Molecular basis of Post-surgical Peritoneal adhesions - An Overview

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Abstract

Post surgical adhesion development remains a frequent occurrence and is often unrecognized by surgeons. Peritoneal adhesions are the leading cause of pelvic pain, bowel obstruction and infertility. The prevention of adhesion till date is speculative due to lack of understanding of mechanisms involved in adhesion development. Adhesions are proposed to the disorder of wound healing and imbalance between fibrinogenesis and fibrinolysis. The unprecedented advancement in Molecular Biology has led us to identify molecules involved in both wound healing and adhesion development. The role of these molecules in peritoneal biological functions is not well understood. Hypoxia is proposed to be major contributing factor for the development of adhesions. The major mechanisms behind adhesion development are increased fibrinogenesis, reduced fibrinolysis, increased Extra Cellular Matrix deposition, increased cytokine production, increased angiogenesis and reduced apoptosis. Better understanding of these events will make efficient management of adhesions possible.
Keywords: Post surgical adhesions, wound healing, extracellular matrix, TGFß, MMP, fibrinogenesis, fibrinolysis.

Introduction

Peritoneal adhesions are well vascularized and innervated bands of tissue, which join together previously normally separated intra-abdominal organs (Gorvy et al., 2005). Whether induced by infection, inflammation, ischemia, and/or surgical injury, peritoneal adhesions occur in overwhelming majority of patients and are leading cause of pelvic pain, bowel obstruction and infertility. Adhesions most of the times go unnoticed because they are difficult to diagnose clinically and require second look laparotomy to assess their degree of severity. It is not clear that why post-surgical peritoneal wounds heal without adhesions in some patients and others develop severe scarring from seemingly equal procedures. In addition, in the same patient, adhesions can develop at one surgical site and not in another.

Though large numbers of surgical procedures are carried out in the world every day, the information available about the mechanisms underlying development of adhesions is scanty. Most studies in human beings and animals that have evaluated the development of postoperative adhesions have been limited to a gross examination for presence or absence of adhesions, as well as the extent of organ or site involvement and the severity of the scarring. Thus to study molecular mechanisms involved in development of adhesions is critical to develop the strategies for prevention of adhesions.

Incidence of Adhesion

Almost every incision is followed by adhesion. Adhesions are believed to contribute to infertility in 40% of infertile couples (Diamond and DeCherney, 1987). Post-surgical abdominal adhesions are most common after gastrointestinal surgery (27%) (Gerehards et al., 1990). Peritoneal dialysis is performed in a substantial number of patients annually and is also a leading cause of adhesion formation in humans (Topley et al., 1996).

Etiology

Besides surgery there are many factors supposed to be responsible for developing adhesions. Different types of injuries like serosal trauma (Granaut et al., 1983), endotoxaemia, intestinal manipulations, intestinal distention, desiccation of serosa, tissue ischemia and traction may lead to adhesions. Foreign

bodies including gauge fragments, sutures (Luijendijk et al., 1996), cotton fibers, lint, glove powder and antibiotic powder (Liakakos et al., 2001) may cause adhesions. Different inflammatory conditions like cystitis, reticulitis (Kopezwski, 1984), appendicitis and pelvic inflammatory disease are linked to the development of adhesions. Radiation, allergic reaction and chemical irritation may lead to adhesions by an unknown mechanism (Thomson and Whawell, 1995).

Grading

Adhesions have been graded on different basis.

Table 1. Grading on the basis of density (Frederick et al., 1986)

0	No adhesions
+(1+)	Localized filmy adhesions
++(2+)	Localized dense adhesions
+++(3+)	Wide spread filmy adhesions
++++(4+)	Wide spread dense adhesions

Table 2. Grading on the basis of traumatized area involved (Guvenal et al., 2001)

0	No adhesions
1	Adhesion on 25% of the traumatized area
2	Adhesion on 50% of the traumatized area
3	Total area involved

Table 3. Grading on the basis of vascularity and density (Canbaz et al., 2005)

0	No adhesion
1	Flimsy adhesions light and easily released with finger
2	Mild adhesions continuous yet avascular, disrupted by gentle blunt dissection
3	Moderate fibrous adhesions, some vascularity, identifiable tissue planes requiring sharp dissection
4	Dense scar with obliteration of tissue planes

Tissue Injury

Any type of tissue injury may lead to one of the four outcomes. The first possible outcome is establishment of tissue equilibrium by normal repair mechanisms. The second outcome is regeneration where exact replacement takes place. There may be a possibility of deficient healing as in case of chronic ulcers. The fourth possibility is excessive healing that occurs specifically in fibrosis and contractures (Diegelmann and Evans, 2004).

Wound Healing

The phenomena of wound healing and adhesion formation are closely linked together. Many cytokines involved in wound healing play a crucial role in development of adhesions. Wound healing is a physiological phenomenon by which body tries to repair damaged tissue by series of regenerative processes. The healing of clean, well perfused, incised surgical wounds and casual wounds where there is minimum destruction of tissue is called healing by first intention. The healing of large wounds, where there has been significant loss or destruction of tissue and infected wound is known as healing by second intention.

Wound healing either surface or internal has three phases-inflammatory, proliferative and maturational phase. Inflammatory phase is characterized by hemostasis and inflammation, activation of clotting cascade, vasodilatation secondary to histamine release and release of vasoconstrictors like thromboxane A2 and prostaglanding-2a. Proliferative phase is marked by epithelialization, angiogenesis, granulation tissue formation and collagen deposition. The maturational phase is characterized by contraction of wound resulting in formation of apparent scar tissue, replacement of type III collagen by stronger type I collagen and removal of blood vessels no longer required by apoptosis.

The Mesothelium

The mesothelium is a tissue consisting of monolayer of mesothelial cells which covers all serosal cavities (pleural, pericardial and peritoneal) (Whitaker et al.., 1982). It is an important player in local fibrin deposition and clearance within serosal cavities. It secretes various factors, such as Plasminogen Activator Inhibitor (PAI) and urokinase Plasminogen Activator Inhibitor (uPAI). Recovery of surgically damaged mesothelium is thus crucial step in prevention of adhesions (Chegini et al., 2001).

Extracellular Matrix (ECM)

The expression of many components of the ECM has been documented in dermal wounds during all phases of healing and their over-expression has been associated with various forms of tissue fibrosis (Holmdahl, 1997). Peritoneal mesothelial cells express both collagen I and III and fibronectin, and this expression is modulated by hypoxia (Saed et al., 1999). Compared with peritoneal fibroblasts, adhesion fibroblasts had a significant increase in the basal mRNA levels for collagen I and fibronectin, and this expression is upregulated under hypoxic conditions. The data suggest that hypoxia modulates ECM production in peritoneal cells through a mechanism that may involve TGF-ß1. In addition, recent data indicates that hypoxia modulates integrin expression in human peritoneal fibroblasts Saed and Diamond, 2004).

Transforming Growth Factor-ß (TGF-ß)

Till date three isoforms of TGF-ß have been identified- TGF-ß1, ß2 and ß3. Elevated level of TGF-ß has been associated with increased incidence of

adhesions. Hypoxia increases Transforming Growth Factor-ß1 (TGF-ß1), which in turn increases PAI-1 and uPA mRNA expression in endothelial cells. Hypoxia via TGF-ß1 has also been shown to decrease the synthesis of collagenase and plasminogen activator. The Q-RT-PCR experiment has been carried out to evaluate the expression of TGF-ß isoforms in normal and adhesion tissue. There was significantly higher level of expression of TGF-ß1 in adhesion tissue as compared to normal peritoneum (Chegini et al., 2001). Although all isoforms are showing increased expression each isoforms display unique pattern of both spatial and temporal expression (Gorvy et al., 2005).

Inflammatory Mediators

During peritoneal injury, coagulation and platelet aggregation are initiated to prevent excessive blood loss. Different inflammatory mediators are released during this process. The processes of clot formation and its degradation are regulated by eicosanoids, antithrombin III, protein C, Plasminogen Activators (PAs) and PAIs. There is documented evidence suggesting increased levels of eicosanoids and their receptors in adhesion tissues (Lijnen, 2001). Also, administration of NSAIDs has proven effective in prevention of adhesions in animal models.

Chemokines

Chemokines are small polypeptides divided into several subgroups according to the spatial arrangement of the first two cysteine residues that include CXC or a, CC or ß, C or Gamma and CX3C or d chemokine (Gerard and Rollins, 2001). Recent in vitro and in vivo studies also point to the critical role for chemokines in peritoneal biology, specifically, the inflammatory response that often leads to adhesion formation (Topley et al., 1996). Gene expression studies and animal model systems point out the importance of complex interaction among cytokines and chemokines in peritoneal inflammatory and immune responses (Orlofsky et al., 2000). Inhibition of chemokines has shown marked decrease in incidence of adhesion (Berkkanoglu et al., 2005).

Angiogenesis

The process of formation of new blood vessels at the site of injury is angiogenesis. Angiogenesis is a self-limited and strictly regulated process that occurs in sequential manner. It involves degradation of the vascular basement membrane and interstitial matrix by endothelial cells, migration and proliferation of endothelial cells and finally tubulogenesis and formation of capillary loops (Szekanecz and Koch, 2001).

The production of proteolytic enzymes in response to angiogenic factors is fundamental to angiogenesis, not only for the degradation of perivascular matrix and tissue stroma, but also for the migration and proliferation of endothelial cells. During angiogenesis, the initial migration and proliferation of endothelial cells occurs in a fibronectin rich ECM, whereas vascular maturation, which takes place at later stages, is laminin rich (Clark, 1996). These processes also involve integrins, the essential components of ECM-cell and cell-cell interactions that promote cell migration, gene expression, cell differentiation and other cellular activities (Clemetson and Clemetson, 2001). At the initial stage of angiogenesis, the induction of proteases such as Matrix Metalloproteinases (MMPs) and serine proteases in endothelial cells is necessary to degrade components of the ECM, including fibronectin and laminin. However, these proteases are produced in inactive forms and must become activated to initiate their local actions. Proteolytic activities of these enzymes are regulated by naturally occurring physiological inhibitors, Tissue Inhibitor of MMPs (TIMPs) and Plasminogen Activator Inhibitors (PAIs).

Human peritoneal capillaries and arteriole endothelial cells express Vascular Endothelial Growth Factor and other angiogenic factors (Wiczyk et al., 1998) that may regulate the proteolytic enzymes and their inhibitors. Since VEGF plays a key role in coagulation fibrinolytic and angiogenic activities, it is considered to be a critical cytokine in the development of peritoneal adhesions. Four species of mRNA encoding VEGFs arising from alternative splicing have been identified (VEGF 188, 165, 145 and 120) of which VEGF188, 165 and 120 are expressed in peritoneal wounds. Up-regulated expression of VEGF188 and 120 occurs during the early stages of peritoneal healing, and down-regulation of VEGF165 expression has been observed 24 to 48 hrs following injury (Rout et al., 2000). Hypoxia, a condition that promotes peritoneal adhesion, alters the expression of several cytokines, chemokines and eicosanoids in the wound, including the expression of VEGF and TGF-ß, shown to cause tissue fibrosis (Howdieshell et al., 2000).

Tissue Remodeling in Adhesion Development

ECM deposition and tissue remodeling are critical to all phases of normal wound healing most notably cell migration, growth and differentiation, angiogenesis and tissue fibrosis (Gallo, 2000). The important step in tissue remodeling is proteolytic degradation of collagens and fibronectin to smaller fragments. These fragments attract inflammatory cells and fibroblasts. Fibronectin fragments but not intact fibronectin also induce the expression of MMPs and PA (Hu et al., 2000). The MMPs degrade more fibronectin to fragments which further increase the MMP

expression and cascade continues.

TGF-ß in a cell specific manner increases the expression of fibronectin and procollagen I in human adhesion fibroblasts, whereas it induces collagen type III, with a limited effect on collagen I in mesothelial cells. Because overproduction of TGF-ß is associated with increased incidence of adhesion formation, modulation of the expression of ECM in adhesion fibroblasts may partly account for TGF-ß induced adhesions (Saed et al., 2000). Peritoneal mesothelial cells and the serosal surface of several peritoneal organs and parietal peritoneum also express integrins, such as av and ß3 (Chegini et al., 2001). These integrins specifically bind fibronectin and vitronectin expressed by peritoneal serosal tissue and mesothelial cells, and are regulated by TGF-ß. The interaction of vitronectin with PAI-1, integrins and MMPs, factors having individual and interactive biological activities critical to the outcome of wound healing, further imply the importance of vitronectin in peritoneal wound healing and adhesion formation.

Matrix Metalloproteinases (MMPs)

MMPs are zinc-dependent endo-peptidases which can degrade essentially all components of the ECM. 28 members of this family have been identified so far. MMPs on the basis of their structure and substrate specificity are classified as collagenases (MMP-1,-8,-13), gelatinases (MMP-2,-9), stromelysins (MMP-3,-7,-10,-11), matrilysin (MMP-9), and the membrane-type MMPs (MT-MMP-1,-2,-3) (Ravanti and Kahari, 2000). The catalytic activity of MMPs is controlled, at least in part, by their physiological inhibitors, Tissue Inhibitors of Metalloproteinases (TIMPs), which are comprised of TIMP-1 –2, -3 and -4 (Vincenti, 2001).

MMPs typically are not constitutively expressed, but are induced in tissues that normally undergo extensive remodeling, such as wound tissue, and in response to various inflammatory conditions. In addition, MMPs are regulated by cytokines, growth factors, hormones, and cell-cell and cell-matrix interactions. In contrast, the expression of TIMPs is widespread in many tissues and is regulated in coordination with MMPs expression. There is documented evidence that peritoneum and adhesion tissues express MMPs and TIMPs with TIMP-1 levels significantly higher in fibrous adhesions than in the peritoneal serosal tissue (Chegini, 2002). This increase in TIMP-1 expression in fibrous adhesions paralleled the expression of TGF-ß1 and integrin (Chegini et al., 2001). Under in vitro conditions, TGF-ß1 inhibits the expression of MMPs and increases TIMPs, decreasing matrix degradation and increasing tissue fibrosis.

Nitric Oxide (NO)

Nitric oxide is a potent antifibrotic effector. In rat models, NO has been shown to inhibit the excessive deposition of collagen, of adhesions (Muscara et al., 2000). Long term inhibition of Nitric Oxide Synthase (NOS) worsens fibrosis in rats. Inducible Nitric Oxide Synthase (iNOS) knocked out mice showed exacerbated experimental fibrosis (Shukla et al., 1999). Addition of NO remarkably increases apoptosis of adhesion fibroblasts but has no effects on normal peritoneal fibroblasts (Ferrini et al., 2002).

Mechanisms of Adhesion

The progenitor to adhesions is the fibrin gel matrix, which develops in several steps, including the formation and insolubilization of fibrin polymer and its interaction with fibronectin and a series of amino acids (Saed and Diamond, 2004). Protective fibrinolytic enzyme systems of the peritoneal mesothelium, such as the tPA system, can remove the fibrin gel matrix. However, surgery, possibly through peritoneal hypoxia, dramatically diminishes fibrinolytic activity (Holmdahl, 1997). This occurs in at least two ways: first, by increasing levels of PAIs, and second, by reducing tissue oxygenation. Tissue hypoxia increases the level of expression of Hypoxia Inducible Factor-1a (HIF-1a) which further causes increased VEGF activity. Tissue hypoxia also increases the proliferation and decreased apoptosis by increasing the levels of Bcl-2, p53 and iNOS. One of the marked outcome is increased TGF-ß1 which contributes to ECM deposition. The ultimate result of tissue hypoxic condition is increased production and deposition and decreased degradation of ECM. The fate of this is adhesion development which may get reorganized and revascularized after span of few years.

Adhesion Phenotype

Collectively, basic molecular biologic characterizations of adhesions and the adhesion fibroblasts (as opposed to the normal peritoneum and normal peritoneal fibroblasts) could be considered as an adhesion phenotype (Saed and Diamond, 2004). Interestingly, the adhesion phenotype can be induced during in vitro culture of human peritoneal fibroblasts by culture under hypoxic (2% O2) conditions. These observations in part have contributed to the formation of hypothesis that adhesions develop as a response to hypoxia, as a means by which the body tries to reestablish a supply of oxygen and nutrients to tissues partially devitalized by the existing pathology and/or the surgical procedure. This hypothesis considers adhesions as more physiologic than pathologic (Saed and Diamond, 2004).

Molecular Biologic Properties of Adhesion Phenotype

Studying the expression of different molecules in

the adhesion fibroblasts, molecular biologic characteristics have been established (Saed and Diamond, 2004).

Conclusion

Post surgical adhesion are the leading cause of pelvic pain, bowel obstruction and infertility and cause a substantial burden during re-operative procedures increasing medical cost. It is unclear, why some patients heal free of adhesions and others develop it following seemingly equal procedures Understanding adhesion formation at molecular level is thus essential and without such information prevention of adhesion will remain an empirical process. Unprecedented advancement in molecular biology has led to identification of many biologically active molecules involved both in wound repair and adhesion development. The list of molecules modifying wound healing process has grown substantially, but their major role in peritoneal biological function and adhesion remain speculative. Elucidation of some of the mechanisms of signaling pathways as well as factors involved in downstream cascades will allow us to identify key molecules involved in wound repair and adhesion.

Future Prospects

The molecular biologic differences between normal peritoneum and adhesions may allow identification of which patients and which sites within patients are most at risk for adhesion development and unique molecules involved in pathology of adhesions. Modification of cellular functions through gene targeting is a promising therapeutic modality. Intra-peritoneal administration of anti-sense oligonucleotides, plasmids and viral vectors has a potential application in adhesion prevention. Intra-peritoneal administration of recombinant therapeutic proteins from a universal microencapsulated cell line as an alternate method of therapy is promising. Retrovirus and adenovirus mediated gene delivery system; a fairly recent approach will be one of the measures used to prevent the adhesions. Cell type and site specific delivery of bioactive molecule will be the focus of future research.

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