomy with a standardized colonic anastomosis as described previously.¹⁵ In brief, after laparotomy and complete transection of the proximal colon, an end-to-end colonic anastomosis was created using a single running layer of 7-0 polypropylene suture. Animals received either 1 intraoperative dose of VPA plus NK-1RA ($n = 6$) or 0.9% saline ($n = 6$). Animals were humanely killed 7 days postoperatively and, after removing a 4-cm segment containing the anastomosis, the burst pressure of the colonic segment was assessed as described previously.¹⁵

Peritoneal fluid collection and the fibrinolytic assay

To evaluate the effects of the drug combination on postoperative peritoneal fibrinolytic activity, rats underwent laparotomy with ischemic button creation with intraoperative administration of VPA plus NK-1RA or saline as described. At 30 minutes and 3 and 6 hours later, animals were anesthetized and the peritoneum lavaged with 600 μ L of phosphate-buffered saline containing 1 IU/mL of heparin. The lavage fluid was carefully removed, mixed an equal volume of 0.2 mol/L sodium acetate (pH 3.9) and centrifuged ($100 \times g$) for 5 minutes to remove cells and debris. The supernatant was immediately flash frozen in liquid nitrogen and stored at -80°C until use. Peritoneal fluid was collected from nonoperated animals for comparison. Fibrinolytic activity was measured using a tissue plasminogen activator (tPA) activity bioassay as described previously.²² In brief, fluid samples were thawed and acidified with 0.2 volumes 0.375 N hydrochloric acid and then diluted 10-fold with distilled water. The fibrinolytic activity of tPA in each sample was assayed in duplicate by adding 50 μ L of the diluted sample to each well of a 96-well microtiter plate containing a 50- μ L tPA stimulator (0.6 mg/mL cyanogen bromide–digested fibrinogen; American Diagnostica, Stamford, CT). Next, 150 μ L of assay buffer (16.7 mg/mL human plasminogen; Athens Research and Technologies, Athens, GA), 667 mmol/L S-2251 substrate [American Diagnostica], and 20 mmol/L Tris, (pH 8.3) was added to each well and gently mixed. Cleavage of the S-2251 substrate by tPA-activated plasmin produces a yellow color that absorbs at 405 and 490 nm (calibration blank). The change in absorbance was measured at 37°C over the course of 10 hours using a spectrophotometric microplate reader (SpectraMax 250; Molecular Devices, Sunnyvale, CA). The fibrinolytic activity of tPA in each sample was determined by extrapolation from a tPA (human; Calbiochem, San Diego, CA) standard curve.

Peritoneal tissue collection and western blot analysis for protein in adhesion tissue

Rats underwent laparotomy with ischemic buttons and intraoperative administration of VPA plus NK-1RA or saline as described. Adhesive button tissue was collected at 30 minutes and 6 hours postoperatively, by resecting each peritoneal ischemic button with any attached adhesions and a 0.5-cm rim of surrounding peritoneal tissue. Peritoneal tissue was also collected from nonoperated rats for comparison. Aliquots of tissues for mRNA and protein were immediately flash frozen in liquid nitrogen and stored at -80°C until use. The tissue was then pulverized with a mortar and pestle in liquid nitrogen, and suspended in 500 μ L RIPA buffer [50 mmol/L Tris, pH 7.4, 1% NP-40, 0.25% deoxycholate, 150 mmol/L NaCl, 1

mmol/L EDTA] supplemented with a mammalian protease inhibitor cocktail used at 100× (Sigma). Lysates were sonicated using a Sonic Dismembrator (Fisher Scientific, Pittsburgh, PA), for 10 seconds on ice. After a 1-hour incubation on ice, the homogenate was centrifuged at $850 \times g$ for 15 minutes at 4°C, and the supernatant (cytosolic fraction) was recovered for Western blot analysis. Total protein content was determined using Bio-Rad protein assay (Bio-Rad, Hercules, CA). Cytosolic extracts (20 µg per sample) were separated on 4–12% Bis-Tris minigels (Invitrogen, Life Technologies, Grand Island, NY) and transferred to Hybond enhanced chemiluminescent membranes (Amersham Pharmacia Biotechnology, GE Healthcare Bio-Sciences, Pittsburgh, PA). The membranes were blocked for 1 hour at room temperature in blotto (1 × 25 mmol/L Tris · HCl [pH 7.4], 2.5 mmol/L KCl, 125 mmol/L NaCl, 0.1% Tween 20, and 5% nonfat dry milk) and incubated with a polyclonal antibody for either fibrinogen (Abcam ab74057, Cambridge, MA), or VEGF (Santa Cruz Biotechnology sc53462, Dallas, TX), diluted 1:5,000 in blotto, for 1 hour at room temperature. Membranes were then subjected to vigorous washes, 4 × 15 minutes in TBS/0.1% Tween 20 (TBS-T) at room temperature, and incubated for 1 hour at room temperature in secondary antibody, peroxidase-conjugated goat anti-rabbit immunoglobulin G (Pierce Chemical, Thermo Fisher Scientific, Rockford, IL) diluted 1:10,000 in blotto. Membranes were then vigorously washed again, 4 × 15 minutes in TBS-T at room temperature. Bands were detected by chemiluminescence with the Pierce Enhanced Chemiluminescent Western Blotting Substrate (Thermo Fisher Scientific, Rockford, IL) according to the manufacturer's instructions. Western blots were scanned and quantified using Scion Image software (Scion Corp, Fredrick, MD). Mean values were determined and expressed as a percentage of nonoperated controls.

Statistical analysis

Data were analyzed either with 1-way analysis of variance or the Student *t* test where appropriate using analytical software (Sigma-Stat; SPSS Inc, Chicago, IL). A log₁₀ transformation was performed if the analyses of the data failed either normality or equivariance testing. If the data still failed these criteria, the nonparametric Dunn test of analysis of variance by ranks followed by the appropriate nonparametric post hoc test was used. When significant effects (*P* .05) were detected, the differences between specific multiple means were determined by post hoc analysis with the Student–Newman–Keuls test. All data are expressed as mean values ± standard error of the mean.

RESULTS

VPA plus NK-1RA reduces adhesions with greater efficacy than either agent alone

To determine whether the combination of VPA plus NK-1RA could provide synergistic adhesion reduction, we compared the efficacy of VPA alone, NK-1RA alone, and VPA plus NK-1RA in the rat ischemic button model (Fig 1). The intraperitoneal administration of VPA alone (*n* = 34) reduces adhesions by 50.3% compared with saline controls (*P* < .001),²⁰ and intraperitoneal NK-1RA alone (*n* = 32) reduced adhesions by 57% (*P* < .001).^{14,23} The combination VPA plus NK-1RA (*n* = 22), given intraperitoneally as a single dose at the time of operation, reduces adhesions by nearly 73% compared with saline controls (*P* < .001; Fig 1). Compared with VPA alone, VPA plus NK-1RA reduces adhesions by an additional 45%

($P < .01$), whereas VPA plus NK-1RA reduces adhesions by an additional 37% compared with NK-1RA alone ($P < .01$).

The most common side effects associated with the use of an NK-1RA or VPA in humans according to the package insert are constipation, diarrhea, loss of appetite, headache, and hair loss, all of which are typically associated with repeated dosing of these compounds. However, the studies reported here utilized a single, 1-time, low dose, after which obvious side effects were not observed.

VPA plus NK-1RA does not compromise intestinal wound healing

Using a rigorous colonic anastomosis model in which a complete colon transection was repaired with an end-to-end, hand-sewn anastomosis, we found that administration of VPA plus NK-1RA significantly increases colonic burst pressure when compared with animals administered saline only (Fig 2). Average colonic burst pressure was 267 ± 16.0 mmHg ($n = 6$) for animals administered VPA plus NK-1RA and 225 ± 7.7 mmHg ($n = 9$) for animals administered saline, values similar those previously reported by our laboratory, including VPA alone²⁰ and NK-1RA alone.²³ Additionally, there was no negative impact on fascial or cutaneous wound healing.

VPA plus NK-1RA increases peritoneal fibrinolytic activity

Our previous studies with a NK-1RA,¹⁴ statins,¹⁵ and *N*-acetyl-L-cysteine¹⁶ showed that these compounds reduced adhesion formation, in part, by upregulating peritoneal fibrinolytic activity. In contrast, VPA alone reduced adhesions, but did not alter peritoneal fibrinolytic activity.²⁰ We therefore tested whether VPA plus NK-1RA administered in combination would modulate the peritoneal fibrinolytic system. In a bioassay measuring peritoneal fibrinolysis, a single dose of VPA plus NK-1RA ($n = 6$) administered intraperitoneally at the time of operation significantly increases tPA activity at 3 ($n = 6$) and 6 hours ($n = 6$) postoperatively compared with 30 minutes ($n = 6$) postoperatively and nonoperated controls ($n = 6$; Fig 3). Interestingly, fibrinolytic activity drops nearly 50% between 3 and 6 hours in the saline control group, and the VPA plus NK-1RA levels remained steadily elevated.

VPA plus NK-1RA reduces fibrinogen levels in adhesive tissue

A key early event in adhesion formation is the plasmin-mediated conversion of fibrinogen to fibrin. Newly formed fibrin in the peritoneum forms the scaffold for new adhesion formation. We previously showed that VPA administered alone significantly reduced fibrinogen protein in adhesive tissue.²⁰ In the present study, we show that within 30 minutes, a single intraperitoneal dose of VPA plus NK-1RA ($n = 5$) significantly reduces adhesive tissue levels of fibrinogen protein compared with saline controls and nonoperated animals (Fig 4, A). Fibrinogen levels continue to drop at 3 hours in the VPA plus NK-1RA treated animals compared with controls ($n = 5$).

VPA plus NK-1RA reduces VEGF levels in adhesive tissue

VEGF plays a key role in regulating the extravasations of adhesiogenic factors, such as fibrinogen and fibrin, from the plasma compartment into the peritoneum. We show that within 30 minutes of a single intraoperative dose of VPA plus NK-1RA ($n = 5$), VEGF

protein levels in adhesive tissue are significantly decreased ($P = .03$) compared with animals administered saline alone ($n = 5$) and remain lower at 3 hours (Fig 4, *B*).

DISCUSSION

The etiology of adhesion formation is multifactorial and, like the treatment of many complex diseases, efficacy can only be achieved using a therapeutic strategy that targets multiple pathways. Using drugs that are approved by the US Food and Drug Administration for other indications, we have shown previously that interference with 2 key pathways that promote adhesion formation can significantly reduce adhesions in a rat model. We first showed that NK-1RAs, currently used to treat nausea and vomiting after chemotherapy and surgery,²⁴ were highly effective in reducing intra-abdominal adhesions by upregulating the peritoneal fibrinolytic system.^{14,23,25} We demonstrated subsequently that histone deacetylation inhibitors, in particular VPA, used for decades to treat epilepsy and psychiatric disorders²⁶ were efficacious in reducing intra-abdominal adhesions by decreasing the accumulation of peritoneal fibrin precursors.²⁰ We now show new data that the intraoperative coadministration of an NK-1RA and VPA at the time of operation reduces adhesion formation by nearly 73% compared with controls, a significantly greater effect than the reduction by either alone. Consistent with the combined effect of these drugs on adhesion reduction, we also show that the unique, beneficial effects of each are retained when they are administered together. Thus, the NK-1RA retains its ability to elevate and maintain peritoneal fibrinolytic activity up to the 6-hour window of therapeutic efficacy²³ and VPA reduces peritoneal adhesion tissue levels of fibrinogen, the precursor of fibrin. By modulating independent pathways that mediate the production and degradation of fibrin, this combined pharmacologic approach is able to more effectively reduce the peritoneal accumulation of fibrin, the scaffolding upon which intra-abdominal adhesions form.

Peritoneal fibrin accumulation is triggered by surgical trauma, an unavoidable consequence of abdominopelvic operations. Manual manipulation, ligation, electrocautery, and vascular clamping can cause mechanical injury and disruption of blood flow to the peritoneum, triggering a localized inflammatory response. During active inflammation, metabolic demands can often exceed supply, resulting in metabolic stress and diminished oxygen levels. Tissue hypoxia, initially localized to the site of injury, rapidly triggers a more widespread inflammatory response that interferes with normal peritoneal wound healing.¹⁸ The hallmark of the wound healing response in the peritoneum is the extravasation of a fibrinous exudate derived from the underlying vasculature. Normally, the fibrin-rich matrix is an integral component of the healing process; however, for adhesion-free healing to occur in the peritoneum, the fibrin-rich exudate must be degraded before remesothelialization.²⁷ The robust inflammatory response in the postoperative peritoneum triggers pathways that not only suppress peritoneal fibrinolysis, but also exacerbate extravasation. Simultaneously, these pathways facilitate the persistence of the fibrinous exudate, laying the foundation for fibrinous²⁸ and permanent adhesion formation.^{13,14} These parallel adhesiogenic events, the so-called vicious triad of trauma, hypoxia, and inflammation,²⁹ set the stage for adhesion formation and demonstrate the complexity and multifactorial nature of adhesiogenesis (Fig 5, *A*). This also explains, in part, why the incidence of adhesions is so ubiquitous in patients

who have undergone an abdominopelvic operation³⁰ and the fact that a site-directed physical barrier or a single therapeutic approach has limited efficacy.²⁹

The peritoneal fibrinolytic system plays an irrefutable role in adhesion-free healing in the abdomen and pelvis.^{18,31–33} Numerous studies have firmly established that peritoneal fibrinolysis is compromised after surgery, either by a reduction in tPA or an increase in its principal inhibitor, plasminogen activator inhibitor-1,³⁴ or both, indicating that peritoneal fibrin accumulation and stabilization is a key underlying event early in adhesiogenesis.^{18,31,32,35} Although our previous studies showed that augmenting the peritoneal fibrinolytic system reduced adhesion formation in a rat model,^{14–16,19} the coadministration of any of these compounds did not increase adhesion prevention, owing in part, to their mechanistic similarities. When we discovered that VPA did not reduce adhesion formation via the fibrinolytic pathway,²⁰ but instead by an alternative mechanism that limited the availability of fibrin substrates, we recognized a potential opportunity to achieve synergistic adhesion reduction by targeting both fibrinolysis with the NK-1RA and fibrin accumulation with VPA (Fig 5, *B*).

Our data also indicate that the 2 drugs had a combinatory effect on extravasation, suggesting that the NK-1RA may be able to modulate more than just fibrinolysis in the context of VPA. VPA alone reduces peritoneal fibrinogen protein levels by 50%, which limits the availability of the fibrin precursor,²⁰ and when combined with the NK-1RA reduced fibrinogen protein even further. A similar effect was observed with VEGF-A. In controls, both fibrinogen protein and VEGF-A levels declined in peritoneal button tissue during the first 30 minutes postoperatively; both were reduced even further in adhesion tissues from the dual treatment group. These data show that NK-1RA may also contribute to the limitation fibrin precursor in the peritoneal space when administered in the presence of VPA.

Several lines of evidence also implicate peritoneal tissue hypoxia in mediating many of the early events that lead to fibrin accumulation. Our laboratory has shown that the quintessential oxygen sensitive transcription factor hypoxia-inducible factor (HIF)-1 α is upregulated by >2-fold within 3 hours of a peritoneal operation.³⁶ Although this finding is consistent with others,^{37,38} evidence showing that downregulating the HIF-1 α gene reduced adhesion formation is far more substantive evidence that hypoxia plays an important role in early adhesiogenic events.³⁹ We have also shown in our rat model that there is a significant increase in the expression pattern of multiple HIF-1 α -mediated genes involved in adhesion formation³⁸ in the early postoperative peritoneum. These include plasminogen activator inhibitor-1,³⁶ VEGF and its receptor,²⁰ and tissue factor.²⁰ In contrast, the intraperitoneal administration of an NK-1RA increased tPA mRNA expression, protein levels, and blunted the increase in plasminogen activator inhibitor-1 mRNA levels in adhesion tissue.³⁶ The NK-1RA also increased fibrinolytic activity in peritoneal fluid^{14,23,25} and delayed the rise HIF-1 α and VEGF-A mRNA expression after surgery (Fig 5, *B*).³⁶

Colonic wound healing was not altered by the coadministration of VPA plus NK-1RA. In fact, the strength of the anastomosis was significantly greater in the animals that received the combination therapy. Because adhesiogenesis and wound healing share common pathways, therapies that alter adhesion formation may compromise anastomotic healing.^{40,41} Any

clinically useful intervention must prevent adhesions without deleterious effects on normal wound healing processes, and the combination of VPA plus NK-1RA meets these criteria.

In conclusion, we have demonstrated that targeting multiple adhesiogenic pathways (Fig 5, *B*) significantly improves adhesion reduction in a rat model. The intraoperative lavage of a drug-containing normal saline solution represents a seemingly ideal delivery mechanism that overcomes the limitations of physical barriers, and may be applied in almost any surgical setting, including laparoscopy. Our data showing that the early reduction in fibrin precursors in concert with increased peritoneal fibrinolysis are not only promising targets for adhesion prevention, but, more important, help to define the molecular events that participate in adhesion formation. We propose that combination pharmacologic interventions targeting multiple mechanisms in adhesiogenesis may prove to have greater efficacy and utility than existing adhesion prevention strategies; we remain optimistic that a solution to this daunting surgical problem can be achieved.

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Drs. Stucchi and Cassidy have filed a patent application no. 61/552,063 for the use of histone deacetylase inhibitors for the treatment of post-surgical adhesions.

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Fig 1.

Effects of combined pharmacotherapy on adhesion reduction. Intraperitoneally administered valproic acid (*VPA*; $n = 34$), a neurokinin-1 receptor antagonist (*NK-1RA*; $n = 32$), and combined *VPA+NK-1RA* ($n = 22$) versus saline controls ($n = 35$), demonstrating the efficacy of synergistic adhesion reduction achieved by the intraoperative co-administration of *VPA+NK-1RA* compared with either *VPA* or *NK-1RA* alone. Adhesions were quantified on postoperative day 7. Data are expressed as mean values $\pm$ standard error of the mean. * $P < .001$ versus saline control; # $P < .01$ versus *VPA* alone and *NK1-RA* alone.

Fig 2. Effects of combined pharmacotherapy on anastomotic wound healing. Intraperitoneal coadministration of valproic acid (*VPA*) plus a neurokinin-1 receptor antagonist (*NK-1RA*; *VPA+NK-1RA*; $n = 6$) increases colonic anastomotic burst pressures compared with saline controls ($n = 9$). Data are expressed as mean values $\pm$ standard error of the mean. $*P = .023$ versus saline controls.

Fig 3.

Effects of combined pharmacotherapy on peritoneal fluid fibrinolytic activity. Intraperitoneal coadministration of valproic acid (VPA) plus a neurokinin-1 receptor antagonist (NK-1RA; VPA+NK-1RA; $n = 6$) increased fibrinolytic activity in peritoneal fluid at 3 ($n = 6$) and 6 hours ($n = 6$) postoperatively compared with 30 minutes ($n = 6$) postoperatively and time 0 or nonoperated (non-op; $n = 6$). Data are expressed as mean values $\pm$ standard error of the mean. * $P < .001$ versus 30 minutes and non-op in both groups. *# $P < .01$ versus 3- and 6-hour saline controls.

Fig 4.

Effects of combined pharmacotherapy on fibrinogen and vascular endothelial growth factor-A protein levels in adhesive tissue. Intraperitoneal coadministration of valproic acid (VPA) plus a neurokinin-1 receptor antagonist (NK-1RA; VPA+NK-1RA) significantly reduced (A) fibrinogen and (B) vascular endothelial growth factor-A (VEGF-A) protein levels at 30 minutes ($n = 5$) postoperatively compared with operated saline controls ($n = 5$) and nonoperated (Non-op) animals ($n = 5$). Data are normalized to GAPDH and expressed as the mean value $\pm$ standard error of the mean. * $P < .001$ versus non-op and VPA+NK-1RA–

treated animals. $^{*}P < .001$ versus 30 minutes, Non-op, and VPA+NK-1RA-treated animals. *GAPDH*, Glyceraldehyde-3-phosphate dehydrogenase.

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Fig 5.

A, Multifactorial etiology of adhesion formation. (1) Surgical injury to peritoneum induces (2) an acute inflammatory response that activates mesothelial and (3) capillary endothelial cells. The subsequent release of proinflammatory cytokines activates platelets that (4) aggregate and adhere to the endothelium triggering the formation of a fibrin thrombus. Hypoxia resulting from interrupted blood flow and inflammation induces the upregulation of the transcription factor (5) hypoxia inducible factor (*HIF*-1 α), which causes activated mesothelial cells to secrete plasminogen activator inhibitor-1 (*PAI*-1) and (6) vascular endothelial growth factor (*VEGF*), 2 key players in the adhesion formation pathway. (7) *PAI*-1 inhibits tissue plasminogen activator (*tPA*), preventing the dissolution of the fibrinous exudate by plasmin and (8) *VEGF* disrupts the mesothelial and endothelial cell junctions facilitating the extravasations of the fibrinous exudate from the vasculature into the peritoneum. (9) We also showed that peritoneal levels of tissue factor (*TF*) are rapidly

upregulated after surgery²⁰ facilitating the maturation of the fibrin matrix and (10) permanent adhesion formation. *B*, Synergistic actions of a NK-1RA and VPA on different pathways of adhesion formation reduces adhesion formation more effectively than either alone. (1) An NK-1RA reduced peritoneal adhesion tissue inflammation,⁴² (2) HIF-1 α , and (3) PAI-1 levels,³⁶ and (4) increased tPA levels effectively (5) increasing total peritoneal fibrinolytic activity.¹⁴ VPA reduced peritoneal adhesion tissue levels of (6) VEGF, (7) fibrinogen, and (8) TF.²⁰ The reduction of fibrin precursors and fibrin in the peritoneum in concert with the increase in peritoneal fibrinolysis facilitate the breakdown of the (9) fibrinous exudate, thus reducing adhesion formation.