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Trametinib prevents mesothelial-mesenchymal transition and ameliorates abdominal adhesion formation

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ABSTRACT

Background: Intra-abdominal adhesions are a major cause of morbidity after abdominal or gynecologic surgery. However, knowledge about the pathogenic mechanism(s) is limited, and there are no effective treatments. Here, we investigated a mouse model of bowel adhesion formation and the effect(s) of an Federal Drug Administration-approved drug (trametinib) in preventing adhesion formation.

Materials and methods: C57BL/6 mice were used to develop a consistent model of intra-abdominal adhesion formation by gentle cecal abrasion with mortality rates of <10%. Adhesion formation was analyzed histologically and immunochemically to characterize the expression of pro-fibrotic marker proteins seen in pathologic scaring and included alpha smooth muscle actin (αSMA) and fibronectin EDA (FN^{EDA}) which arises from alternative splicing of the fibronectin messenger RNA resulting in different protein isoforms. Trichrome staining assessed collagen deposition. Quantitative polymerase chain reaction analysis of RNA isolated from adhesions by laser capture microscopy was carried out to assess pro-fibrotic gene expression. To block adhesion formation, trametinib was administered via a subcutaneous osmotic pump.

Results: Adhesions were seen as early as post-operative day 1 with extensive adhesions being formed and vascularized by day 5. The expression of the FN^{EDA} isoform occurred first with subsequent expression of αSMA and collagen. The drug trametinib was chosen for *in vivo* studies because it effectively blocked the mesothelial to mesenchymal transition of rat mesothelium. Trametinib, at the highest dose used (3 mg/kg/d), prevented adhesion formation while at lower doses, adhesions were usually limited, as evidenced by the presence of FN^{EDA} isoform but not αSMA.

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Conclusions: Cecal abrasion in mice is a reliable model to study abdominal adhesions, which can be ameliorated using the MEK1/2 inhibitor trametinib. While blocking adhesion formation at the cell and molecular levels, trametinib, at the therapeutic doses utilized, did not impair the wound healing at the laparotomy site.

Introduction

Although peritoneal adhesions may be caused by infection, inflammation, or ischemia, surgical procedures are the primary cause since greater than 90% of patients will develop adhesions after abdominal surgery.[1] In the United States between 1998 and 2002, over 18% of hospital admissions were secondary to abdominal adhesions alone resulting in approximately 948,000 d of inpatient hospital care at an estimated cost of 1.18 billion dollars.[2] Such adhesions are responsible for pelvic pain, bowel obstruction, and infertility. Although modern advances in surgical technique, including laparoscopy, have led to a decrease in their incidence, intestinal adhesions still pose a very significant medical as well as economic problem.[3,4] Unfortunately, adequate therapeutic solutions have proven elusive. While there are some studies using therapeutics to attempt to prevent adhesion formation, these have largely not gained wide acceptance.[5-10] Similarly, the use of barriers has also produced mixed results.[11] Once formed, adhesions are removed by adhesiolysis surgery. More recent studies have shown that adhesiolysis procedures in the United States account for 967,332 d of care at a cost of 2.3 billion dollars.[12]

Adhesion formation has a complex pathogenesis and can be broken down into several stages which are as follows.

(1) An inflammatory response, whose blockage has largely proven to be unsuccessful. It is characterized by an influx of multiple cell types and production of a variety of cytokines and factors.[13,14]
(2) The coagulation cascade and clot formation whose characterization is critical to understand adhesion pathogenesis. Multiple studies provide a rational basis for enhancing clot lysis as a therapeutic strategy. However, in practice, this has proven difficult. These events occur early postoperatively, and the failure of fibrinolysis allows cellular infiltration of the initial fibrinous matrix.[15-18] Although, in many cases, the formation of a clot is essential to limit injury, resolution of the clot, in a timely manner, is necessary to prevent adhesion formation. Thus, the balance between fibrin clot formation and its lysis is critical.
(3) The final stage in the adhesion process is formation of a connective tissue scar. By and large, this stage causes the most severe complications and has many common features with fibrotic reactions found elsewhere in the body, including systemic ones (e.g., scleroderma) and those affecting individual organs including lung, heart, liver, and kidney.

Because the pathophysiology of fibrotic reactions has received insufficient attention, there exists an urgent need for cellular and molecular characterization of adhesion formation. The critical cell in the formation of an adhesion is the myofibroblast which produces increased amounts of fibrillar collagens as well as other matrix proteins and which expresses alpha smooth muscle actin (αSMA), a molecular marker of activated myofibroblasts.[19,20] Although the origins of myofibroblasts may differ depending on the affected organ and the initiating event, in the abdominal cavity, they may arise through a process of *trans-differentiation* of mesothelial cells in which these cells lose their specific epithelial phenotypic markers such as expression of E-cadherin and acquire a mesenchymal or myofibroblast phenotype. This change in mesothelial phenotype has been termed mesothelial-mesenchymal transition (MMT).

Since its first identification, it has been known that transforming growth factor-β (TGF-β), a pleiotropic growth factor with a wide and diverse spectrum of biological activities, plays a key role in fibrotic diseases by mediating the formation of myofibroblasts and stimulating the production of extracellular matrix (ECM,[21-24]). In addition to TGF-β, interleukin-6, another pleiotropic cytokine with a diverse range of biological activities,[25-27] was also found to be elevated in peritoneal fluid during and/or after abdominal surgeries thus potentially implicating it in the cascade of events which lead to adhesion formation.[28] Significantly, the levels of these cytokines correlated with the severity of abdominal adhesion formation.[29,30] The complex signaling pathways activated by TGF-β involve both canonical and noncanonical signaling pathways. In the present context, the critical downstream event elicited by noncanonical signaling is the mitogen activated protein kinase (MEK) activation of Erk1/2, which, when activated by phosphorylation, enters the nucleus and, in association with other factors, mediate the transcription of pro-fibrotic genes and cell cycle regulatory proteins (Fig. 1,[31]).

We have previously found that U0126, a MEK1/2 inhibitor not in clinical use, blocked the rat peritoneal MMT induced by TGF-β.[32] To extend these findings to a drug with clinical potential, in the present study we have evaluated the effect of the MEK1/2 inhibitor, trametinib, currently in clinical use in the treatment of malignant melanoma in humans, on the TGF-β–induced rat peritoneal MMT and abdominal adhesion formation in a mouse model. Trametinib effectively blocked the MMT *in vitro* and markedly diminished adhesion formation *in vivo*, likely by inhibiting the activation of Erk1/2.[32] Taken together, these findings indicate that trametinib may be a useful drug for the inhibition of adhesion formation and warrant human clinical studies.

The goals of the present study were to use the mouse cecal abrasion model to characterize the temporal pro-fibrotic