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Efficacy of barriers and hypoxia-inducible factor inhibitors to prevent CO_2 pneumoperitoneum-enhanced adhesions in a laparoscopic mouse model

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KEYWORDS:
Barriers;
Flotation;
HIF;
Hypoxia;
Intraperitoneal adhesion formation;
Laparoscopy;
Pneumoperitoneum;
Prevention;
Surfactant

Abstract

STUDY OBJECTIVE: To investigate the effects of hypoxia-inducible factor (HIF) inhibitors, flotation agents, barriers, and a surfactant on pneumoperitoneum-enhanced adhesions in a laparoscopic mouse model.

DESIGN: Prospective randomized trial (Canadian Task Force classification I).

SETTING: Department of Obstetrics and Gynecology, University Hospital Gasthuisberg, Catholic University of Leuven.

SUBJECTS: One hundred fourteen female BALB/c mice.

INTERVENTIONS: Adhesions were induced during laparoscopy in BALB/c female mice. Pneumoperitoneum was maintained for 60 minutes with humidified CO_2. In 3 experiments the effects of HIF inhibitors such as 17-allylamino 17-demethoxygeldanamycin, radicicol, rapamycin, and wortmanin, flotation agents such as Hyskon and carboxymethylcellulose, barriers such as Hyalobarrier gel and SprayGel, and surfactant such as phospholipids were evaluated.

MEASUREMENTS AND MAIN RESULTS: Adhesions were scored after 7 days during laparotomy. Adhesion formation decreased with the administration of wortmannin ($p < .01$), phospholipids ($p < .01$), Hyalobarrier Gel ($p < .01$), and SprayGel ($p < .01$).

CONCLUSIONS: These experiments confirm the efficacy of barriers and phospholipids to separate or lubricate damaged surfaces. They also confirm the role of mesothelial hypoxia in this model by the efficacy of the HIF inhibitor wortmannin.

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One of the complications of abdominal surgery is intra-abdominal adhesion formation. Adhesions can cause intestinal obstruction, chronic pain, and infertility. Although postoperative adhesion formation remains an important clinical problem, its prevention is still inadequate and overall poorly understood.

Over recent years, CO_2 pneumoperitoneum became known as a cofactor in postoperative adhesion formation.[1] Mesothelial hypoxia was suggested as a mechanism, because adhesions increased with duration of CO_2 pneumoperitoneum and with insufflation pressure, because similar effects were observed with helium pneumoperitoneum and because the addition of 2% to 4% of oxygen to both CO_2 and helium pneumoperitoneum decreased adhesion formation.[1] This hypothesis was supported by the absence of pneumoperitoneum-enhanced adhesions in mice deficient for factors which are up-regulated during hypoxia, such as plasminogen activator inhibitor 1 (PAI-1),[2] vascular endothelial growth factor (VEGF), placental growth factor,[3] and hypoxia-inducible factor 1α and 2α (HIF-1α and HIF-2α).[4]

HIF is an α/β heterodimeric DNA-binding complex that directs an extensive transcriptional response to hypoxia. HIF activity is induced during hypoxia through the stabilization and activation of its subunit HIF-1α whereas during normoxia subunit HIF-1α is rapidly degraded by the ubiquitin-proteosome system.[5] Inhibition of HIF activity can be achieved by decreasing heat shock protein 90 (Hsp-90) or by blocking the phosphatidylinositol 3-kinase (PI3K) pathway. The molecular chaperone Hsp-90 is important to maintain the appropriate folding and conformation and to regulate the balance of synthesis and degradation of its clients such as HIF-1α.[6] Therefore Hsp-90 inhibitors, such as radicicol, geldanamycin, and its derivated 7-allylaminogeldanamycin (17-AAG), decrease HIF-1α activity.[6] In addition, 17-AAG and radicicol bind to the intrinsic ATPasa activity in the N-terminal site of Hsp-90, resulting in degradation of Hsp-90 client proteins by the ubiquitin proteosome pathway. Another approach to decrease HIF activity is the inhibition of the PI3K/Akt pathway with inhibitors such as wortmannin and rapamycin[7] because phosphorylation is involved in the HIF-1α subunit stabilization, as well as in the regulation of HIF-1 transcriptional activity.[8]

Prevention of adhesion formation after laparoscopic surgery has been poorly addressed. Several agents have been tested, such as antibodies against VEGFR1,[9] crystalloids,[10,11] 4% icodextrin,[11] ferric hyaluronate gel,[11–13] Sepracoat,[14] a cross-linked hyaluronan solution,[15] and hyaluronate membrane,[16] which were effective in different animal models. These observations, however, were generally reported with only 1 drug in different models, different species, and with different scoring systems. A comprehensive quantitative evaluation of efficacy in 1 model is still lacking.

These experiments are the second part of a series of experiments to evaluate most known substances in 1 model to obtain quantitative and comprehensive information on adhesion prevention. In this article, the effects of HIF inhibitors, flotation agents, barriers, and a surfactant were investigated.

Materials and methods

The laparoscopic mouse model for adhesion formation

Experimental setup, that is, animals, anesthesia, and ventilation, laparoscopic surgery, induction, and scoring of intraperitoneal adhesions, has previously been described in detail.[1–4,9,10,17–19,27] Briefly, in the pneumoperitoneum-enhanced adhesions model, adhesions were induced during laparoscopy by creating a mechanical lesion. Pneumoperitoneum was maintained for 60 minutes with pure and humidified CO_2 at 15 mm Hg insufflation pressure. Gas and body temperatures were kept strictly at 37°C with a heated chamber (Figure 1).

Animals

One hundred fourteen 9- to 10-week-old female BALB/c mice weighing 20 g were used. Animals were kept under standard laboratory conditions, and they were fed a standard laboratory diet with free access to food and water at any time. The study was approved by the Institutional Review Animal Care Committee.

Anesthesia and ventilation

Mice were anesthetized with intraperitoneal 0.08 mg/g pentobarbital, intubated with a 20-gauge catheter, and mechanically ventilated (Mouse Ventilator MiniVent, Type 845; Hugo Sachs Elektronik-Harvard Apparatus GmbH,

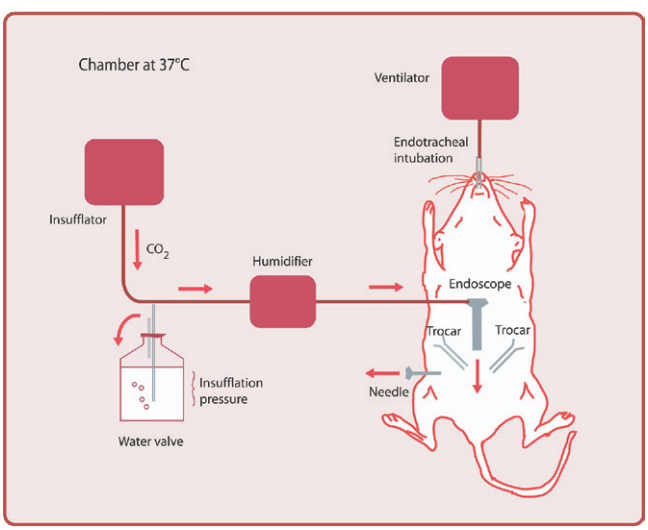

Figure 1 Laparoscopic mouse model.

March-Hugstetten, Germany) by use of humidified room air with a tidal volume of 250 μL at 160 strokes/min. Humidified air for ventilation was used to prevent cooling, as occurs during ventilation with non-humidified air.[17]

Laparoscopic surgery

A midline incision was performed caudal to the xiphoid process, a 2-mm endoscope with a 3.3-mm external sheath for insufflation (Karl Storz, Tuttlingen, Germany) was introduced into the abdominal cavity, and the incision was closed gas tight around the endoscope to avoid leakage.

Pneumoperitoneum was created with pure CO_2 at 15 mm Hg insufflation pressure using the Thermoflator Plus (Karl Storz) and a water valve to damper pressure changes. The gas was humidified (Storz Humidifier 204320 33; Karl Storz), and the whole setup was kept in a chamber at 37°C to obtain CO_2 at 37°C and with 100% relative humidity. We used, as described previously, a controlled flow of CO_2 through the abdominal cavity of 23 mL/min with a 26-gauge needle, to ascertain a continuously 100% CO_2 environment by removing constantly any oxygen that might have diffused from the capillaries.

Induction of intraperitoneal adhesions

Pneumoperitoneum-enhanced adhesion formation was induced by maintaining the pneumoperitoneum for 10 or 60 minutes and by performing standardized 10-mm × 1.6-mm lesions in the antimesenteric border of both right and left uterine horns and pelvic sidewalls with bipolar coagulation (BICAP, bipolar hemostasis probe, BP-5200A, 5F, 200 cm; IMMED Benelux, Linkebeek, Belgium) at 20 W (Autocon 200; Karl Storz; standard coagulation mode).

Scoring of adhesions

Adhesions were qualitatively and quantitatively scored. Scoring was done blindly (the investigator was not informed of the group being evaluated) after 7 days during laparotomy under microscopic vision. The qualitative scoring system assessed the following: extent (0: no adhesions; 1: 1%–25%; 2: 26%–50%; 3: 51%–75%; 4: 76%–100% of the injured surface involved, respectively), type (0: no adhesions; 1: filmy; 2: dense; 3: capillaries present), tenacity (0: no adhesions; 1: easily fall apart; 2: require traction; 3: require sharp dissection), and total (extent + type + tenacity).

The quantitative scoring system assessed the proportion of the lesions covered by adhesions with the following formula: adhesion (%) = (sum of the length of the individual attachments/length of the lesion) × 100. The results are presented as the average of the adhesions formed at the four individual sites (right and left visceral and parietal peritoneum), which were individually scored. Because the initial measurements are in millimeters (thus with an error of 0.5 mm), the precision of the division will be around 1%. According to the law of error propagation (where $\Delta Z/Z = \Delta X/X + \Delta Y/Y$) the accuracy yields some 4% CV or 1% for the sum of 4 estimates. Therefore only 1 digit becomes significant.[20]

Products

HIF inhibitors

The 17-allylamino 17-demethoxygeldanamycin (17-AAG) was donated by Kosan Biosciences (Hayward, CA). Radicicol, wortmannin, and rapamycin were bought from A.G. Scientific, Inc., (San Diego, CA). The doses administered were 17-AAG 20 mg/kg, radicicol 25 mg/kg, wortmannin 0.31 mg/kg, and rapamycin 3 mg/kg. Radicicol, rapamycin, 17-AAG, and wortmannin were dissolved in pure dimethylsulphoxide (DMSO) at final concentrations of 7.5 mg/mL, 0.9 mg/mL, 6 mg/mL, and 5 mg/mL, respectively. Afterward, wortmannin stock was diluted to 0.093 mg/mL in saline solution. Stocks were kept at −20°C until they were used.

The doses used in this experiment were those proven effective or nontoxic in the in vivo models. Rapamycin at the dose of 3 mg/kg showed an immunosuppressive effect in mice and rats.[21,22] Wortmannin at the dose of 0.31 mg/kg (MTD/2) showed an antitumor effect in mice.[23] Different MTD doses of 17-AAG were found in the literature, that is, 50 mg/kg and 80 mg/kg, and they both showed an antitumor effect in mice.[24,25] We tried 40 mg/kg, but it was toxic in our model; therefore the dose used was 20 mg/kg. Although the MTD of radicicol did not show any antitumor effect in mice,[26] the dose of 25 mg/kg (MTD/4) was used because it was nontoxic.

Flotation agents

Carboxymethylcellulose 2% was prepared and sterilized by the pharmacists of the Hospital Gasthuisberg. Hyskon (32% dextran 70) was donated by Gynotec (Malden, The Netherlands).

Mechanical barriers

Hyalobarrier Gel is a sterile, transparent, and highly viscous gel obtained by condensation of hyaluronic acid (HA) through an auto-cross-linking process and is indicated for laparoscopic and hysteroscopic or open surgical procedures. It was kindly provided by Fidia Advanced Biopolymers SRL (Abano Terme, Padova, Italy). SprayGel Adhesion Barrier System consists of 2 liquids which cross-link to form a biocompatible absorbable hydrogel; it can be used for laparoscopic and laparotomy procedures. SprayGel (Confluent Surgical, Inc., Waltham, MA) was donated by Medical International AG (Kaltenbach, Switzerland).

Surfactant

Phospholipids solution (9%), donated by Dr. Marc Jansen (Department of Surgery, University Clinic, RWTH Aachen, Germany), was diluted to 3% in saline solution before use.

Experimental design

Because anesthesia and ventilation can influence body temperature, the timing was strictly controlled. The time of anesthesia injection was considered time 0 (T_0). The animal preparation and ventilation started after exactly 10 minutes (T_{10}). The pneumoperitoneum started at 20 minutes (T_{20}) and was maintained for 10 or 60 minutes, until T_{30} or $T_{80,}$ respectively.

We used a sample size of 8 mice because, taking into account the coefficient of variability of 30% for adhesion formation in our experiments in Balb/c mice[27] and the power of the experiment of 70%, a decrease of 40% in adhesions formation can be detected. A decrease of less than 40% in adhesion is not clinically relevant.

Experiment 1 was designed to evaluate the effect of radicicol, rapamycin, 17-AAG, and wortmannin on adhesion formation. Pneumoperitoneum-enhanced adhesions were induced, and 0.1 mL of the HIF pathway inhibitors (17-AAG, rapamycin, radicicol, and wortmannin) was injected immediately before performing the lesions under laparoscopic vision (17-AAG, rapamycin, radicicol and wortmannin groups, respectively). Two control groups for pneumoperitoneum-enhanced adhesions were used, the first without any treatment, the second with injection of 0.1 mL of the vehicle used to dissolve the drugs (control 60 minutes pneumoperitoneum and control vehicle, respectively). Another control group was performed maintaining the pneumoperitoneum for 10 minutes (control 10 minutes pneumoperitoneum or basal adhesion), and no treatment was administered (7 groups, 8 mice per group, n = 56).

Experiment 2 was designed to evaluate the effect of flotation agents, Hyskon and carboxymethylcellulose 2% (CMC 2%) and a barrier Hyalobarrier gel, on adhesion formation. After performing the lesion, a volume of 0.5 mL of Hyskon or CMC 2% were injected intraperitoneally (Hyskon and CMC 2% groups, respectively) and approximately 1 mL of Hyalobarrier gel was applied on the lesions (Hyalobarrier gel group) under laparoscopic vision. The control group received intraperitoneally saline solution 0.5 mL. Injection of Hyskon in the abdominal cavity was associated with 20% of deaths. Because 2 of 8 mice died within the 24 hours after the surgery, they were replaced (2 of 10 mice = 20% mortality rates) (4 groups, 8 mice per group, 10 mice for Hyskon group; n = 32 mice).

Experiment 3 was designed to evaluate the effect of a mechanical barrier (SprayGel) and a surfactant (phospholipids) on adhesion formation. After performing the lesions, a small incision was made, and the 5-mm SprayGel applicator was introduced in the abdominal cavity. SprayGel was applied immediately following the instructions for use, and 2 stitches were made to close the incision. After application of the product, SprayGel-coated tissues were rinsed with 0.5 mL of saline solution (SprayGel group). Phospholipids 3% solution 0.5 mL was applied intraperitoneally after performing the lesion (Phospholipids group). The control group received saline solution 0.5 mL (3 groups, 8 mice per group, n = 24).

Each experiment was performed with block randomization by day to avoid day-to-day variability. Therefore, 1 block of mice comprising 1 animal of each group was operated on the same day, and within a block the animals were operated in a random order.

Statistics

Statistical analyses were performed with the SAS System (SAS Institute, Cary, NC). Since adhesions scores were not normally distributed (Kurtosis test), medians and ranges are shown and differences between groups were evaluated by the nonparametric Wilcoxon rank-sum test. Because several products were used to test the same hypothesis, a Bonferroni correction[28] was used to exclude spurious significances, that is, the alpha value for significance ($\alpha = 0.05$) was divided by the number of products tested.

The p values for all the comparisons were included in the Results section. To make Table 1 and Figure 2 clearer, only the significant values were included.

Results

The results of all 3 experiments are listed in Table 1 and Figure 2. In experiment 1, the effect of HIF inhibitors was evaluated. We confirmed as shown previously that adhesion formation was higher after 60 minutes than after 10 minutes of pneumoperitoneum (proportion: p = .02, total: p = .08, extent: p = .01, type: p = .19, tenacity: p = .15, Wilcoxon rank-sum test). The administration of the vehicle DMSO did not have any effect on adhesion formation (proportion: p = .93; total: p = .96; extent: p = 1.0; type: p = .64; tenacity: p = .85). Wortmannin reduced pneumoperitoneum-enhanced adhesion formation in comparison with both controls groups, that is, the untreated (proportion: p = .04; total: p = .08; extent: p = .04; type: p = .02; tenacity: p = .19) or vehicle-treated (proportion: p = .01; total: p = .03; extent: p = .01; type: p = .07; tenacity: p = .22) control groups. After wortmannin treatment in the 60-minute pneumoperitoneum group, adhesions were no longer different from basal adhesions, that is, 10 minutes of pneumoperitoneum (proportion: p = .43, total: p = .75, extent: p = .41, type: p = .83, tenacity: p = .70). The 17-AAG, rapamycin, and radicicol did not reduce adhesions neither in comparison with the untreated-control group (proportion: p = .49; total: p = .89; extent: p = .72; type: p = .89; tenacity: p = 1.0; proportion: p = .48; total: p = .54; extent: p = .48;

Table 1 Prevention of pneumoperitoneum-enhanced adhesions in a laparoscopic mouse model

Experiment	Group	Concentration; volume of the dose	Qualitative scoring (total)
1	Control 10 min PP: basal	—	1.5 (0–2.5)
	Control 60 min PP: untreated	—	2.3 (1.3–7.0)
	Control: 60 min PP: DMSO	0.1 mL	2.3 (1.8–3.0)
	17-AAG	7.5 mg/mL; 0.1 mL	2.3 (1.0–5.5)
	Radicicol	9 mg/mL; 0.1 mL	3.0 (0.0–6.0)
	Rapamycin	5 mg/mL; 0.1 mL	2.8 (0.0–6.3)
	Wortmannin	93 μg/mL; 0.1 mL	1.3 (0.0–3.0)†
2	Control (saline solution)	0.5 mL	3.5 (3.3–5.3)
	Hyskon	0.5 mL	3.3 (2.5–5.3)
	CMC	2%; 0.5 mL	3.1 (1.8–3.8)
	Hyalobarrier Gel	Around 1 mL	0.5 (0.0–2.0)*
3	Control (saline solution)	0.5 mL	3.8 (3.0–4.8)
	SprayGel	Quantity necessary to cover the lesions	2.8 (1.0–2.8)*
	Phospholipids	3%; 0.5 mL	3.0 (3.0–4.5)

17-AAG = 7-allylaminogeldanamycin; CMC = carboxymethylcellulose; DMSO = dimethylsulphoxide; PP = CO_2 pneumoperitoneum.

CO_2 pneumoperitoneum was maintained for 60 minutes (humidified gas, 15 mm Hg insufflation pressure). Adhesions were induced during laparoscopy by performing a bipolar lesion. Three experiments were performed evaluating the effects of HIF inhibitors (17-AAG, radicicol, rapamycin, and wortmannin), surfactants (phospholipids 3%), flotation agents (Hyskon and CMC), and barriers (Hyalobarrier gel and SprayGel). Adhesions were scored after 7 days during laparotomy.

The qualitative scoring system (total) is represented (median, range). To make the table clearer, only the significant comparisons to the control groups were placed.

*p <.05 intraexperiment comparisons (each group compared to its own control group).

†p <.05 intraexperiment comparisons (each group compared to the vehicle control group for experiment 1).

type: p = .53; tenacity: p = .41; proportion: p = .79; total: p = .83; extent: p = .79; type: p = .71; tenacity: p = .65; respectively) nor with the vehicle treated-control group (proportion: p = .86; total: p = .96; extent: p = .93; type: p = .85; tenacity: p = .85; proportion: p = .36; total: p = .38; extent: p = .25; type: p = .55; tenacity: p = .21; proportion: p = .46; total: p = .40; extent: p = .54; type: p = .70; tenacity: p = .42; respectively).

In experiment 2, the effect of Hyskon, CMC 2%, and Hyalobarrier gel were evaluated on 60 minutes of pneumoperitoneum-enhanced adhesions. In comparison with the control group, adhesion formation decreased strongly with Hyalobarrier gel (proportion: p <.01; total: p <.01; extent: p <.01; type: p <.01; tenacity: p <.01), but not significantly with Hyskon (proportion: p = .18; total: p = .23; extent: p = .17; type: p = .19; tenacity: p = .26) or CMC 2% (proportion: p = .08; total: p = .07; extent: p = .09; type: p = .06; tenacity: p = .12). Hence, it is not surprising that adhesion formation scores were lower in the Hyalobarrier gel group than in the Hyskon (proportion: p <.01; total: p <.01; extent: p <.01; type: p <.01; tenacity: p <.01) or CMC 2% (proportion: p <.01; total: p <.01; extent: p <.01; type: p <.01; tenacity: p <.01) groups.

In experiment 3, the effect of a mechanical barrier (SprayGel) and a surfactant (phospholipids 3%) were analyzed. Adhesion formation decreased with SprayGel (proportion: p <.01; total: p <.01; extent: p <.01; type: p = .08; tenacity: p <.04) and with phospholipids 3% (proportion: p <.01; total: p = .12; extent: p <.02; type: p = .78; tenacity: p = .65). SprayGel was more effective in reducing

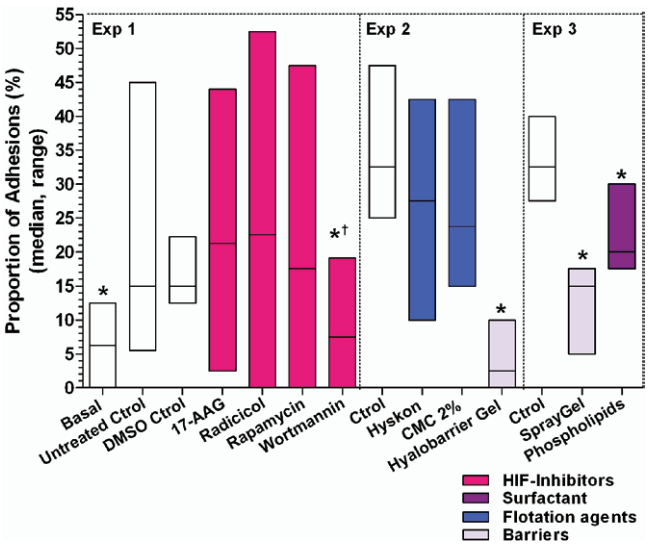

Figure 2 Prevention of pneumoperitoneum-enhanced adhesions in a laparoscopic mouse model. CO_2 pneumoperitoneum was maintained for 60 minutes (humidified gas, 15 mm Hg insufflation pressure). Adhesions were induced during laparoscopy by performing a bipolar lesion. Three experiments were performed evaluating the effects of HIF inhibitors (17-AAG, radicicol, rapamycin, and wortmannin), surfactants (phospholipids 3%), flotation agents (Hyskon and CMC), and barriers (Hyalobarrier gel and SprayGel). Adhesions were scored after 7 days during laparotomy. The quantitative scoring (proportions of adhesions) is indicated (median and range). To make the figure clearer, only the significant comparisons to the control groups were placed. *p <.05 intraexperiment comparisons (each group compared with its own control group). †p<.05 intraexperiment comparisons (each group compared to the vehicle control group for experiment 1).

adhesions than phospholipids 3% (proportion: $p < .01$; total: $p < .01$; extent: $p < .01$; type: $p = .05$; tenacity: $p = .05$).

Discussion

These experiments are part of a series of experiments designed to evaluate most known and new substances in 1 model to obtain quantitative and comprehensive information on adhesion prevention. These experiments aimed to confirm the role of HIF up-regulation as a mechanism of pneumoperitoneum-enhanced adhesion formation by blocking HIF through the inhibition of the Hsp-90 (17-AAG and radicicol) or of the PI3K signaling pathway (wortmannin and rapamycin). Taking into account these 2 mechanisms involved in HIF inhibition and also the Bonferroni correction, we can define 2 hypotheses. If the hypothesis was that HIF inhibitors decrease pneumoperitoneum-enhanced adhesion formation, 4 products can be considered leading to an α of 0.0125. If, however, the hypothesis was that inhibition of PI3K decreases adhesions through HIF, only 2 similar products (wortmannin and rapamycin) can be considered leading to an α of 0.025. Comparing with the vehicle-treated control group, wortmannin reduced pneumoperitoneum-enhanced adhesions either considering α of 0.0125 or of 0.025. Surprising, the comparison of wortmannin with the untreated control group was not significant considering both α values, although there were no differences between both control groups. This may be explained by the higher SE obtained in this control group.

If wortmannin decreases adhesion formation through HIF inhibition, it might be surprising that 17-AAG, rapamycin, and radicicol did not. First, these were screening experiments, and it cannot be excluded that different doses or way of administration could become effective. Specifically, radicicol is known to be very unstable,[26] and 1 injection could be insufficient. Second, the variability of adhesion formation in this experiment was surprisingly highly possible related to the use of DMSO as a solubilizing agent. Third, because 14-AAG and radicicol act through inhibition of Hsp-90 whereas wortmannin and rapamycin act through inhibition of the PI3K pathway, the later pathway could be more effective for adhesion reduction. Finally, the PI3K pathway is not only effective in HIF inhibition but also has other effects. PI3K pathway is involved in cell survival and proliferation and in many aspects of angiogenesis[29] and fibrinolysis, such as VEGF,[30] uPA,[31] and PAI-1[32] up-regulations. Each one of these factors is involved in the adhesion formation.[2,3] PI3K signaling is involved in the inflammatory response because blocking its activity reduces neutrophil influx by diminishing their attachment and migration[33] and reduces the adherence and spreading of the macrophages.[34] The inhibition of PI3K/Akt contributes to Hsp synthesis in addition to attenuating HIF-1α translation.[35] Specifically, wortmannin inhibits the superoxide release by the polymorphonuclear leukocytes (PMNs)[36] and during myocardial ischemia reperfusion injury, it can attenuate PMN infiltration into the myocardium and suppress superoxide release by PMNs.[37] Therefore a beneficial effect of wortmannin in reducing the toxic effect of reactive oxygen species produced during ischemia/reperfusion process can also be postulated.

In conclusion, the effect of wortmannin could be considered as supporting the hypothesis that HIF is up-regulated during pneumoperitoneum-enhanced adhesions. The absence of effect of the other products does not refute the hypothesis explained. Moreover, it cannot be excluded that wortmannin might be effective through many other mechanisms. To answer this, a detailed experiment should be done.

Flotation agents and barriers are the most well-known substances to reduce adhesions. For the barriers, we can consider that 4 barriers (flotation agents are barriers) were tested correcting the α value to 0.0125, or that 2 flotation agents and 2 mechanical barriers were tested leading an α of 0.025 for significance. In this experiment, Hyalobarrier gel was the most effective in decreasing adhesion, even significant compared with the smaller α. Moreover, in the Hyalobarrier gel–treated group, 50% (4 of 8 mice) of mice did not develop any adhesion. It should be emphasized that this is exceptional and that the incidence of adhesion formation in the other groups (control and noncontrol groups) was 100%. These results are consistent with previous observations in a laparoscopic model[13] and in an open surgery model[38,39] in rabbits and in rats.[40] Hyalobarrier gel was also proven to be effective in clinical trials, that is, in laparoscopic myomectomy[41,42] and in hysteroscopic surgery.[43,44] The ability of the Hyalobarrier gel in preventing adhesion formation may be explained, in addition to being a barrier, by its inflammatory modulating activity, for example, by induction of interleukin-1,[45,46] interleukin-8,[47] interleukin-12,[48] and tumor necrosis factor alpha[46] production. HA also improved wound healing.[49] On the other hand, HA can act as a reactive oxygen species scavenger.[50,51] It was recently demonstrated that HA increases the proliferation rate of human peritoneal mesothelial cells[52] and increases the fibrinolytic response.[53]

Although some decrease in adhesion formation was observed with CMC 2% in our laparoscopic model, the differences were not statistically significant. CMC 2% was shown to reduce intraabdominal adhesions in rats[54–56] and in rabbits,[57] but the reports were not consistent. No effect of CMC was seen in rats[58] and in rabbits.[59] In conclusion, CMC probably has some effectiveness, but the effect is small when used as a single product.

We failed to demonstrate effectiveness of Hyskon in our model. Hyskon was shown to decrease adhesions in a rabbit model,[60,61] whereas in other reports no effect on adhesion formation was observed. No effects were seen in rabbits,[59,62,63] in hamsters,[64] and in rats.[58] Injection of Hyskon 0.5 mL in the mouse abdominal cavity (25 mL/kg) was, moreover, associated with a mortality rate of 20%. This was

also observed in rats in which 20 mL/kg produced a mortality rate of 75%.[55]

SprayGel was effective in our model, even also comparing the $\alpha = 0.0125$, which is consistent with previous observations during open surgery in rats, rabbits,[65] and pigs[66] and in the human being after a laparoscopic ovarian surgery[67] and laparoscopic and open myomectomy.[68]

Phospholipids were effective in adhesion prevention in our laparoscopic mouse model, as previously demonstrated in a rabbit during open surgery.[69,70] They were, however, not effective in an open mouse model.[71] This is the first time that phospholipids were tried during laparoscopic surgery. The composition of the phospholipids solution used in this experiment was phosphatidylcholine 70% by weight, phosphatidylethanolamine 15% by weight, neutral lipids 8% by weight, sphingomyelin <3% by weight and lysophosphatidylcholin <3% by weight[72]; the large concentration of phosphatidylcholine, the lipid more predominant of the peritoneal cavity[73] may be helping to prevent adhesions. The ability of the phospholipids in preventing adhesion formation can be explained by its induction of lubricity, antiwear, and release or antistick properties.[74]

Conclusion

In conclusion, wortmannin at a dose of 0.31 mg/kg body weight clearly prevents pneumoperitoneum-enhanced adhesion. The mechanism involved probably is the prevention of HIF up-regulation, but other mechanisms as inhibition of angiogenesis, inflammation, oxidative stress, and fibrinolysis inhibition, cannot be ruled out. It is premature to exclude effectiveness of the other HIF inhibitors on pneumoperitoneum-enhanced adhesion. Barriers such as Hyalobarrier gel and SprayGel were confirmed to be highly effective, and phospholipids 3% were also shown to be effective. These results should not be viewed as stand-alone observations but could help to develop an overall strategy to reduce adhesions by combining treatments aiming at the different pathophysiological mechanisms of adhesion formation.

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