Effect of intestinal ischemia-reperfusion on expressions of endogenous basic fibroblast growth factor and transforming growth factor β in lung and its relation with lung repair

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Subject headings: lung; intestinal ischemia-reperfusion injury; basic fibroblast growth factor; transforming growth factor β

INTRODUCTION
Our previous investigations have shown that basic fibroblast growth factor (bFGF) and transforming growth factor β (TGF β) play important roles in organ injury and repair after ischemia and reperfusion insult, and that there was a significant relationship between gene expression of bFGF or TGF β and lung repair[1,2]. Because many growth factors are involved in wound repair by their mitogenic and non-mitogenic effects, we have further investigated the alteration of endogenous bFGF and TGF β in the lung tissue following intestinal ischemia-reperfusion injury and explored their effects on lung repair as well.

MATERIALS AND METHODS
Animal model and tissue preparation
Sixty male, pathogen-free Wistar rats, weighing 250 g ± 10 g were used in this study. They were divided into 5 groups, which underwent sham-operation, ischemia (45 minutes), and reperfusion (6, 24 and 48 hours, respectively) after ischemia (45 minutes). Immunohistochemical method was used to observe the localization and amounts of both growth factors.

RESULTS
Positive signals of both growth factors could be found in normal lung, mainly in alveolar cells and endothelial cells of vein. After ischemia and reperfusion insult, expressions of both growth factors were increased and their amounts at 6 hours were larger than those of normal control or of 24 and 48 hours after insult.

CONCLUSION
The endogenous bFGF and TGF β expression appears to be up-regulated in the lung following intestinal ischemia and reperfusion, suggesting that both growth factors may be involved in the process of lung injury and repair.

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Project supported by the National Grant for Outstanding Young Researchers of China, No.39525024

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Received 2000-01-15 Accepted 2000-03-01
after antigen repair. Biotinylated IgG was added as second antibody. Horseradish peroxidase labeled streptomycin-avidin complex was used to detect second antibody. Slides were stained with diaminobenzidine, and examined under light microscope. The brown or dark brown stained cytoplasm and/or cell membrane was considered as positive. The phosphate-buffered saline (PBS) solution was used as negative control.

**Statistical analysis**
The slides from 5 animals in each group were used for observation and statistical analysis. One visual field in each slide was randomly selected and observed under light microscope with 400-fold magnification. The percentage of positive immunohistochemical staining cells was expressed as mean ± SD. Statistical analyses were performed using paired Student’s t test. $P<0.05$ was considered significant.

**RESULTS**

**Pathological alternations of lung tissue**
The histological structure of alveolar and mesenchymal cells was normal in healthy lungs, while the lung tissues from ischemia and reperfusion rats were significantly damaged, with pulmonary edema and inflammatory cell infiltration.

**Expression of bFGF and TGF β**
Both bFGF and TGF β were expressed in alveolar epithelial cells and microvascular endothelial cells of normal lung tissues. The positive signals were of immunohistochemical staining in brown or dark brown color and localized in cytoplasm and/or membrane when observed under light microscopy (Figure 1A and B). After ischemia, the expressions of both bFGF and TGF β were increased, especially in the area of alveolar epithelial cells and capillary endothelial cells (Figure 2A and B). At 6 hours postinjury, the expression of bFGF was the same as that in the early injury, while that of TGF β was increased significantly. Many positive cells were type I alveolar cells (Figure 3A and B). Up to 24 hours and 48 hours postinjury, the expression of both growth factors returned to basal levels. By quantitative analysis, the expressions of both bFGF and TGF β were quite different in the early injury when compared with those of control group ($P<0.01$, Table 1).

| Groups          | Animals | bFGF   | TGF β  |
|-----------------|---------|--------|--------|
| Sham-operated   | 5       | 15.4 ± 3.4 | 20.0 ± 5.1 |
| Ischemia 45min  | 5       | 61.8 ± 7.5  | 63.4 ± 7.0  |
| Reperfusion 6h   | 5       | 42.4 ± 10.1 | 50.6 ± 7.1  |
| Reperfusion 24h  | 5       | 29.0 ± 5.5  | 32.8 ± 8.7  |
| Reperfusion 48h  | 5       | 15.6 ± 3.3  | 19.4 ± 7.1  |

*P<0.05, **P<0.01, vs sham-operated.
In severe cases, the animal would die of damage, enhanced permeability of microcirculation, content of ATP in tissue, alveolar endothelial cell manifested as increased inflammatory reaction, low (IL) and immunocytokines. The tissue damage was such as tumor necrosis factor (TNF) and interleukin including bacteriotoxin, inflammatory mediators, activation of systemic inflammatory mediators endogenous endotoxin. This process is associated with progressively impaired and invaded by bacteria or mucosal blood flow, the gut barrier function can be insult, etc. Under the condition of an inadequate indirect injury such as shock, gut ischemia, reperfusion insult, etc. The condition of an inadequate mucosal blood flow, the gut barrier function can be progressively impaired and invaded by bacteria or endogenous endotoxin. This process is associated with activation of systemic inflammatory mediators including bacteriotoxin, inflammatory mediators, such as tumor necrosis factor (TNF) and interleukin (IL) and immunocytokines. The tissue damage was manifested as increased inflammatory reaction, low content of ATP in tissue, alveolar endothelial cell damage, enhanced permeability of microcirculation, etc. In severe cases, the animal would die of pulmonary failure. But most commonly, these changes are maintained brief because the lung has the ability of self-repair. Recent studies demonstrated that one of the important mechanisms of self-protection and self-repair was the effect of endogenous growth factor and/or nitric oxide synthetase. Therefore, the localization and quantitation of endogenous growth factors play important roles in lung repair.

Both bFGF and TGF β are important growth factors involved in tissue repair. They are involved in dermal and epidermal wound healing via their chemotactic effects for inflammatory cells and mitogenic effects for tissue cells, such as epidermal cells, fibroblasts and endothelial cells. Normally, TGF β is stored and released from platelets and macrophages, while bFGF combined with heparin is stored in endothelial cells in an inactive form. Both the growth factors are involved in the process of capillary reconstruction and tissue regeneration by their mitogenic and non-mitogenic effects. At the same time, they can also be relieved from injured tissues. Our previous researches have indicated that severe trauma results in histological damage, further decreasing the endogenous growth factors. Thus, it is necessary to supply exogenous growth factors to promote internal organ repair. We have also found that slight ischemia can induce the expression of endogenous factors, and these growth factors participate in the process of wound healing. We also investigated the gene expression of both growth factors in the same animal mode l, and found that the changes of these gene expressions were consistent with the changes of their proteins. On the basis of these studies, we came to a conclusion that there is a positive relationship between growth factors and tissue repair, and induction of endogenous bFGF and TGF β by ischemia is necessary for tissue repair. By the end of tissue repair, they are restored in tissue again. This result demonstrates that growth factors are involved in organ repair by their increased synthesis or released from damage cells after ischemia-reperfusion insult.

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