Giant to Small Emphysematous Bullae Induced by Sephadex Beads and Carrageenan

EMPHYSEMATOUS BULLAE have been described as large cystic structures filled with air, most frequently found in the subpleural parenchyma of the apical and anterior regions of the lung, although they have been found throughout the lung. Occasionally they may fill more than half of one chest cavity ("giant emphysematous bullae"), and, more rarely, little lung substance remains ("vanishing lung"). The pathogenesis and disease process in such disorders are largely unknown, because specimens from humans are limited in number. Some experimental animal models have been reported. Previous work has shown that in rabbits given one intrapulmonary injection of methylcholanthrene suspended in a solution of carrageenan cysticike develop structures in the lung parenchyma that closely resemble pulmonary emphysematous bullae in man. We think that vasospasms of medium to small pulmonary arteries and ischemic necrosis of the lung parenchyma in an early stage of the disorder could be important in the development of these cysticike structures. To explore circulation disturbances in the pulmonary arterial system, we have been using Sephadex beads to cause the same condition. Intrapulmonary administration of carrageenan solution causes pneumonia, so carrageenan-induced pneumonia may make the alveoli fragile. We tried to cause cysticike structures in the lungs of experimental animals using pulmonary embolization instead of methylcholanthrene and the experimental pneumonia induced by a carrageenan solution. Our results showed giant and small emphysematous bullae in the treated lobes of many of these animals.

Materials and Methods

Animals

Sixty-four healthy male albino rabbits that weighed 2.0–2.3 kg were used. They were kept under the usual conditions. There were nine groups (Table 1).

Preparation of Embolus

One gram of Sephadex beads (Sephadex G-50 coarse; particle size, 100–300 μ, Pharmacia, Uppsala, Sweden) that had been separated from smaller beads with a 150-μ
A mesh was suspended in 100 ml of physiologic saline. The average number of beads per milliliter was about 2000.

Procedures

The rabbits to be given carrageenan later were divided into five groups and were given, injected into an ear vein, 0.3, 0.25, 0.2, or 0.1 ml or none of the Sephadex suspension. About 3 hours later, all of the animals were anesthetized with an intravenous injection of Nembutal (sodium pentobarbital), 20 mg/kg body wt. The trachea was exposed through a midline neck incision by an aseptic technique. A firm but flexible catheter was inserted into the trachea and gently lowered into the lung until it was wedged into the lower (diaphragmatic) lobe. The tip of the catheter was bent slightly to the left, which caused it to slide into the lung. The outer diameter of the catheter was 4 mm; so it reached only the bronchial level, not the bronchioles. It was then withdrawn slightly, and 7 ml of a solution of 0.75% carrageenan (lambda carrageenan, Sigma Chemical Co., St. Louis, Mo, Lot C3889) in physiologic saline was injected. The catheter was gently withdrawn. The skin was sutured and the animals were left to recover. Surviving animals were killed at 2 months. For studies of the disorder process, two rabbits that had received 0.3 ml and four that had received 0.2 ml of the Sephadex suspension and then the carrageenan were killed at 1 week, 2 weeks, and 1 month. The control animals were divided into four groups and given the same doses (0.1–0.3 ml) of the Sephadex suspension. Then they received 7 ml of physiologic saline instead of the carrageenan solution. Surviving animals were killed 2 months later.

Rabbits were killed by 20 ml of an intravenous injec-

tion of 2.5% glutaraldehyde fixative in 0.1 M cacodylate buffer, pH 7.4, under light anesthesia. A tracheal cannula was inserted, the same fixative was instilled into the trachea under slight pressure (about 10 cm H₂O), and the trachea was securely tied. The lungs, thymus, and heart were taken out together. The affected and unaffected lobes in all of the rabbits were fixed in 20% neutral buffered formalin. Tissue blocks of sizes suitable for light microscopy were cut. The blocks were processed in paraffin, and 3- or 6-μ sections were stained with hematoxylin and eosin (H&E).

Results

Evaluation of Cystic Lesions in Treated Lobes of Animals Killed After 2 Months

The term “bulla" here means a cystlike structure with thin walls, filled with air, and 0.31 cm or more across (an emphysematous bulla in man has been defined as 1 cm or more in diameter,11 and since the weight ratio of these rabbits to man was about 2/60, we set the diameter of these cystlike structures in rabbits at 0.31 cm for reasons of convenience). The term “giant bullous lesion" means a lesion that occupies more than half,12 or, in another report,13 more than 40%, of one chest cavity. Six rabbits (2, 2, 1, and 1 animals given carrageenan solution after 0.3, 0.25, 0.2, or 0.1 ml, respectively, of the Sephadex suspension) of the 64 rabbits died within 3 days and were omitted from this study.

Groups Given Carrageenan Solution in the Lung

The lobes treated with carrageenan solution looked different grossly, and we could identify them easily. The other lobes were normal in appearance grossly, but pulmonary embolism was found microscopically. Findings
of bullous lesions and of giant bullae are in Table 1. Representative giant bullous lesions found in some rabbits given the larger doses of Sephadex are shown in Figure 1. The lesions were multiple (five or more) in the pathologic lobes of two rabbits treated with 0.3 ml of the Sephadex suspension and in two treated with 0.25 ml (Figure 2). We did not find giant or multiple lesions in the lung parenchyma of animals treated with 0.2 or 0.1 ml or none of the Sephadex suspension. The walls of smaller bullous lesions were composed of compressed alveolar septa, slightly thickened pleura, or, sometimes, connective tissue bands (Figure 3A; also 4C). The epithelium lining the connective tissue wall could not be identified clearly by microscope. Severe irregular emphysema (Figure 3B and C) was seen in many animals in these groups given carrageenan. The walls of the giant bullous lesions were thin and composed remnants of disrupted and destroyed lung tissue (Figure 4A, B, and D). Large or medium-sized aseptic bland abscesses were found in the pathologic lobes of one animal in each of the groups given 0.3, 0.25, or 0.2 ml of Sephadex suspension and carrageenan.

Groups Given Saline in the Lung (Controls)

In 5 animals in the control group given 0.3 ml of Sephadex and in 3 in each other group, the lobes treated with physiologic saline were grossly normal in appearance, as were the other lobes. However, both the affected
Figure 3—Sections showing single bullous lesions and focal irregular emphysema in the lungs of rabbits given one of the lower doses (0.2 or 0.1 ml) of the Sephadex suspension and then carrageenan, being killed 2 months later. A—Typical bullous lesion at the subpleural portion in the lower lobe. B and C—We define bullous lesions as being 0.31 cm or more in diameter; so the lesions in both B and C are not bullous, but focal emphysematous lesions. B, bronchus; L, lumen of the bullous lesion. (H&E, A, x 30; B and C, x 60) Bar = 5 mm (A) and 2.5 mm (B and C).

Figure 4—Sections of walls of bullous lesions in the lungs of rabbits given carrageenan and killed 2 months later. In A and B, the pleura retains its connection with the underlying alveoli but is stretched over the expanding bulla. In C, the lining membrane and wall are composed of compressed alveoli only, and in D, of connective tissue. P, pleura; L, lumen of the bullous lesion. (H&E, A, x 103; B, x 171; C, x 103; D, x 171).
and unaffected lobes had pulmonary embolisms, as in the rabbits given carrageenan.

To estimate the numbers of emboli caused by the Sephadex, the average number per 4-sq cm area in a section stained with H&E from the right diaphragmatic lobe was calculated. The Sephadex beads were observed at the level of the medium-sized to small pulmonary arteries as an amorphous chromophobic material (Figure 5). They were surrounded by a few foreign body giant cells, or fibroblasts, or by fibrous connective tissue, but little infiltration by neutrophils or lymphocytes was seen. The average number of emboli per 4-sq cm area in rabbits given 0.3, 0.25, 0.2, or 0.1 ml of the Sephadex suspension before carrageenan was 1.3, 1, 0.2, or 0.3, respectively. In groups not given carrageenan, these averages were 1.3, 0.7, 0.7, and 0, respectively. It was difficult to estimate the number of beads in sections from the pathologic lobes, because there are large air spaces in some sections, and we could not find whether there were a relationship between the bullous lesion and the beads or not.

Evaluation of the Progress of the Disorder

Observations at 1 Week

The pathologic lobes in all rabbits examined, 4 in the group given 0.2 and 2 in that given 0.3 ml of the Sephadex suspension, had severe pneumonia but no bullous lesions (Figure 6A). Affected alveoli were filled with macrophages that had phagocytosed the carrageenan. Bronchi and bronchioles were edematous, and emboli were seen in the branches of the pulmonary arteries. Small, localized ischemic necrosis of the lung parenchyma in the areas affected by pneumonia was sometimes present.

Observations at 2 Weeks

In 2 of the 4 rabbits in the group given 0.2 ml and in both rabbits examined in the group given 0.3 ml of the Sephadex suspension, cystlike structures were found. These lesions were often in the subpleural lung parenchyma, surrounding carrageenan-induced pneumonia in the lower lobes. In the lumens of the lesions there were air spaces, necrotic tissues, and many macrophages that had phagocytosed the materials given or the necrotic tissue and cell debris (Figure 6B). Foci of aseptic ischemic necrosis were also seen in the areas of pneumonia. The cystlike structures seen in the rabbits treated with 0.3 ml of the Sephadex suspension were larger than...
in rabbits treated with 0.2 ml. In the affected lobes, carrageenan-induced pneumonia with microscopic necrotic foci and pulmonary embolism with the Sephadex particles were noted.

Observations at 1 Month

Bullous lesions, pneumonia, and pulmonary embolism were found in the pathologic lobes of 3 of the 4 rabbits given 0.2 ml and in 1 of the 2 rabbits given 0.3 ml of the Sephadex suspension. A giant bullous lesion was present in 1 animal treated with 0.2 ml of the Sephadex suspension. The lumens of the bullous lesions contained air and macrophages; sometimes necrotic tissue or cell debris was seen at the intraluminal surface. The walls of the lesions were thin, consisting of a band of connective tissue, alveolar septa, or pleura. The pneumonia in the pathologic lobes was less severe than in the earlier stages, but foci of emphysematous lesions in the areas of pneumonia were noted.

Discussion

Our results show that giant to small thin-walled cystlike structures filled with air were present in the affected lobes of many rabbits in experimental groups killed 2 months after receiving carrageenan. It is difficult to distinguish cystic lung changes from saccular bronchiectatic or emphysematous changes. Most bronchiectatic sacs, however, are either blind, without leading distally into small airways, or else severely stenosed. Cystic lesions are located more centrally than bullous ones and seldom touch the pleural surface.4 Cysts, including bronchogenic cysts and other types, were lined with thick fibrous tissue, and the bullae with only alveolated tissue or alveolated and thin connective tissue. Cavitary masses have thick, nodular, irregular, or poorly defined walls. Inflammatory cells such as neutrophils, lymphocytes, and plasma cells often infiltrate these walls. Thus, the cystlike structures described here are probably emphysematous bullae.

We found that the proportion of rabbits developing bullous lesions in the groups given carrageenan and killed 2 months later were higher when 0.3, 0.25, or 0.2 ml of Sephadex suspension was used than with 0.1 ml or none of the suspension. Giant bullous lesions were found in two groups, those that received 0.3 or 0.25 ml of Sephadex suspension and carrageenan and were killed 2 months later. There were no rabbits with giant bullous lesions in the other groups that received carrageenan and were killed 2 months later. These results suggest that the size and number of bullous lesions formed in the lung parenchyma are associated with the amount of Sephadex suspension used. Emphysematous bullae are associated with pulmonary emphysema or interstitial fibrosis. An experimental animal model has been induced by the administration of β-aminopropionitril and CaCl₂ or elastase; β-aminopropionitril is an agent that interferes with the synthesis of collagen and elastin. A deficiency of copper also affects the maturation and repair of connective tissue. Soskel and coworkers⁵ reported an animal model for emphysematous bullae induced by feeding the diet. Our animal model is different because we did not use the β-aminopropionitril or a copper-deficient diet. Pulmonary bullae are often demonstrated in young patients with spontaneous pneumothorax but no other demonstrable pulmonary lesions. In older patients the commonest predisposing factor is chronic bronchitis.¹ Six some 30 years ago, Crenshaw⁶ hypothesized that diffuse hypertrophic emphysema, emphysematous bullae, and "vanishing" or "cotton-candy" lungs are all stages of one disease, namely, obliterative vascular disease of both the bronchial and pulmonary systems in humans. He called this "vascular pathogenesis." He reported that in areas of bullous disease there is little or no bleeding, and that these areas involve actual destruction of lung parenchyma, not simply pleural or superficial parenchymal changes. Fain et al¹² agree with this concept of vascular pathogenesis and suggest that cigarette smoking decreases the blood flow to the alveoli, resulting in loss of elasticity of the alveolar wall and finally its rupture. Welch¹⁷ thinks that a bulla may arise both from a disruption of the alveolar walls and from compression of the alveoli and their periphery. Studies of the early stages of experimental emphysematous bullae caused by methylcholanthrene and carrageenan found stenotic lumens of the pulmonary arterial branches (probably due to vasoconstriction), ischemic necrosis of the lung parenchyma, and pneumonia.⁴ Our results here support the concept of vascular pathogenesis. The affected lobes of the rabbits in the groups given carrageenan and killed 1 week later had severe pneumonia with patchy infarcted foci. Necrotic foci of the infarcts, sometimes with air spaces, were seen in the same groups in rabbits killed after 2 weeks, and typical bullous lesions were seen in rabbits killed after 2 months. We think that the necrotic tissues of the infarct, phagocytosed macrophages, and foreign matter were expectorated into the airway, since necrotic tissue masses encapsulated with fibrous connective tissue were present at the peritracheal soft tissues near the tracheostomy scar in many animals receiving carrageenan.

There is general agreement that infarction as a sequela of experimental pulmonary embolism rarely occurs in healthy animals. Even repeated embolization using Sephadex in healthy rabbits did not result in pulmonary infarction.⁹ To cause infarction in animals, two of the three routes of oxygenation, the airway and
the pulmonary and bronchial arteries, must be affected. Roach and Laufman\textsuperscript{18} found that infarction was caused by embolization only in areas where atelectasis or pneumonia already existed. However, even in dogs with such disorders, in 3 of the 13 (23\%) infarction did not develop. In humans, simple occlusion of a pulmonary artery will not usually cause infarction in an otherwise healthy lung; if the pulmonary circulation is impaired, occlusion of a branch of a pulmonary artery may cause hemorrhagic infarction. However, most instances of clinical infarction actually represent congestive atelectasis, not tissue death.\textsuperscript{19} In our experiments, there were 1 or 2 rabbits in each group given carrageenan in which no bullous lesions were found.

There are few case reports of pulmonary infarcts developing into emphysematous bullae in man.\textsuperscript{5,20,21} The discrepancy between clinical observations and bullous lesions brought about in experimental animals is not understood. It is, however, known that chronic inflammatory diseases of the lung, such as tuberculosis,\textsuperscript{22} chronic bronchitis,\textsuperscript{15} bronchiolitis,\textsuperscript{23} and pulmonary fibrosis,\textsuperscript{17} are not infrequently complicated by bullous lesions. Perhaps the patients with these diseases tend to suffer from ischemic pulmonary infarct in the inflammatory lesions, caused by circulation disturbance. In our experiments, bullous lesions were found in rabbits killed at 2 weeks, but not at 1 week. There seems to be a latent period for development of these disorders. There might be a latent period in man like that in rabbits; so association pulmonary infarcts with bullous lesions may be masked.

References


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