

CARLOS ROGER MOLINAS, M. MERCEDES BINDA
AND PHILIPPE KONINCKX DISCUSS THE
ROLE OF VEGF IN ADHESION FORMATION

Vascular endothelial growth factor and postoperative adhesion formation

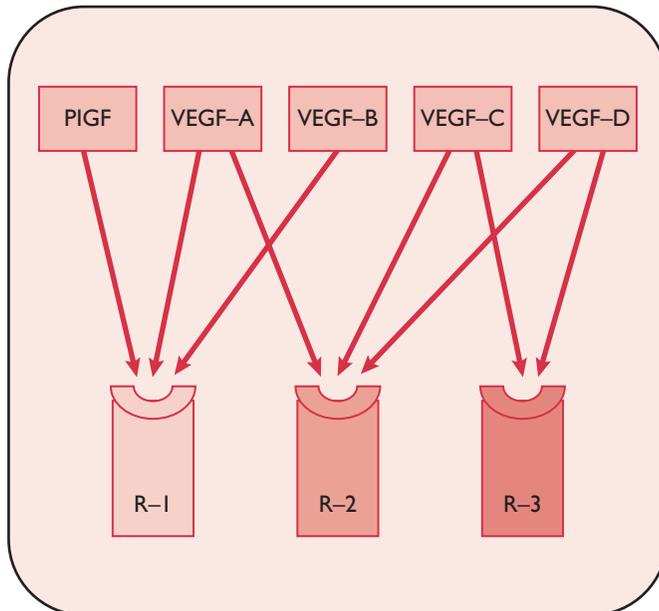


Figure 1. The VEGF family.

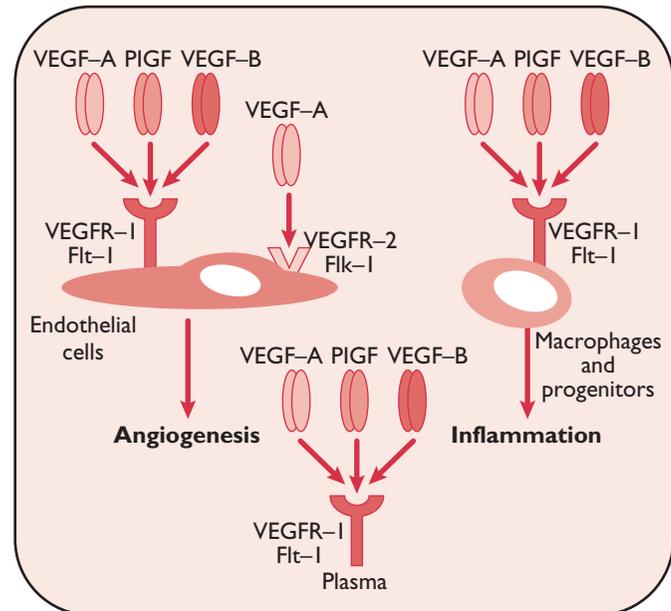


Figure 2. Stimulation of inflammation and angiogenesis.

Peritoneal trauma determines an inflammatory reaction with fibrin deposition on the injured surface, fibroblast growth, extracellular matrix (ECM) deposition and angiogenesis, leading to adhesion formation. The roles of fibrin and the plasminogen system, fibroblasts and ECM in adhesion formation are well known,¹⁻⁴ whereas the role of angiogenesis has not yet been fully explored.

Angiogenesis, i.e. the formation of new blood vessels from existing vessels, occurs when the distance from the nearest capillary exceeds an efficient diffusion range for adequate supply of oxygen and nutrients to cells. It is regulated by cellular hypoxia through the modulation of angiogenic factors such as vascular endothelial growth factor (VEGF).^{5,6}

The VEGF family

The VEGF family includes VEGF-A, VEGF-B, VEGF-C, VEGF-D and placental growth factor (PIGF) (Figure 1). VEGF-A, VEGF-B and PIGF are transcribed from single genes and processed by alternative splicing into different isoforms. For VEGF-A, there are four isoforms in humans (VEGF-A₁₂₁, VEGF-A₁₆₅, VEGF-A₁₈₉ and VEGF-A₂₀₆) and three in mice (VEGF-A₁₂₀, VEGF-A₁₆₄ and VEGF-A₁₈₈). For VEGF-B, there are two isoforms in humans and in mice (VEGF-B₁₆₇ and VEGF-B₁₈₆). For PIGF, there are three isoforms in humans (PIGF-1, PIGF-2 and PIGF-3) and one in mice (PIGF-2).^{7,8} These factors bind to two high-affinity transmembrane tyrosine kinase receptors with seven immunoglobulin-like extracellular domains and a kinase intracellular domain, i.e. VEGFR-1/Flt-1 (for VEGF-A, VEGF-B and PIGF) and VEGFR-2/Flk-1 (for VEGF-A). These receptors are selectively but not exclusively expressed on endothelial cells. A truncated soluble form of VEGFR-1, resulting from alternative splicing and retaining its binding activity, is present in serum. VEGFR-1 is, unlike VEGFR-2, also expressed on inflammatory cells. Therefore, VEGF-A, VEGF-B and PIGF can stimulate inflammation in addition to angiogenesis (Figure 2).^{9,10}

The role of VEGF in adhesion formation

Because of the presence of VEGF in endothelial cells of blood vessels supplying pelvic adhesions, a role for VEGF in adhesion formation has been suggested.¹¹ These observations were supported by the reduction of adhesion formation after treatment with antibodies against VEGF in an open surgery mouse model¹² and are consistent with the reported altered expression of VEGF isoforms in an open surgery rat model.¹³

The role of VEGF in adhesion formation after laparoscopic surgery has also recently been addressed by using transgenic mice and monoclonal antibodies in a laparoscopic mouse model (Figure 3, overleaf).¹⁴ Adhesions were induced by standardised peritoneal lesions during laparoscopy and scored after 7 days at laparotomy. Since previous data indicate that adhesions increase with the duration of the pneumoperitoneum and the insufflation pressure,¹⁵⁻¹⁷ the CO₂ pneumoperitoneum was maintained at 14 mmHg for the minimum time needed to induce the lesions (10 minutes) or for a longer period (60 minutes) to evaluate basal adhesions and pneumoperitoneum-enhanced adhesions, respectively.^{14,18,19}

Carlos Roger Molinas, MD, PhD, Post-doctoral Researcher, M. Mercedes Binda, PhD, Post-doctoral Researcher, and Philippe Koninckx, MD, PhD, Head, Division of Endoscopic Surgery, Department of Obstetrics and Gynecology, University Hospital Gasthuisberg, Katholieke Universiteit Leuven, Leuven, Belgium

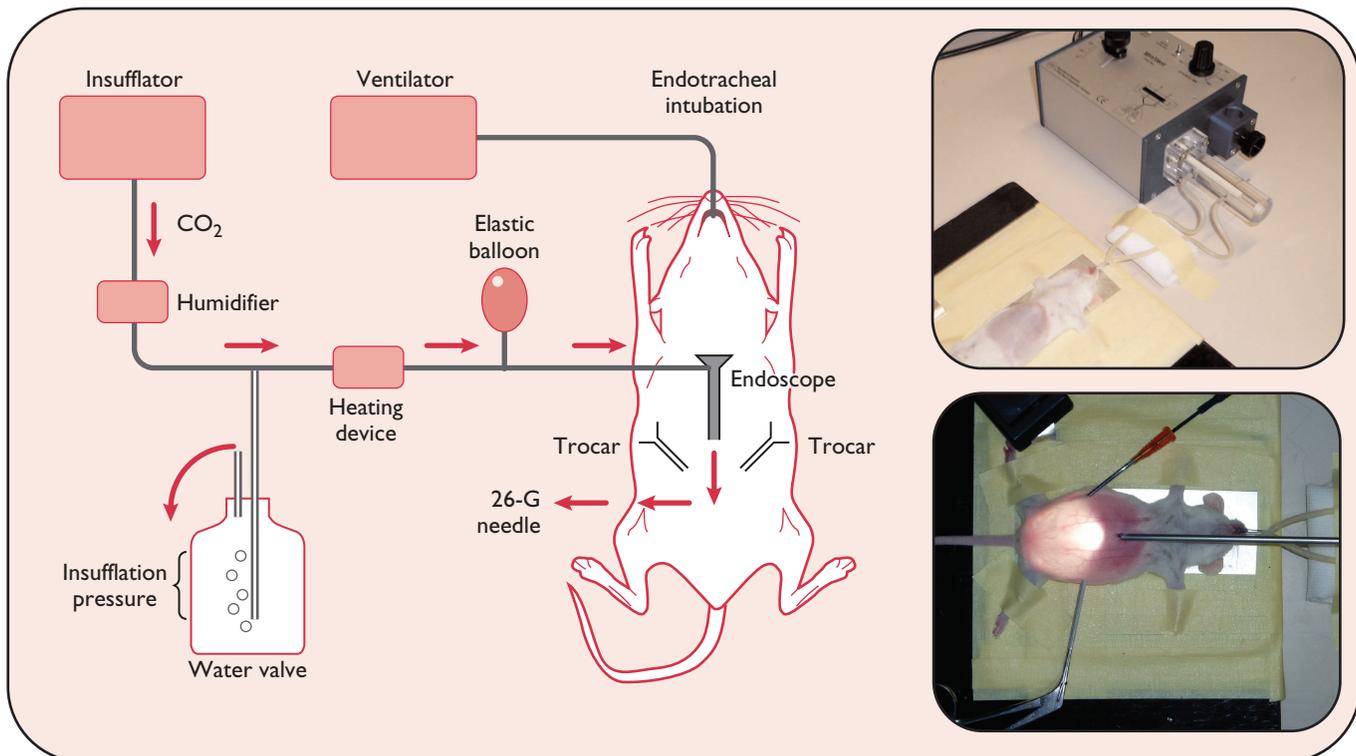


Figure 3. Laparoscopic mouse model.

The role of VEGF-A was evaluated in wild-type mice (VEGF-A^{+/+}) and transgenic mice deficient for VEGF-A₁₂₀ and VEGF-A₁₈₈ and expressing exclusively VEGF-A₁₆₄ (VEGF-A^{164/164}). In VEGF-A^{+/+} mice, pneumoperitoneum increased adhesions. In VEGF-A^{164/164} mice, basal adhesions were higher than in VEGF-A^{+/+} mice, whereas pneumoperitoneum only slightly increased adhesions. In comparison with VEGF-A^{+/+} mice, pneumoperitoneum-enhanced adhesions were higher in VEGF-A^{164/164} mice (Figure 4).¹⁹

The role of VEGF-B was evaluated in wild-type mice (VEGF-B^{+/+}) and transgenic mice deficient for VEGF-B (VEGF-B^{-/-}). In VEGF-B^{+/+} mice, pneumoperitoneum was found to increase adhesions. In VEGF-B^{-/-} mice, basal adhesions were similar to VEGF-B^{+/+} mice, whereas pneumoperitoneum did not increase adhesions. In comparison with VEGF-B^{+/+} mice, pneumoperitoneum-enhanced adhesions were lower in VEGF-B^{-/-} mice (Figure 5).¹⁹

The role of PIGF was evaluated in wild-type mice (PIGF^{+/+}) and transgenic mice deficient for PIGF (PIGF^{-/-}) and by using monoclonal antibodies with differing neutralising capacities of the binding of PIGF to its receptor. In PIGF^{+/+} mice, pneumoperitoneum increased adhesions. In PIGF^{-/-} mice, basal adhesions were slightly lower than in PIGF^{+/+} mice, whereas pneumoperitoneum did not increase adhesions. In comparison with PIGF^{+/+} mice, pneumoperitoneum-enhanced adhesions were lower in PIGF^{-/-} mice (Figure 6).¹⁹

In the experiment with PIGF antibodies, mice were treated either with IgG or non-neutralising antibodies (Ab-A), with neutralising antibodies (Ab-B and Ab-C) or with semi-neutralising antibodies (Ab-D). In the control groups, pneumoperitoneum increased adhesions. In mice treated with neutralising antibodies, basal adhesions were lower than in the control groups, whereas pneumoperitoneum did not increase adhesions. In these mice pneumoperitoneum-enhanced adhesions were lower than in the control groups. In semi-neutralising antibodies-treated mice, an intermediate effect was observed (Figure 7).¹⁹

For basal adhesions, the data clearly demonstrate a role for VEGF-A₁₆₄, which is consistent with the presence of VEGF-A in peritoneal adhesions^{11,13} and the reduction of adhesions following VEGF-A antibodies administration after open surgery.¹² However, no role for VEGF-B was observed, whereas a role for PIGF remains controversial.

For pneumoperitoneum-enhanced adhesions, the data indicate that pneumoperitoneum increases adhesions through VEGF-B and PIGF up-regulation and probably also through VEGF-A₁₆₄ up-regulation. Indeed, pneumoperitoneum-enhanced adhesions are absent in VEGF-B^{-/-} and PIGF^{-/-} mice because the pneumoperitoneum cannot up-regulate these non-existent factors. This is fully consistent with the observations in mice treated with PIGF antibodies. The slight increase in adhesions following 60 minutes

of pneumoperitoneum in VEGF-A^{164/164} mice does not rule out VEGF-A₁₆₄ up-regulation because adhesion formation could already be near maximal due to the over-expression of this factor.

The role of VEGFR-1

The role of the common receptor of VEGF-A, VEGF-B and PIGF, i.e. VEGFR-1, was evaluated by using monoclonal antibodies against VEGFR-1. In the control group, i.e. IgG-treated mice, pneumoperitoneum was found to increase adhesions. In VEGFR-1 antibodies-treated mice, basal adhesions were found to be similar in IgG-treated mice, whereas pneumoperitoneum did not increase the formation of adhesions. In VEGFR-1 antibodies-treated mice, pneumoperitoneum-enhanced adhesions were found to be less than in IgG-treated mice (Figure 8).²⁰ These data indicate that the effects of the VEGF family are mediated to a large extent by this receptor. This is supported by the recently reported reduction of peritoneal fibrosis after soluble VEGFR-1 gene transfer in mice,²¹ since this isoform, by retaining its binding capacity, reduces the binding of the ligands to the functional cellular receptors.

Relative contribution of VEGF-driven angiogenesis and inflammation to adhesion formation

Since VEGFR-1 is expressed on endothelial cells and on inflammatory cells; it remains unclear whether these effects are related mainly to stimulation of angiogenesis and/or stimulation of inflammation.

Several complementary mechanisms have been proposed for VEGF-driven angiogenesis and inflammation.^{9,10,22} VEGF-A induces angiogenesis by activating VEGFR-2, while VEGFR-1 might function as an inert 'decoy' regulating the availability of VEGF-A to activate VEGFR-2. PIGF stimulates angiogenesis by several mechanisms:

- PIGF displaces VEGF-A from VEGFR-1, increasing the fraction of VEGF-A available to activate VEGFR-2;
- PIGF up-regulates the expression of VEGF-A;
- PIGF transmits its own intracellular angiogenic signals through VEGFR-1;
- PIGF activates receptor cross-talk between VEGFR-1 and VEGFR-2, enhancing VEGFR-2-driven angiogenesis;
- PIGF forms heterodimers with VEGF-A.

VEGF-A and PIGF stimulate inflammation through VEGFR-1 by increasing mobilisation of bone marrow-derived myeloid progenitors into peripheral blood, by increasing myeloid cell differentiation, mobilisation and activation, and by increasing cytokine production by macrophages.

To what extent angiogenesis and inflammation each contribute to adhesion formation remains to be elucidated. Regardless of the main mechanism of action, the available data point to peritoneal hypoxia as the trigger factor.^{15,16} This is supported by the absence of pneumoperitoneum-enhanced adhesions in mice deficient for hypoxia inducible factors (HIFs) 1 or 2,¹⁴ which is consistent with the effects of the VEGF family.²⁰ Indeed, VEGF-A is up-regulated at the transcriptional level by HIF-1²³ with the consequent regulation of VEGF-A/PIGF and VEGF-A/VEGF-B heterodimers. It is also supported by the absence of pneumoperitoneum-enhanced adhesions in mice deficient for plasminogen activator inhibitor-1 (PAI-1),¹⁸ since PAI-1 is up-regulated by hypoxia through HIF-1.²⁴⁻²⁶

Relevance to human surgery

The relevance of the mouse data for human surgery has still to be proven. There is evidence that the mechanisms involved in adhesion formation following laparoscopic surgery are much more complex than originally believed. The mouse model has the advantage of being suitable for a thorough investigation of the roles of different pneumoperitoneum-related factors and of the relative importance of cells, molecules and processes involved. The use of this model in adhesion-formation studies will permit a more comprehensive understanding of the intrinsic underlying mechanisms, in order to enable the design of more specific experiments in humans.

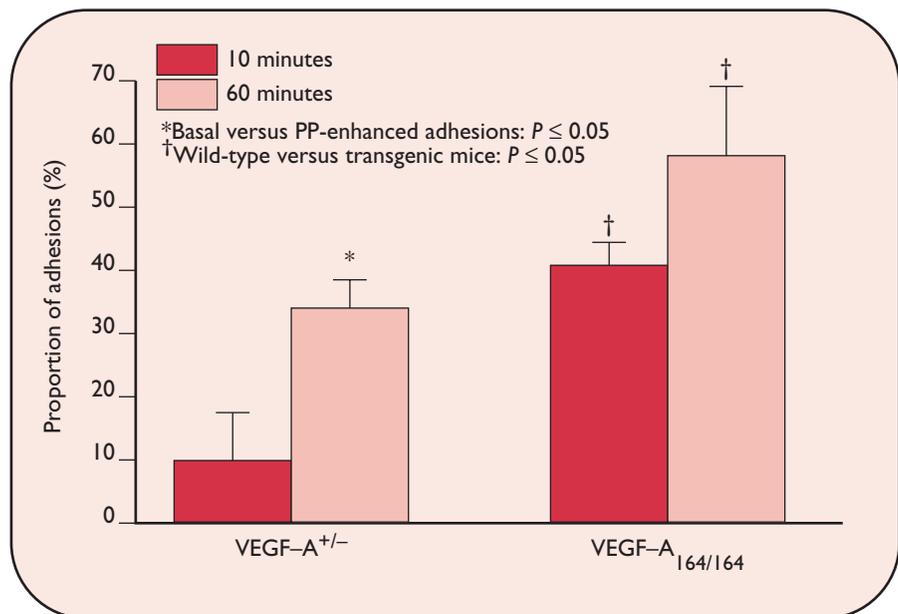


Figure 4. Adhesion formation and VEGF-A.

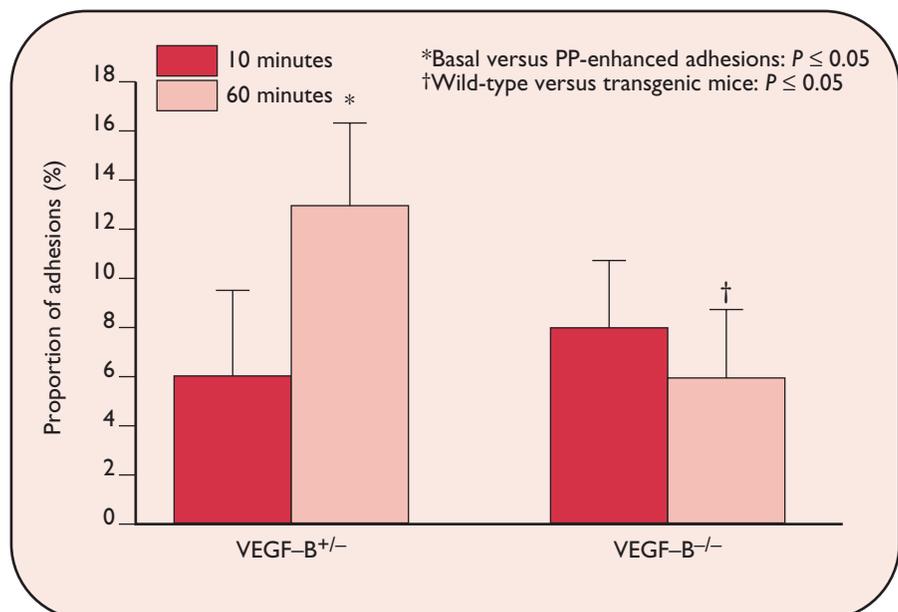


Figure 5. Adhesion formation and VEGF-B.

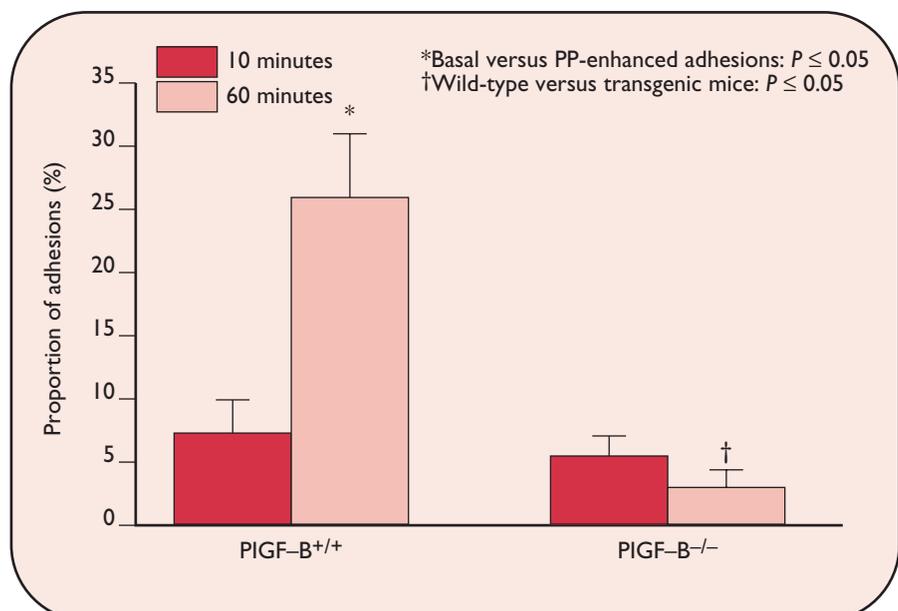


Figure 6. Adhesion formation and PIGF.

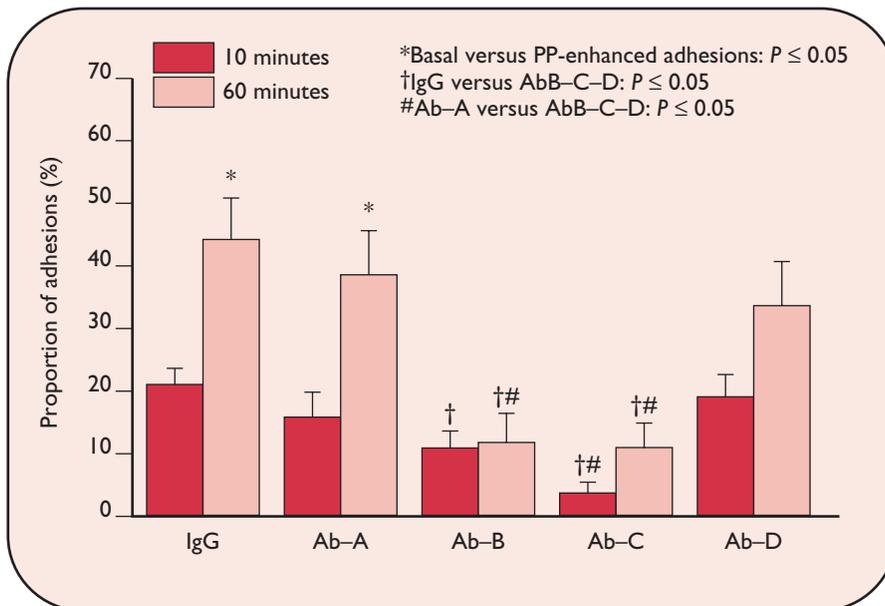


Figure 7. Adhesion formation and PIGF antibodies.

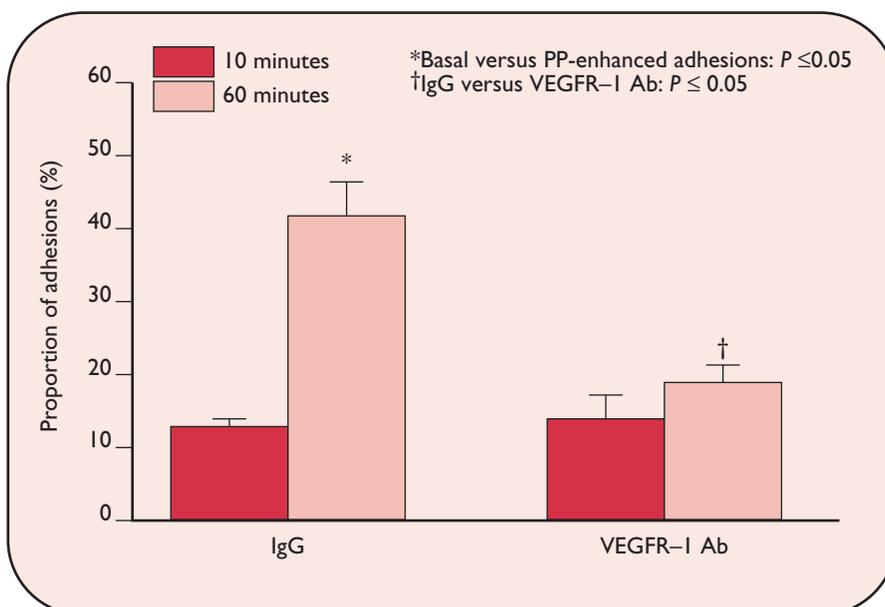


Figure 8. Adhesion formation and VEGFR-1 antibodies.

Summary

These data confirm that CO₂ pneumoperitoneum is a co-factor in adhesion formation, suggesting VEGF-A, VEGF-B and PIGF up-regulation as mechanisms and pointing to the critical role of VEGFR-1. These observations open new insights in the pathogenesis of adhesion formation and might lead to new methods for adhesion prevention after surgery.

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SUMMARY

- CO₂ pneumoperitoneum is a co-factor in adhesion formation, suggesting that there is VEGF-A, VEGF-B and PIGF up-regulation and highlighting the critical role of VEGFR-1
- These observations open new insights in the pathogenesis of adhesion formation and might lead to new methods for adhesion prevention following surgery