Bioglass as a pleurodesing agent as effective as talc in rabbits

Bioglass: Tavşanda talk kadar etkin bir plörodezik ajan

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THORACIC SURGERY

Background: Bioglass is a silica based biomaterial which is widely used for filling and augmentation of bone defects, in drug delivery systems, as a scaffold in tissue engineering, and for covering the surfaces of implant materials. With the hypothesis that silica content of Bioglass might cause pleural irritation similar to that of talc, we investigated the pleurodesing effect of Bioglass in an experimental rabbit model.

Methods: Thirty New Zealand male rabbits weighing 2.5-3 kg were randomly divided into three groups. Doses of 70 mg/kg Bioglass particulates and talc in 1 ml/kg saline solution were given through a left chest tube to two groups, while the third group only had a chest tube insertion. At the end of 28 days, pleural adhesions and lung parenchyma were evaluated by gross observation and histological examination.

Results: Pleurodesing effect was significantly greater in talc- and Bioglass-administered rabbits compared to the controls (p=0.0001). Compared to Bioglass, talc had a more irritative effect on pleura, but its pleurodesing effect was not superior (p=0.971). There were no significant differences between talc and Bioglass with respect to parenchymal inflammation and fibrosis (p=0.075).

Conclusion: Bioglass may prove to be an effective pleurodesing agent with comparable effectiveness to that of talc.

Key words: Biocompatible materials; ceramics/pharmacology; glass; pleura; pleurodesis/methods; rabbits; talc/pharmacokinetics.

Amaç: Bioglass kemik defektlerinin doldurulmasında ve güçlendirilmesinde, ilaç salınım sistemlerinde, bir iskelet yapı olarak doku kültürlerinde ve implant materyallerinin yüzeylerinin kaplanmasında yaygın olarak kullanılan silika bazlı bir biyomateryaldir. Bu deneysel çalışmada, Bioglassın silika içeriğinin talkta olduğu gibi plevral irritasyona neden olabileceği varsayılarak, Bioglassın plörodezik etkisi araştırıldı.

Çalışma planı: Çalışmada 30 adet 2.5-3 kg ağırlığında, erkek, Yeni Zelanda cinsi tavşan rastlantısal olarak üç gruba ayrıldı. İlk iki gruba 1 ml/kg izotonik serum içerisinde 70 mg/kg Bioglass veya talk sol göğüs tüpü içerisinden verildi. Kontrol grubuna sadece göğüs tüpü takıldı. Yirmi sekizinci günde plevral yapışıklıklar ve akciğer parenkimi makroskobik ve histolojik olarak değerlendirildi.

Bulgular: Talk ve Bioglass grubunda, kontrol grubuna göre anlamlı olarak daha fazla plörodezik etki görüldü (p=0.0001). Bioglass ile karşılaştırıldığında, talkın plevral irritatif etkisi daha fazlaydı; ancak, plörodezik etkide bir üstünlüğü yoktu (p=0.971). İki materyal arasında parenkimal inflamasyon ve fibrosis açısından anlamlı bir fark yoktu (p=0.075).

Sonuç: Bulgularımız, Bioglassın etkili bir plörodezik ajan olduğunu ve talk ile benzer etkinlik gösterdiğini ortaya koymuştur.

Anahtar sözcükler: Biyouyumlu materyal; seramik/farmakoloji; cam; plevra; plörodezi/yöntem; tavşan; talk/farmakoloji.

Pleurodesis, the creation of a fibrous adhesion between the visceral and parietal layers of the pleura, has been widely used for recurrent and persistent pleural effusions and air leaks. However, an ideal pleurodesing agent has yet to be found. Several chemical and biological agents have been studied, all of which have some degree of advantages and disadvantages.

Bioglass is a bioactive ceramic composed of sodium and calcium salts, phosphates, and silicon dioxide.[1] It is hemostatic,[2] radiopaque,[3] biocompatible and absorbable,[1] and also has antibacterial activity against some bacteria[4] in bone tissue. It has been widely used experimentally and clinically for filling bone defects and for bone augmentation,[1] in drug delivery systems,[5]

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as a scaffold in tissue engineering,[6] and for covering the surface of implant materials.[7]

Bioglass is a silica based biomaterial and has a crystalline structure as talc.[1] Because of the structural similarity of these materials, we hypothesized that bioglass may have pleural inflammatory effects and cause pleurodesis when administered intrapleurally. To our knowledge, there is no report of intrapleural administration of this biomaterial for pleurodesis in the literature. The purpose of this study was to evaluate the pleurodesing potency of bioglass.

MATERIALS AND METHODS

Animals. Thirty New Zealand white male rabbits weighing 2.5-3.0 kg were used for the study. The experiment was approved by the ethics committee of the institution.

Pleurodesing agents. Sterilized talc powder (Steritalc, Novatech, France) and Bioglass particulate (PerioGlas, USBiomaterials Corporation, Florida, USA) were commercially supplied. Perioglas with particle size ranging from 90 to 700 microns, was pestled in a ceramic pot and sifted with a 100-micron filter (Emperor Aqautics, USA). Thus, Bioglass powder was obtained with particle size smaller than 100 microns. Both materials were packaged and sterilized with dry autoclave before using for pleurodesis.

Surgical procedure. The rabbits were anesthetized with intramuscular ketamine 35 mg/kg, and xylazine 5 mg/kg. The left hemithorax was shaved and cleaned with povidone-iodine solution. A 1-cm skin incision was made midway between the spine and the sternum. A 14 F aspiration cannula was inserted into the thorax with a blunt dissection at the sixth or seventh intercostal space and was fixed to the skin with a 3-0 silk suture. The cannula was then connected to a water-seal drainage system. The lung was re-expanded by transient negative suction (–5 cm-H_2O).

The rabbits were randomly divided into three groups. Equal doses of (70 mg/kg) Bioglass or talc in 1 ml/kg saline solution were given through the chest tube in two groups. Ten control animals had only chest tubes inserted and the tubes were immediately removed. In the Bioglass and talc groups, the tubes were removed after administration of the agents into the thoracic cavity and the skin was closed. All the animals received paracetamol 2 mg/ml in drinking water for two days.

At the end of 28 days, all the rabbits were sacrificed and *en bloc* removal of the thoracic cage was performed. The lungs were expanded by intratracheal injection of 10% formalin. The entire thorax was submerged in a 10% formalin solution.

Histopathologic examination. Necropsy was performed by one of the investigators (N.O.F.) who was blinded to the randomization of the animals. Each pleural cavity was exposed carefully by a bilateral incision through the diaphragm. The sternum and the medial portions of the anterior ribs were removed so that the lungs and pleural cavities could be evaluated.

Gross pleurodesis was graded according to the following scheme: 1– no adhesions; 2– rare adhesions with no symphysis; 3– a few scattered adhesions with no symphysis; 4– many adhesions with no symphysis; 5– many adhesions with symphysis involving less than 5% of the thoracic cavity; 6– many adhesions with symphysis involving 5% to 25% of the thoracic cavity; 7– many adhesions with symphysis involving 25% to 50% of the thoracic cavity; 8– many adhesions with symphysis involving more than 50% of the thoracic cavity.[8]

For microscopy, the samples obtained from the visceral pleura were placed in neutral buffered 10% formalin. Tissue samples were processed routinely and sections in 4-micrometer thickness were prepared. The sections were stained with hematoxylin-eosin (H-E). Microscopic slides were evaluated for the presence of pleural and parenchymal inflammation and fibrosis. Inflammation and fibrosis were graded from 0 to 4 for absent, equivocal, mild, moderate, or marked degrees, respectively.

Statistical analysis. All the data were expressed as median (minimum-maximum) values. The scores for pleurodesis, microscopic fibrosis and inflammation in the groups were compared using the Kruskal-Wallis test. When significance was obtained, the Mann-Whitney U-test was used for further comparison. A p value of less than 0.05 was accepted as significant.

RESULTS

No animal died prematurely. No signs of distress were observed after the intrapleural administration. All the animals rapidly regained a normal feeding pattern and resumed normal activities after the procedure.

Pleurodesis effects. Varying degrees of pleurodesis were found in the talc and Bioglass groups (Fig. 1a, b), which was significantly more than the control group (p=0.0001). Talc had no superior pleurodesing effect to Bioglass (p=0.971), but was significantly more irritant for pleura (p=0.0001). There were no significant differences between the Bioglass and talc groups with respect to parenchymal inflammation and fibrosis (p=0.075). Statistical analyses are shown in Table 1.

Table 1. Statistical analysis of the groups (Mann-Whitney U-test)

Groups (n=10)	Pleurodesis effect	Pleural inflammation	Parenchymal inflammation
Talc *vs* Bioglass	p=0.971	p=0.0001	p=0.075
Talc *vs* control	p=0.0001	p=0.0001	p=0.0001
Bioglass *vs* control	p=0.0001	p=0.004	p=0.0001

Parenchymal deposition. Bioglass and talc particles were seen in the pleural and lung parenchymal tissues under light microscopy and polarized light (Fig. 2).

DISCUSSION

Our rabbit model of pleurodesis showed that Bioglass provided an effective pleurodesis which was very similar to that of talc. Similar doses of Bioglass and talc produced nearly an equivalent degree of pleurodesis in rabbits. The effect of Bioglass may be attributable to its silicon dioxide content similar to talc's. Silicon dioxide, the major content of Bioglass, is a crystalline type of silica. Silica exposure causes lung damage by the following mechanisms: direct cytotoxicity, activation of

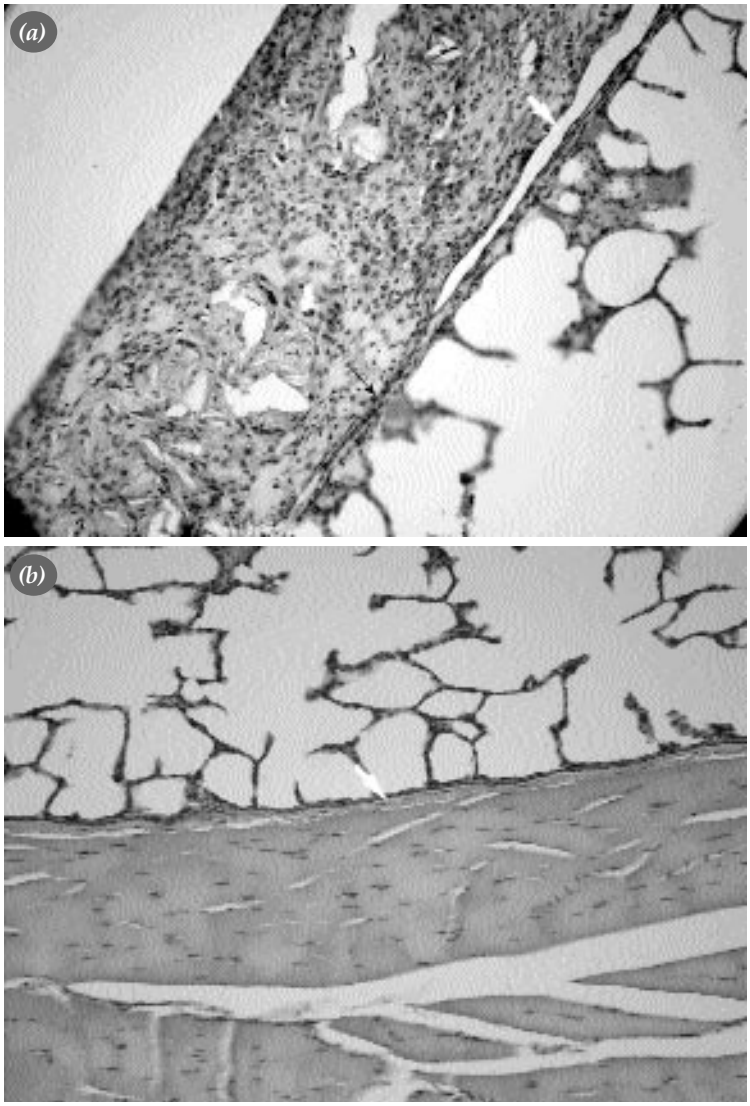

Fig. 1. (a) Talc-induced pleurodesis. Excessive thickening of the parietal pleura and partial adhesions (grade 6) between the visceral and parietal pleura (black arrow). Thickening of the visceral pleura is observed (white arrow) (H-E x 40). **(b)** Bioglass-induced pleurodesis. A thin adhesion line (grade 7) between the parietal and visceral pleura is seen (white arrow) (H-E x 40).

oxidant generation by alveolar macrophages, stimulation of the secretion of inflammatory cytokines and chemokines from alveolar macrophages and/or alveolar epithelial cells, and stimulation of secretion of fibrogenic factors from alveolar macrophages and/or alveolar epithelial cells.[9] Another possible inflammatory effect of Bioglass is likely to be due to the fact that Bioglass presents as a particulate. Particles of size that is smaller than 1 mm, when phagocytized by cells, are held to be responsible for the cytokine response. Those particles that are greater in size may not be phagocytized. However, they may induce inflammatory mediator release. Previous studies have documented the secretion of inflammatory mediators, such as prostaglandins, upon exposure to particles greater than 10 mm. Large particles may directly irritate cells to release inflammatory substances.[10]

Bioglass can bond the living bone and soft tissues. The basis of this bonding depends on its chemical reactivity with body fluids. Three general processes- leaching, dissolution, and precipitation- occur. Sodium is leached from the glass and replaced with protons from the solution. An important aspect of this reaction is that the local pH is driven from an acidic value to a neutral or slightly basic pH, which can be more supportive of healing. Silicic acid is released into the solution, silanol groups form a hydrated layer at the glass surface. The silanol groups produce a silica gel, which serves as a nucleation site for a calcium phosphate (CaP) layer. Organic species in the local environment such as collagen, chondroitin sulphate, and glycoseaminoglycans, are incorporated into this bioactively forming layer. Osteoblasts are attracted to the hydroxcarbonate apatite and release organic constituents, followed by mineralization. The end result is a strong glass-silica gel-hydroxycarbonate apatite-bone.[1]

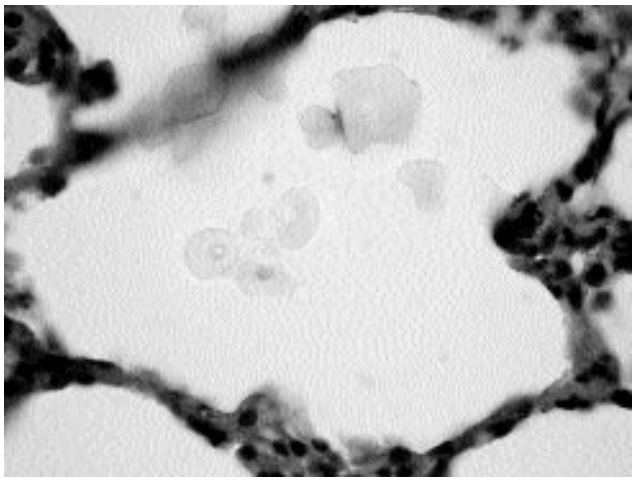

Fig. 2. Parenchymal deposition. Bioglass particles are seen in the lung parenchyma (H-E x 80).

Bioactive materials may influence attachment, proliferation and differentiation of cells, and subsequent integration in a host tissue. Additionally, bioactive materials are capable of releasing ions, which may affect cellular responses such as local increases in osteoblastic and osteoblast-like cells, chondrocyte activities, minimal inhibitory effect on the proliferation of sinoviocyte, and increase in macrophage activation. It was shown by cell culture studies that Bioglass could change intracellular ions, resulting in increases in pH, calcium, potassium, small decreases in sodium, and increases in lactate production and ATP generation by stimulation of glycolysis.[11] As a result of these metabolic changes, collagen and cytokine release from the cells mentioned above are stimulated by Bioglass. We could not find any information in the literature on the effects of Bioglass on mesothelial cells. Although Bioglass is a biocompatible, nontoxic, and noninflammatory bioactive material for hard and soft tissues, it was reported that peritoneal injection of massive amounts of Bioglass caused acute nephrotoxic death in mice and rats, and a single injection of furosemide prevented acute nephrotoxicity of Bioglass.[12] However, there is not any report about toxic or lethal dose of Bioglass in human beings.

Although the mechanisms underlying drug-induced pleural symphysis are not well understood, increasing evidence suggests that an inflammatory reaction may play an important role. Instillation of a sclerosing substance causes injury to mesothelial cells ranging from cuboidal transition to total cell desquamation. In addition, a dramatic increase was observed in pleural neutrophil counts. Cytokines may be released from pleural macrophages and mesothelial cells. Intrapleural neutrophils may release proteolytic enzymes and toxic oxygen radicals, thereby damaging the mesothelial cells. Finally, intense proliferation of fibroblasts can be seen on the pleural surface within three days of pleurodesis. Inflammatory processes are accompanied by fibrin deposition on both the visceral and parietal pleural surfaces. Fibrin, fibrinogen, and their degradation peptides induce fibroblast adherence, proliferation, and collagen production.[13]

Many chemical (talc, tetracyclines, antineoplastics, povidone-iodine, silver nitrates, polidocanol) and biologic (autologous blood, biologic glues, collagen) agents have been tried for pleurodesis.[14-16] Nowadays, talc and tetracyclines are the most commonly used agents for pleurodesis. Both have some side effects. Talc is a hydrated magnesium silicate, $Mg_3Si_4O_{10}(OH)_2$, and is one of the most popular pleurodesing agents because of its high effectiveness, low cost, and wide availability. However, it has been well

documented that talc is associated with adult respiratory distress syndrome (ARDS) in 3% to 9% of cases after intrapleural administration.[16] Systemic distribution and progressive deposition of talc particles after intrapleural administration have been shown in animal studies.[16] Other reported side effects include fever, pain, infection, hypotension, arrhythmia, arterial desaturation syndrome, and sclerosis.[17] In addition, talc causes carcinogenesis in mice.[18] On the other hand, the most common adverse effects of tetracyclines are chest pain and fever.

It is questionable whether Bioglass may be an alternative to talc in pleurodesis. It has the following disadvantages: *(i)* parenchymal deposition. This is an undesired characteristic for a pleurodesing agent due to risk of ARDS. Although Bioglass has been widely used in bone and soft tissue studies, we could not find any report about its pleural use and its effects on mesothelial cells, and we do not know its effect on pulmonary tissue. Furthermore, it is not known whether it results in systemic dissemination, and if so, what effects are produced. *(ii)* Bioglass is not cheaper than talc (nearly $200 *vs* $75) and, at present, there is not any commercial form of bioglass for pleurodesis. Its advantages are; *(i)* Bioglass is biocompatible and nontoxic; (ii) it is easily available; *(iii)* it causes less pleural fibrosis; *(iv)* it has an antibacterial activity, and *(v)* there is no evidence that it has carcinogenic properties.[19]

It is debatable whether Bioglass creates an extra-and intracellular alkaline media. This effect may occur in the pleural cavity and may cause better pleurodesis in patients with pleural effusions with low pH. Another debatable issue is that Bioglass may increase collagen release in mesothelial cells like it causes in some other cells. Indeed, talc and tetracyclines also induce collagen mRNA synthesis, and they also stimulate a significant amount of collagen release in pleural inflammatory processes.[20] This causes excessive pleural inflammation and pain.[20] In our study, we did not observe excessive pleural fibrosis, and it is plausible to speculate that Bioglass may create pleurodesis by increasing collagen synthesis without excessive pleural inflammation, and thus, may cause less pain in pleurodesis. Further studies are needed to shed light on the mechanism of action of Bioglass in pleurodesis.

In conclusion, Bioglass is as an effective pleurodesing agent and its effectiveness is comparable to that of talc. Bioglass is absorbed by the pleura and causes parenchymal inflammation and fibrosis of the lung, which is not significantly higher than that of talc. Nevertheless, whether Bioglass may be used as an alternative for pleurodesis warrants further studies.

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