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Role of hypoxia inducible factors 1α and 2α in basal adhesion formation and in carbon dioxide pneumoperitoneum-enhanced adhesion formation after laparoscopic surgery in transgenic mice

Carlos Roger Molinas, M.D., M.Sc.,[a,b] *Rudi Campo, M.D.,*[c] *Osama Ali Elkelani, M.D.,*[a,b] *Maria Mercedes Binda, Ph.D.,*[a] *Peter Carmeliet, M.D., Ph.D.,*[d] *and Philippe Robert Koninckx, M.D., Ph.D.*[a,b]

University Hospital Gasthuisberg, Katholieke Universiteit Leuven, Leuven, Belgium

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Reprint requests: Carlos Roger Molinas, M.D., M.Sc., Department of Obstetrics and Gynecology, University Hospital Gasthuisberg, Herestraat 49, 3000 Leuven, Belgium (FAX: 32-16 344205; E-mail: roger.molinas@uz.kuleuven.ac.be).

[a] Center for Surgical Technologies, Katholieke Universiteit Leuven.

[b] Department of Obstetrics and Gynecology, University Hospital Gasthuisberg, Katholieke Universiteit Leuven.

[c] Leuven Institute for Fertility and Embryology, Leuven, Belgium.

[d] Center for Transgene Technology and Gene Therapy, Flanders Interuniversity Institute for Biotechnology, Katholieke Universiteit Leuven.

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Objective: To evaluate the role of hypoxia inducible factors (HIFs) 1α and 2α in adhesion formation after laparoscopic surgery.

Design: Prospective, randomized study.

Setting: Academic research center.

Animal(s): Forty Swiss/129SvJ wild-type mice and transgenic mice partially deficient for the genes encoding for HIF-1α (HIF-1$\alpha^{+/-}$) or HIF-2α (HIF-2$\alpha^{+/-}$).

Intervention(s): Adhesions were induced by standardized lesions during laparoscopy. To evaluate "basal adhesions" and "pneumoperitoneum-enhanced adhesions," the pneumoperitoneum was maintained for a minimum (10 minutes) or prolonged (60 minutes) period, respectively.

Main Outcome Measurement(s): Adhesions were blindly scored after 7 days.

Result(s): In both HIF-1α and HIF-2α wild-type mice, pneumoperitoneum enhanced adhesion formation. In comparison with wild-type mice, basal adhesions were lower in HIF-1$\alpha^{+/-}$ and similar in HIF-2$\alpha^{+/-}$ mice. Pneumoperitoneum did not enhance adhesion formation in HIF-1$\alpha^{+/-}$ or in HIF-2$\alpha^{+/-}$ mice. Therefore, in comparison with the correspondent wild-type mice, pneumoperitoneum-enhanced adhesions were lower in HIF-1$\alpha^{+/-}$ and HIF-2$\alpha^{+/-}$ mice.

Conclusion(s): These data confirm that CO_2 pneumoperitoneum enhances adhesion formation and indicate that this effect is mediated, at least in part, by an up-regulation of HIF-1α and HIF-2α. (Fertil Steril® 2003; 80(Suppl 2):795–802. ©2003 by American Society for Reproductive Medicine.)

Key Words: Adhesion formation, laparoscopy, CO_2 pneumoperitoneum, HIF-1α, HIF-2α, transgenic mice

Surgical peritoneal trauma initiates an inflammatory reaction determining fibrin deposition on the peritoneal injured surface and migration, proliferation, and differentiation of several cell types that modulate the subsequent peritoneal healing. The local fibrinolytic activity is critical because fibrin, if not completely degraded, will provide a scaffold for fibroblasts growth, extracellular matrix (ECM) deposition, and angiogenesis, leading to adhesion formation. The roles of fibrin and other members of the plasminogen system, fibroblasts, and ECM in adhesion formation are well known (1–6). The role of angiogenesis and angiogenic factors, however, remains largely unknown.

Angiogenesis, the formation of new blood vessels extending from existing vessels, occurs when the distance between cells and the nearest capillary exceeds an efficient diffusion range to maintain an adequate supply of oxygen and nutrients. Angiogenesis is highly regulated by cellular hypoxia (7–12).

The importance of hypoxia in adhesion formation has been postulated for several reasons besides its role in angiogenesis. First, surgical trauma induces tissue necrosis and an inflam-

matory reaction involving local hypoxia, as was recognized in adhesion formation (13–17). Second, hypoxia modulates the expression of several molecules involved in different stages of adhesion formation, such as plasminogen activator inhibitor-1 (PAI-1), tissue-type plasminogen activator (tPA), transforming growth factor-β (TGF-β), matrix metalloproteinases, and tissue inhibitors of metalloproteinases (3–5, 18–24). Third, we recently demonstrated that the pneumoperitoneum during laparoscopic surgery is a cofactor enhancing adhesion formation. Since adhesions increase with the duration of pneumoperitoneum and with the insufflation pressure and decrease with the addition of oxygen and since similar effects were found with CO_2 and helium pneumoperitoneum, mesothelial hypoxia has been postulated as the driving mechanism (25–28). It is unknown whether the mechanisms of adhesion formation after a peritoneal lesion only ("basal adhesions") and of the increased adhesion formation after a peritoneal lesion with the additional effect of the pneumoperitoneum ("pneumoperitoneum-enhanced adhesions") are similar or different.

The hypoxic response is not restricted to specific specialized cell types, and a general similar mechanism might act in a variety of cell types. Most mammalian cells can respond to alterations in oxygen levels by increasing or decreasing the expression of specific genes (29, 30). The hypoxic regulation of many of these genes, such as vascular endothelial growth factor (VEGF), takes place at both transcriptional and post-transcriptional levels. The transcriptional regulation is mediated by transcription factors known as hypoxia inducible factors (HIFs) (31–33).

HIFs are nuclear proteins that bind to hypoxia response elements (HREs) in the promoter or enhancer regions of hypoxia inducible genes, activating gene transcription in response to reduced cellular oxygen concentration (30). HIFs are members of the basic helix-loop-helix (bHLH) periodic (Per) aryl hydrocarbon receptor nuclear translocator (ARNT) single-minded (Sim) (PAS) domain protein family. Several proteins have been identified in this bHLH-PAS family that belong to the α class or the β class. Each member of the α class is able to heterodimerize with a member of the β class to form a stable activation complex. Whereas β class members are constitutively expressed in a ubiquitous or a tissue-specific way, α class members are often inducible by environmental stimuli such as light or hypoxia (34, 35).

HIF-1, the first HIF identified, is a heterodimer composed of HIF-1α and HIF-1β subunits (36–38). HIF-2 is a heterodimer composed of HIF-2α and HIF-1β subunits (39). HIF-1α and HIF-2α, the specific hypoxia-regulated subunits, are structurally very similar and share the same heterodimerization partner. Therefore, both HIF-1 and HIF-2 are highly similar in structure and regulatory domains and are able to bind to the same HRE of target genes.

This study was performed to evaluate the role of HIF-1α and HIF-2α in both basal adhesions and CO_2 pneumoperitoneum-enhanced adhesions in a laparoscopic mouse model using transgenic mice partially deficient for the genes encoding for these factors.

MATERIALS AND METHODS

Animals

The study was performed in 40 female, 10- to 12-week-old mice weighing 30–40 g. For the first experiment, 20 50% Swiss/50% 129SvJ wild-type mice (HIF-1$\alpha^{+/+}$) and transgenic mice partially deficient for the gene encoding for HIF-1α (HIF-1$\alpha^{+/-}$) were used. For the second experiment, 20 87.5% Swiss/12.5% 129SvJ wild-type mice (HIF-2$\alpha^{+/+}$) and transgenic mice partially deficient for the gene encoding for HIF-2α (HIF-2$\alpha^{+/-}$) were used. All wild-type and transgenic mice were obtained from the Center for Transgene Technology and Gene Therapy of the Katholieke Universiteit Leuven (KUL). The transgenic mice were generated as described elsewhere (40, 41).

No phenotypic differences were observed between wild-type and transgenic mice of the same strain. It has been reported, however, that HIF-1$\alpha^{+/-}$ mice exposed to chronic hypoxia present significantly delayed development of polycythemia, right ventricular hypertrophy, pulmonary hypertension, and pulmonary vascular remodeling and significantly greater weight loss than HIF-1$\alpha^{+/+}$ mice (42). The animals were kept under standard laboratory conditions (temperature 20°C–22°C, relative humidity 50%–60%, 14 hours light and 10 hours dark) at the animal facilities of the KUL. They were fed with a standard laboratory diet (MuraconG, Carsil Quality, Turnhout, Belgium) with free access to food and water at any time. The study was approved by the Institutional Review Animal Care Committee.

Anesthesia and Mechanical Ventilation

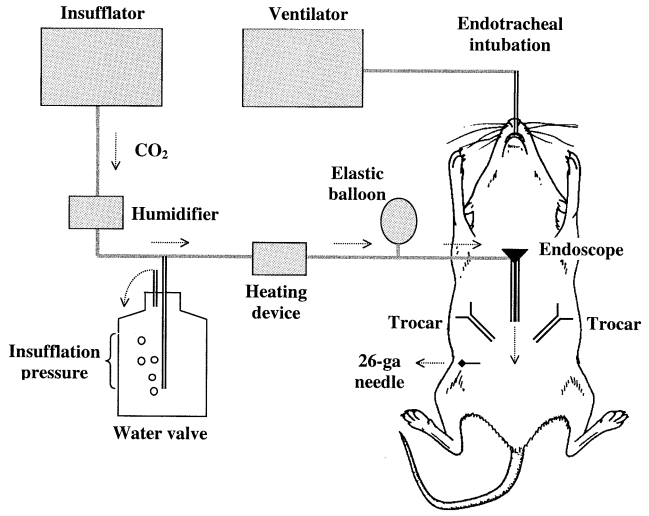
After the IM anesthesia with pentobarbital (Nembutal; Sanofi Sante Animale, Brussels, Belgium; 0.07 mg/g), the abdomen was shaved and the animal was secured to the table in the supine position. Endotracheal intubation was performed as described elsewhere (27). Briefly, a 22-gauge catheter (Insyte-W, Vialon, Becton Dickinson, Madrid, Spain) was introduced in the trachea by transillumination of the vocal cords. The catheter was connected to a mechanical ventilator (Rodent Ventilator, Harvard Apparatus, Holliston, MA), and the animal was ventilated with room air (tidal volume of 500 μL, 85 strokes/minute). In other studies, blood samples from the carotid artery during the pneumoperitoneum showed that this ventilation pattern is enough to maintain the oxygen saturation (sO_2) at 98%–99% (Molinas et al., submitted).

Laparoscopic Surgery for Induction of IP Adhesions

Laparoscopy and induction of adhesions were performed as described elsewhere (27). A 3.5-mm midline incision was made caudal to the xyphoides appendix, and a 2-mm endo-

FIGURE 1

The laparoscopic mouse model. The mouse is intubated and mechanically ventilated. CO_2 pneumoperitoneum is maintained using an insufflator together with a water valve with a free escape of excess of CO_2 to limit insufflation pressure and an elastic balloon to dampen pressure changes. The gas is humidified and heated. A 26-gauge needle with free escape of gas from peritoneal cavity maintains a constant composition of the pneumoperitoneum. Endoscopic surgery is performed using a 2-mm endoscope and two 14-gauge secondary ports.



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scope with a 3.3-mm external sheath for insufflation (Karl Storz, Tüttlingen, Germany) was introduced into the abdominal cavity. The endoscope, connected to a video camera (Karl Storz) and light source (Karl Storz), was secured in a holder. Because the mouse abdominal wall is very thin, a variable gas leakage, and thus a variable flow, through the abdomen occurred. Therefore, the incision was closed gas tight around the endoscope with 6/0 polypropylene suture (Prolene; Ethicon, Johnson and Johnson Intl., Brussels, Belgium).

For the pneumoperitoneum, the gas was insufflated through the main port with the Thermoflator Plus (Karl Storz) using heated (37°C; Optitherm, Karl Storz) and humidified (Aquapor, Dräger, Lübeck, Germany) CO_2 as insufflation gas. An insufflation pressure of 17 mmHg and a flow rate of 1.5 L/minute together with a water valve and an elastic balloon were used to maintain a continuous insufflation pressure of 20 cm H_2O (approximately 15 mmHg). The water valve and the balloon are necessary to adapt the flow rate to a mouse and to dampen the pressure changes during insufflation. Indeed, any excess of CO_2 freely escapes from the water valve, whereas pressure is maintained accurately in the water valve and pressure changes are minimized (Fig. 1).

Since the peritoneum has a large surface and high exchange capacity, theoretically some oxygen could diffuse from the circulation to the abdominal cavity. To have a constant 100% CO_2 concentration in the abdominal cavity, the gas was continuously replaced. This was achieved by inserting a 26-gauge needle (BD Plastipak, Becton Dickinson) through the abdominal wall, giving a continuous flow through the abdominal cavity of 10 mL/minute at 20 cm H_2O.

After the establishment of the CO_2 pneumoperitoneum, two 14-gauge catheters (Insyte-W, Vialon, Becton Dickinson) were inserted under laparoscopic vision in both the right and left flank for the working instruments. The uterus was grasped in the midline with a 1.5-mm grasper and standardized 10-mm × 1.6-mm lesions were performed in the antimesenteric border of both the right and left uterine horns by monopolar or bipolar coagulation (10 W, standard coagulation mode, 10 seconds) (Autocon 350, Karl Storz). In addition, identical lesions were made in right and left pelvic sidewalls. The type of lesion in each side was randomly determined. Monopolar coagulation was performed with a homemade 1.6-mm ball electrode, whereas bipolar coagulation was performed with a 1.6-mm probe (Bicap, Circon Corporation, Santa Barbara, CA).

To evaluate postoperative basal adhesion formation and pneumoperitoneum-enhanced adhesion formation, the pneumoperitoneum was maintained for the minimum time needed to induce the peritoneal lesions (standardized at 10 minutes) or for a longer period (60 minutes), respectively. The secondary ports were removed after finalizing the peritoneal lesions, and the incisions were closed. The first incision was closed at the end of the surgery. All incisions were closed in a single layer with 6/0 polypropylene suture (Prolene; Ethicon, Johnson and Johnson Intl.).

Scoring of Adhesions

A xyphopubic midline incision and a bilateral subcostal incision were made, and the whole abdominal cavity was explored during laparotomy 7 days after the induction of adhesions as described elsewhere (27). After the evaluation of port sites and viscera, the pelvic fat tissue was carefully removed and adhesions were blindly scored under microscopic vision using a qualitative and a quantitative scoring system.

In the qualitative scoring system, the following characteristics were assessed (modified from Leach et al.) (43): 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). In addition, a quantitative scoring system was used as described by Holmdahl et al. (44). This system has the advantage of being devoid of any

TABLE 1

Adhesion scores in wild-type mice (HIF-1$\alpha^{+/+}$) and transgenic mice partially deficient for HIF-1α (HIF-1$\alpha^{+/-}$).

Genotype (n)	Adhesions	Lesions	Scores			
			Extent	Type	Tenacity	Total
HIF-1$\alpha^{+/+}$ (n = 10)	Basal	M	0.9 ± 0.2	1.4 ± 0.4	1.3 ± 0.3	3.6 ± 0.8
		B	0.4 ± 0.2	0.6 ± 0.2	0.6 ± 0.2	1.6 ± 0.7
		M-B	0.6 ± 0.1	1.0 ± 0.2	1.0 ± 0.2	2.6 ± 0.5
	Pneumoperitoneum-enhanced	M	1.3 ± 0.2	1.5 ± 0.3	1.4 ± 0.2	4.2 ± 0.6
		B	1.1 ± 0.2	0.9 ± 0.2	1.2 ± 0.2	3.2 ± 0.4
		M-B	1.2 ± 0.1[a]	1.2 ± 0.2	1.3 ± 0.1	3.7 ± 0.4
HIF-1$\alpha^{+/-}$ (n = 10)	Basal	M	0.4 ± 0.2	0.5 ± 0.2	0.6 ± 0.2	1.5 ± 0.6
		B	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.2	0.4 ± 0.4
		M-B	0.3 ± 0.1[b]	0.3 ± 0.1[b]	0.4 ± 0.2	1.0 ± 0.4[b]
	Pneumoperitoneum-enhanced	M	0.4 ± 0.4	0.4 ± 0.4	0.4 ± 0.4	1.2 ± 1.2
		B	0.3 ± 0.2	0.4 ± 0.2	0.4 ± 0.2	1.1 ± 0.7
		M-B	0.4 ± 0.2[b]	0.4 ± 0.2[b]	0.4 ± 0.2[b]	1.2 ± 0.6[b]

Note: Adhesions were induced during laparoscopy by monopolar (M) and bipolar (B) coagulations and scored after 7 days during laparotomy. Basal and pneumoperitoneum-enhanced adhesions were evaluated, maintaining the pneumoperitoneum for 10 or 60 minutes, respectively. Means ± SE are indicated together with significance for the means of M and B coagulations (M-B).

[a] $P<.05$: pneumoperitoneum-enhanced adhesions vs. basal adhesions.

[b] $P<.05$: HIF-1$\alpha^{+/-}$ mice vs. HIF-1$\alpha^{+/+}$ mice.

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subjective interpretation. It measures the proportion of the lesions covered by adhesions using the following formula: adhesions (%) = (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 with lesions inflicted by monopolar or bipolar coagulation), which were individually scored.

Experimental Design

All experiments were performed using block randomization by days. Therefore, one block of mice, comprised of one animal from each group, was operated on during the same day. Within a block, the animals were operated on in random order.

In the first experiment (n = 20), basal adhesions and pneumoperitoneum-enhanced adhesions were assessed in HIF-1$\alpha^{+/+}$ mice (n = 5 and n = 5, respectively) and HIF-1$\alpha^{+/-}$ mice (n = 5 and n = 5, respectively).

In the second experiment (n = 20), basal adhesions and pneumoperitoneum-enhanced adhesions were assessed in HIF-2$\alpha^{+/+}$ mice (n = 5 and n = 5, respectively) and HIF-2$\alpha^{+/-}$ mice (n = 5 and n = 5, respectively).

Statistics

Statistical analysis was performed with the SAS System (SAS Institute, Cary, NC) using the Kruskal-Wallis test to compare individual groups. All data are presented as the mean ± SE.

RESULTS

All animals survived the surgical procedures and were available for adhesion scoring after 7 days. Adhesions formed between the injured visceral site and the pelvic fat and/or between the injured parietal site and the pelvic fat. No adhesions were observed at the site of the laparoscopic ports or at other sites.

Monopolar lesions systematically induced more adhesions than bipolar lesions. The proportion of adhesions for monopolar and bipolar lesions were, respectively, as follows. In HIF-1α wild-type mice, 13% ± 3% and 6% ± 3% for basal adhesions and 23% ± 4% and 21% ± 5% for pneumoperitoneum-enhanced adhesions. In HIF-1$\alpha^{-/-}$ mice, 7% ± 4% and 1% ± 1% for basal adhesions and 7% ± 7% and 4% ± 3% for pneumoperitoneum-enhanced adhesions. In HIF-2α wild-type mice, 17% ± 4% and 13% ± 7% for basal adhesions and 41% ± 4% and 35% ± 5% for pneumoperitoneum-enhanced adhesions. In HIF-2$\alpha^{-/-}$ mice, 15% ± 5% and 2% ± 2% for basal adhesions and 20% ± 7% and 7% ± 5% for pneumoperitoneum-enhanced adhesions. Similar effect was observed for the extent, type, tenacity, and total adhesion scores (Tables 1 and 2). To maximize statistical significance, only the means of both lesions were used for further analysis.

In HIF-1α wild-type mice, pneumoperitoneum enhanced adhesion formation (proportion: $P=.01$; extent: $P=.01$). In comparison with HIF-1α wild-type mice, basal adhesions were lower in HIF-1$\alpha^{+/-}$ mice (extent: $P=.03$; type: $P=.04$; total: $P=.05$). In HIF-1$\alpha^{+/-}$ mice, pneumoperitoneum did

TABLE 2

Adhesion scores in wild-type mice (HIF-2$\alpha^{+/+}$) and transgenic mice partially deficient for HIF-2α (HIF-2$\alpha^{+/-}$).

Genotype (n)	Adhesions	Lesions	Scores			
			Extent	Type	Tenacity	Total
HIF-2$\alpha^{+/+}$ (n = 10)	Basal	M	1.0 ± 0.3	0.7 ± 0.2	1.2 ± 0.2	2.9 ± 0.7
		B	0.9 ± 0.5	0.6 ± 0.3	0.9 ± 0.5	2.4 ± 1.2
		M-B	1.0 ± 0.3	0.7 ± 0.2	1.1 ± 0.3	2.7 ± 0.9
	Pneumoperitoneum-enhanced	M	2.1 ± 0.3	2.0 ± 0.2	2.0 ± 0.2	6.1 ± 0.5
		B	1.8 ± 0.2	1.8 ± 0.3	1.8 ± 0.3	5.4 ± 0.7
		M-B	2.0 ± 0.2	1.9 ± 0.2[a]	1.9 ± 0.2	5.8 ± 0.5[a]
HIF-2$\alpha^{+/-}$ (n = 10)	Basal	M	0.9 ± 0.2	1.3 ± 0.4	1.2 ± 0.4	3.4 ± 1.0
		B	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.3 ± 0.3
		M-B	0.5 ± 0.2	0.7 ± 0.3	0.7 ± 0.2	1.9 ± 0.6
	Pneumoperitoneum-enhanced	M	1.0 ± 0.4	1.0 ± 0.4	1.1 ± 0.4	3.1 ± 1.1
		B	0.4 ± 0.2	0.5 ± 0.3	0.4 ± 0.2	1.3 ± 0.8
		M-B	0.7 ± 0.2[b]	0.8 ± 0.3[b]	0.8 ± 0.3[b]	2.2 ± 0.8[b]

Note: Adhesions were induced during laparoscopy by monopolar (M) and bipolar (B) coagulations and scored after 7 days during laparotomy. Basal and pneumoperitoneum-enhanced adhesions were evaluated, maintaining the pneumoperitoneum for 10 or 60 minutes, respectively. Means ± SE are indicated together with significance for the means of M and B coagulations (M-B).

[a] $P<.05$: pneumoperitoneum-enhanced adhesions vs. basal adhesions.

[b] $P<.05$: HIF-2$\alpha^{+/-}$ mice vs. HIF-2$\alpha^{+/+}$ mice.

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not enhance adhesions. Therefore, in comparison with HIF-1α wild-type mice, pneumoperitoneum-enhanced adhesions were obviously lower in HIF-1$\alpha^{+/-}$ mice (proportion: $P=.04$; extent: $P=.02$; type: $P=.02$; tenacity: $P=.01$; total: $P=.01$) (Fig. 2, Table 1).

In HIF-2α wild-type mice, pneumoperitoneum enhanced adhesion formation (proportion: $P=.02$; type: $P=.01$; total: $P=.03$). In comparison with HIF-2α wild-type mice, basal adhesions were similar in HIF-2$\alpha^{+/-}$ mice. In HIF-2$\alpha^{+/-}$ mice, pneumoperitoneum did not enhance adhesions. Therefore, in comparison with HIF-2α wild-type mice, pneumoperitoneum-enhanced adhesions were obviously lower in HIF-2$\alpha^{+/-}$ mice (proportion: $P=.01$; extent: $P=.01$; type: $P=.01$; tenacity: $P=.01$; total: $P=.01$) (Fig. 3, Table 2).

DISCUSSION

A laparoscopic mouse model was used to evaluate basal adhesion formation and pneumoperitoneum-enhanced adhesion formation. "Basal adhesions" in our model, however, do not result from the electrosurgical peritoneal lesion only since CO_2 pneumoperitoneum was present, albeit for 10 minutes only. Theoretically, basal adhesions without any additional effect of CO_2 pneumoperitoneum would require the shortest duration possible, the minimum insufflation pressure, and some 3% of oxygen added to the CO_2 pneumoperitoneum, since adhesion formation decreases with shorter duration, lower pressure, and the addition of oxygen (25–28). In these experiments, for the evaluation of basal adhesions the pneumoperitoneum was maintained for the minimum time needed to perform the lesions (standardized at 10 minutes). We used, however, 100% CO_2 at 20 cm H_2O because a lower pressure and the addition of oxygen, although theoretically better, would introduce two additional

FIGURE 2

Proportion of adhesions in wild-type mice (HIF-1$\alpha^{+/+}$) and transgenic mice partially deficient for HIF-1α (HIF-1$\alpha^{+/-}$). Adhesions were induced during laparoscopy and scored after 7 days during laparotomy. Basal adhesions (*open bars*) and pneumoperitoneum-enhanced adhesions (*filled bars*) were assessed, maintaining the pneumoperitoneum for 10 or 60 minutes, respectively. Means ± SE are indicated. [a]$P<.05$: pneumoperitoneum-enhanced adhesions vs. basal adhesions. [b]$P<.05$: HIF-1$\alpha^{+/-}$ mice vs. HIF-1$\alpha^{+/+}$ mice.

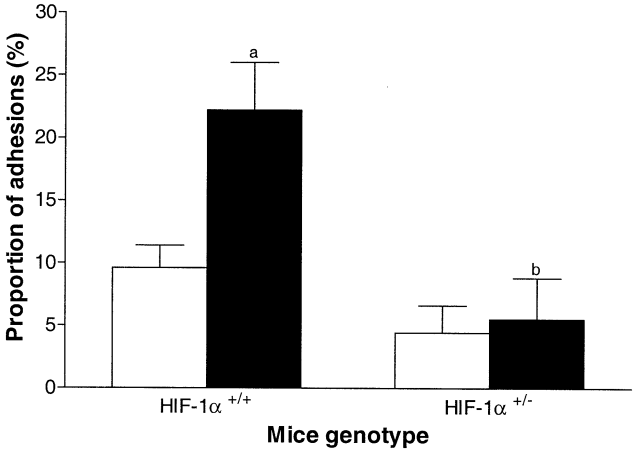

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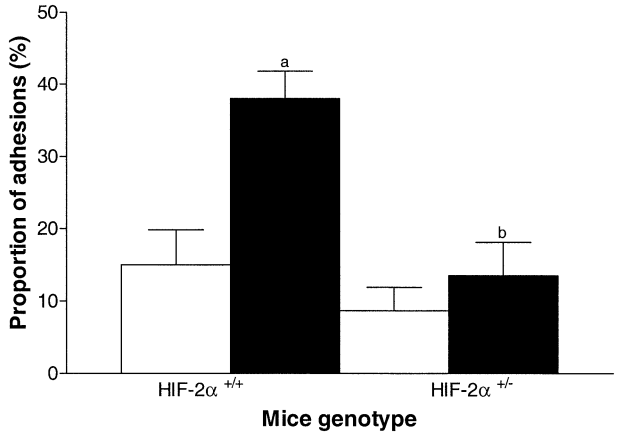
FIGURE 3

Proportion of adhesions in wild-type mice (HIF-2$\alpha^{+/+}$) and transgenic mice partially deficient for HIF-2α (HIF-2$\alpha^{+/-}$). Adhesions were induced during laparoscopy and scored after 7 days during laparotomy. Basal adhesions (*open squares*) and pneumoperitoneum-enhanced adhesions (*filled squares*) were assessed, maintaining the pneumoperitoneum for 10 or 60 minutes, respectively. Means ± SE are indicated. [a]$P<.05$: pneumoperitoneum-enhanced adhesions vs. basal adhesions. [b]$P<.05$: HIF-2$\alpha^{+/-}$ mice vs. HIF-2$\alpha^{+/+}$ mice.



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variables, i.e., pressure and oxygen. Doing both controls was unfortunately not feasible because of the limited availability of transgenic animals.

This study confirms that CO_2 pneumoperitoneum is a cofactor in adhesion formation since pneumoperitoneum-enhanced adhesions were observed in all wild-type mice.

To the best of our knowledge, this is the first study demonstrating the role of HIFs in postoperative adhesion formation. In mice partially deficient in HIF-1α or HIF-2α, adhesion formation did not increase after 60 minutes of CO_2 pneumoperitoneum, demonstrating that the mechanism of CO_2 pneumoperitoneum-enhanced adhesions involves HIF-1α and HIF-2α, which obviously cannot be up-regulated in these mice. In mice partially deficient in HIF-1α, but not in HIF-2α, basal adhesions were lower than in wild-type animals, which suggests that HIF-1α, but not HIF-2α, also has a role in basal adhesion formation.

Our results can be explained by postulating that CO_2 pneumoperitoneum enhances adhesion formation, at least in part, through an up-regulation of HIF-1α and HIF-2α. Since HIF-1α and HIF-2α are expressed in response to hypoxia, this study confirms that CO_2 pneumoperitoneum-enhanced adhesion formation is mediated by mesothelial hypoxia as suggested (27). This is also consistent with the reported role of hypoxia in adhesion formation (3–5, 13–24). This hypothesis is, moreover, supported by the similarity in partial oxygen tensions regulating HIF-1 expression and adhesion prevention after laparoscopic surgery. Indeed, HIF-1 levels increase exponentially with lower intracellular oxygen tensions (evaluated in human cervical carcinoma HeLa S3 cells in culture), with a half maximal expression of HIF-1 around 1.5%–2% of oxygen and a maximal response at 0.5% of oxygen (45). This is consistent with our observation that adhesion formation decreases with the addition of oxygen to the CO_2 pneumoperitoneum with a half maximal effect around 1.5%–2% of oxygen and a maximal effect from 3% of oxygen onward (27).

We recently evaluated the role of VEGF, i.e., VEGF-A, VEGF-B, and placental growth factor (PlGF) (46) and of the plasminogen system, i.e., PAI-1, uPA, tPA (47), in adhesion formation in transgenic mice using the same model, demonstrating that pneumoperitoneum-enhanced adhesions involve up-regulation of PAI-1, VEGF-A, VEGF-B, and PlGF. These findings and the role of HIFs are consistent with the hypothesis of mesothelial hypoxia as the driving mechanism of pneumoperitoneum-enhanced adhesions. Indeed, it is known that VEGF-A expression is up-regulated by hypoxia through HIF-1α (31–33). Similarly, PAI-1 is known to be up-regulated by hypoxia, and recently an HRE capable of binding HIF-1 was described in the gene encoding for this protein (18–20).

It is too early for a comprehensive model integrating the relative impact of HIFs, of the VEGF family, and of the plasminogen system on basal adhesions and on pneumoperitoneum-enhanced adhesions. This model has to integrate the effects on normal and damaged peritoneum together with the effects of the duration of pneumoperitoneum, the insufflation pressure, and the addition of oxygen. The available data, however, derived from experiments with transgenic mice for PAI-1, VEGF-A, VEGF-B, and PlGF and now for HIF-1α and HIF-2α, show clearly that the mechanisms involved in basal adhesions and in pneumoperitoneum-enhanced adhesions are, at least partially, different.

An effect of the mouse strain in adhesion formation was also observed. Indeed, the more Swiss and the less C57Bl/6J genetic background, the more adhesions were observed. These strain differences were obvious for both basal adhesions and pneumoperitoneum-enhanced adhesions. This observation is consistent with our previous studies (47) and was not surprising since strain differences have been reported for fibrosis and healing responses, e.g., for hepatic fibrosis (48), lung fibrosis (49), colorectal fibrosis (50), ear wound healing (51), myocardial healing (52), and bone regeneration (53).

In conclusion, our data confirm the role of the CO_2 pneumoperitoneum as a cofactor in adhesion formation and demonstrate a role of HIF-1α in basal adhesions and of both HIF-1α and HIF-2α in pneumoperitoneum-enhanced adhesions, suggesting HIF-1α and HIF-2α up-regulation as a mechanism for this pneumoperitoneum-enhanced adhesion

formation. This is fully consistent with the up-regulation of these factors by hypoxia and with the concept that CO_2 pneumoperitoneum causes mesothelial hypoxia and confirms the role of hypoxia in adhesion formation. These observations give new insights into the pathophysiology of adhesion formation. Since the mechanisms involved in basal adhesions and in pneumoperitoneum-enhanced adhesions are, at least partially, different, new methods for adhesion prevention after laparoscopic surgery could be developed.

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