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Preliminary Study of Percutaneous Alcohol Injection into the Lung

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Although percutaneous ethanol injection is widely used to treat hepatic tumors, this technique has not been applied to lung tumors. We performed a preliminary experiment with percutaneous ethanol injection into the rabbit lung, and evaluated the local and systemic effects of absolute ethanol injection on pulmonary structures in order to assess the feasibility and safety of this technique as a local treatment for human lung tumors. Percutaneous injection of absolute ethanol into the rabbit lung was performed under CT guidance. The volume of ethanol injected ranged from 0.6 to 1.0 ml (approximately 0.2–0.5 ml/kg). Follow-up CT scans were performed 1, 2, 7 and 30 days after the injection. The animals were killed at intervals (range: 3 h–30 days), and the lung was examined histologically. The ethanol was well tolerated and did not induce significant systemic side-effects. All doses induced necrosis in the injected lung, but none was lethal. Although ethanol spilling into the thoracic cavity induced effusion and pleuritis, these reactions were manageable. Alcohol injection produced an area of necrosis surrounded by pulmonary edema associated with polymorphonuclear cells invasion within 24 h; moreover, granulation change, epithelial regeneration, and alveolar septal fibrosis had appeared by one week. The necrosis was sometimes multifocal, probably due to transbronchial spread of the injected ethanol. In conclusion, the feasibility and safety of absolute ethanol injection were confirmed. Neither severe systemic side effects nor lethal extensive necrosis were observed with injected ethanol; however, an unexpected side effect, multifocal necrosis, was seen. The latter reaction suggests that careful observation and care would be essential after alcohol injection into the lung.

Key words: Lung damage — Ethanol — Percutaneous — Injection — Complication

Percutaneous ethanol injection is widely used for the treatment of small hepatic tumors. Although controversy persists, previous studies have demonstrated excellent long-term survival rates in patients treated by this procedure.[1, 2] On the other hand, percutaneous ethanol injection of lung tumors has never been tried, probably because other local therapies such as surgery and radiation are effective, or serious side effects are anticipated owing to ethanol spillage into the pleural cavity.

Radiation and surgery are well accepted modalities for the treatment of localized lung cancer. Recently, small lung carcinomas, which had been undetectable with conventional radiography, have been found due to the introduction of spiral CT screening for lung cancer in Japan.[3] Although controversy persists,[4–6] limited surgical resection or limited field radiation might be adequate for such small lesions.[7, 8] In treating such lesions, surgical resection may be too invasive, especially because many of these small lesions are expected to be found in elderly people. Although radiation therapy may be indicated for such patients, it has some disadvantages, such as radiation pneumonitis and the requirement of a complex and expensive facility for administration. Thus, another therapeutic modality for small lung carcinomas will be needed in the near future. Therefore, we evaluated the feasibility of ethanol injection into the lung as an alternative to conventional local therapies.

We performed a preliminary study of percutaneous absolute ethanol injection into the lung to assess the feasibility of its clinical application as another local treatment modality for human lung cancers.

MATERIALS AND METHODS

Ten rabbits, weighing 2.3 to 3.0 kg, were used. Six were used in a pilot study to determine the proper volume and mode of ethanol injection, and the other four were used in a protocol study as described below. We employed two injection velocities: slow, which meant the injection duration was more than three minutes, and rapid, i.e., less than 30 s.

All experiments were performed under anesthesia by intraperitoneal administration of 40–50 mg/kg sodium pentobarbiturate (Nembutal, Abbott Laboratories, Abbott Park, IL). Percutaneous injection of absolute (99.5%; w/v) ethanol into the intact rabbit lung was performed under CT guidance. Puncture was performed with a 23-gauge needle in the supine position. The volume of injected ethanol was approximately 0.2 ml/kg. The CT experiments were carried out on an X-vision/SP (Toshiba, Tokyo). Ten-millimeter-thick contiguous sections were used to evaluate the effects of injected ethanol. CT scan of the whole lung was per-

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formed to decide the optimal site to be injected prior to each injection. Just after the ethanol injection, the injected site was evaluated by CT. Follow-up CT scans were performed 1, 2, 7 and 30 days after the injection. If a pleural effusion was detected, thoracentesis was performed. The electrocardiogram was monitored at the time of injection to detect acute influences on the heart. The rabbits were killed and dissected at 1, 3, 7, and 30 days after the injection. Macroscopic findings were obtained and the lungs were excised and fixed in 3.7% formalin. Hematoxylin and eosin staining was performed and microscopic changes in the lungs were examined.

The two-sided paired *t* test was used for statistical analysis (Stat View-J 4.11, Macintosh). A P value of less than 0.05 was considered significant.

RESULTS

Pilot study (Table I) Six rabbits were used in a pilot study. The rabbits were killed at 3 h, 24 h, 8 days, or 30 days after the alcohol injection. The volume of injected ethanol ranged from 0.6 to 1.0 ml. The injection of 1.0 ml of ethanol induced a large area of necrosis, probably because of a pulmonary infarction due to damage sustained by proximal arteries. It was essential to avoid such events, as well as other complications such as pneumothorax and pleural effusion. We tried to control such complications by changing the volume and velocity of the injection. Slow injection, which meant the injection duration was more than three minutes, successfully avoided pneumothorax and/or effusion. Finally, slow injection of 0.6 ml of absolute ethanol was chosen as the optimal mode of injection based on the pilot study results.

The protocol study Four rabbits were used in a protocol study as described above.

Acute and chronic complications: Pneumothorax and pleural effusion were observed in one of the four rabbits on the day of injection; however, these reactions were not lethal and had disappeared from the CT image by day 3. Acute arrhythmias were not observed during ethanol injection (Fig. 1). No other systemic complications were observed.

Radiographic findings on CT scan (Table II): The injected ethanol was localized in all cases on the CT obtained on the injection day (Fig. 2). Nodule sizes ranged from 9.8 to 15.0 mm in maximum dimension, with an average of 12.6 mm. The next day, the nodules had expanded in all cases, and the increase was statistically significant ($P = 0.018$). Thereafter, nodules tended to remain unchanged or to decrease. Radiologically, pneumothorax and effusion were observed as mentioned above; moreover, lobar atelectasis of the injected lobe was observed in two of four rabbits, and did not resolve. One was allowed to persist for a month, and then become a calcified lesion. Air bronchograms were observed in one rabbit, probably due to acute pulmonary edema. None of the lesions was lethal. No other abnormalities were noted.

Histopathological findings of the lung with absolute ethanol injection: The injected ethanol was localized (Fig. 3a), and four main histopathological findings were observed on day 1 (24 h after the injection) (Table III); severe focal necrosis (injected region), the infiltration of polymorphonuclear cells (PMNs), alveolar edema, and peribronchial edema. Three layer formation was observed, which consisted of a central necrotic area surrounded by PMN infiltration and alveolar edema (Fig. 3b). In other regions, multifocal necrotic areas were found along the bronchial

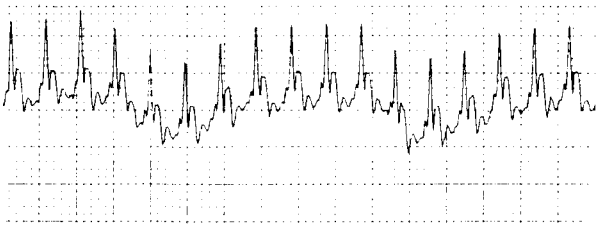

Fig. 1. Representative examples of ECG during the injection of ethanol. No acute arrhythmia was observed.

Table I. Results of the Pilot Study

No.	Vol. of ethanol (ml)	Velocity of injection	Pneumothorax	Pleural effusion
1	0.6	rapid[a)]	severe[b)]	moderate[c)]
2	0.6	rapid	none	severe[c)](40 ml)
3	0.8	rapid	none	severe (30 ml)
4	1.0	rapid	none	moderate
5	1.0	slow[a)]	none	slight
6	1.0	slow	none	none

a) Rapid means the injection time was less than half a minute; slow means it was more than three minutes.
b) Severe means lethal.
c) Moderate and severe means that the effusion needed aspirating.

Table II. Radiographic Findings of the Injected Ethanol in Rabbit Lung

Rabbit	No. 1	No. 2	No. 3	No. 4
Vol. of ethanol (ml)	0.6	0.6	0.6	0.6
Acute systemic complications	none	none	none	none
Interval to termination (day)	1	3	8	30
Radiographic findings				
Pneumothorax				
0 h	none	none	none	slight[a)]
24 h	none	none	none	slight
48 h	—	none	none	none
7 day	—	—	none	none
30 day	—	—	—	none
Pleural effusion				
0 h	none	none	none	slight[a)]
24 h	none	none	none	slight
48 h	—	none	none	none
7 day	—	—	none	none
30 day	—	—	—	none
Maximum size of the nodules (mm)				
0 h	11.6	14.0	9.8	15.0
24 h	28.4	21.2	17.6	25.0
48 h	—	23.4	12.3	31.0
7 day	—	—	12.3	14.0
30 day	—	—	—	13.4
Other observed findings				
Atelectasis	observed	none	none	observed
Air bronchogram	none	observed	none	none

a) Not lethal, and thoracentesis was not needed.

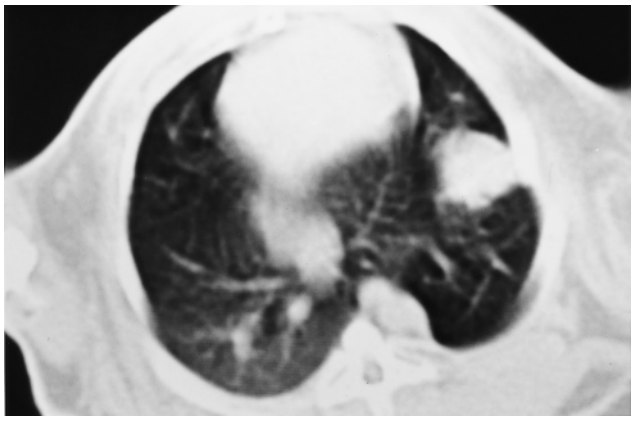

Fig. 2. Radiographic findings of the lung just after the injection of ethanol. The injected ethanol was localized unless it spilt out of the lung.

tree, suggesting transbronchial spread of the injected ethanol (Fig. 3c). Epithelia of the bronchioles exfoliated in such regions. The presence of peribronchial edema is also possible evidence of this mode of spread of the injected ethanol.

On day 3, no significant changes were observed as compared to day 1, except in the fibrin exudation around the necrotic area and the greater nodule size (see above). The increase in size was pathologically due to enlargement of the area of inflammation and alveolar edema.

On day 8, significant differences were found as compared to the earlier phase. Although the necrotic area persisted, edema and PMN infiltration had nearly disappeared. The “three layer” formation had changed to a four layer formation, as follows; central necrotic area, surrounding granulation change, epithelial regeneration, and alveolar septal fibrosis (Fig. 4a). Regions of granulation change and epithelial regeneration appeared to correspond to the area of PMN infiltration on days 1 to 3, and septal fibrosis to the alveolar edema on days 1 to 3. Other characteristic findings were the appearance of foreign giant cells and calcification, both of which were recognized in the granulation regions. Furthermore, intrabronchial granulation was present in the damaged bronchioles. Re-epithelialization of the exfoliated bronchioles was also observed (Fig. 4b).

On day 30, while the necrotic area had become obscure, calcification was prominent (Fig. 5). Other pathological findings were essentially the same as those of day 8. Epithelial regeneration persisted.

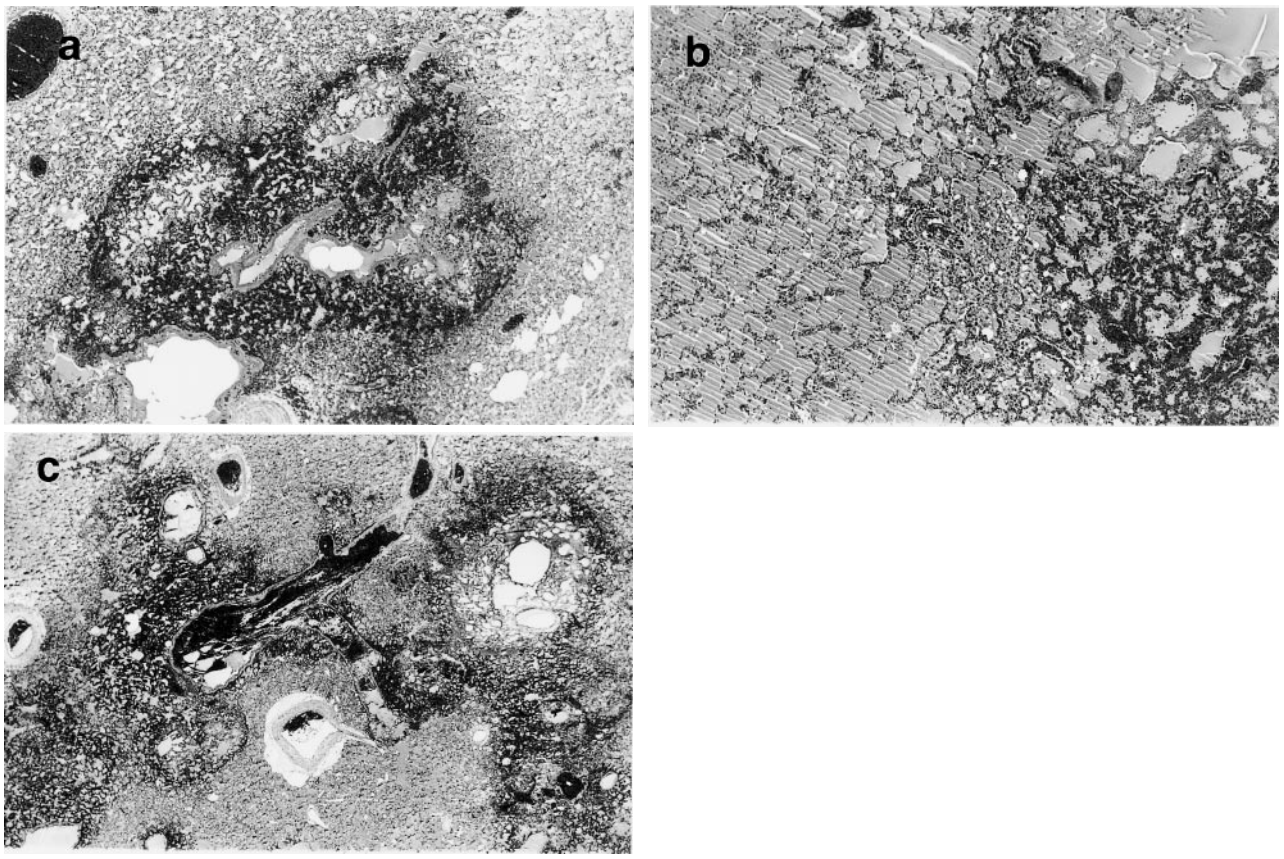

Fig. 3. Histopathological findings of the lung 24 h after the injection of absolute ethanol (hematoxylin-eosin stain). a, Focal necrosis surrounded by alveolar edema was observed. b, Three layer formation was observed; severe necrosis (right side of this figure), the infiltration of polymorphonuclear cells (central part), and alveolar edema (left side). c, Multiple small necrotic areas were found along the bronchial tree, which would suggest transbronchial spread of the injected ethanol. Intrabronchial hemorrhage was also seen. (original magnifications (a) ×15, (b) ×25, (c) ×5)

Table III. Histopathological Findings of the Lung into Which Absolute Ethanol was Injected

Time course	1 day	3 days	8 days	30 days
Necrosis[a)]	yes	yes	yes	no
PMN cell infiltration	yes	yes	no	no
Alveolar edema	yes	yes	no	no
Peribronchial edema	yes	yes	no	no
Epithelial regeneration	no	no	yes	yes
Granulation formation	no	no	yes	yes
Alveolar septal fibrosis	no	no	yes	yes
Calcification	no	no	yes	yes
Foreign body giant cell	no	no	yes	yes
Pleuritis	no	yes	no	no

a) Multifocal necrosis was observed one day after the injection.

Pleurisy was observed in only one rabbit, indicating minimal ethanol spillage into the pleural cavity (Fig. 6). Once ethanol had spilled out, severe pleurisy was experienced in the pilot study. However, none of them was so severe as to be lethal.

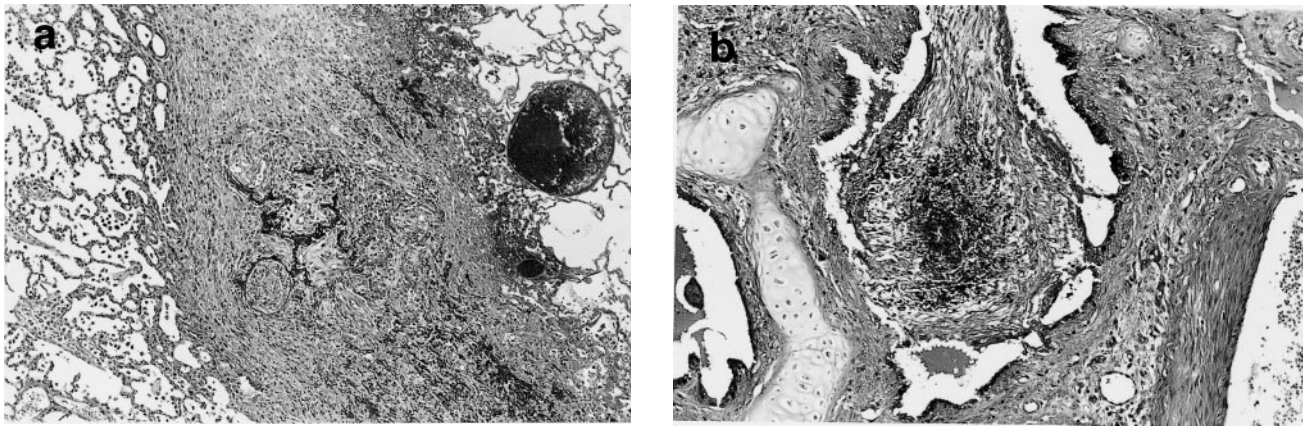

Fig. 4. Histopathological findings of the lung 8 days after the injection of absolute ethanol (hematoxylin-eosin stain). a, Four layer formation was observed; focal necrosis (right side of this figure), granulation (central part), epithelial regeneration (central part), and septal fibrosis (left side). b, Intrabronchial granuloma and reepithelialization of the damaged bronchus were found. (original magnifications (a) ×25, (b) ×37)

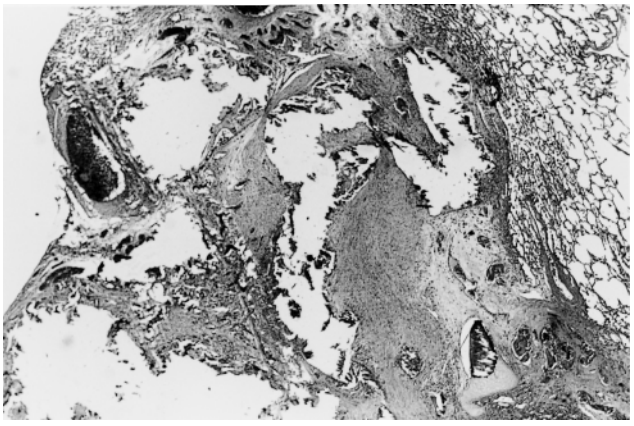

Fig. 5. Histopathological findings of the lung 30 days after the injection of absolute ethanol (hematoxylin-eosin stain). Multiple calcifications were observed. (original magnifications ×10)

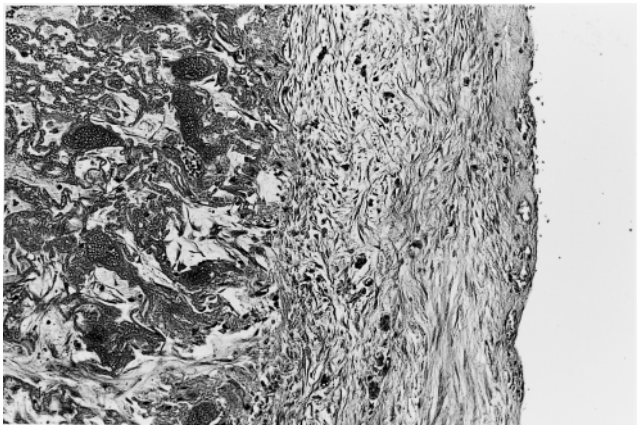

Fig. 6. Histopathological findings of the pleura affected by the spilt ethanol on day 8 (hematoxylin-eosin stain). Pleural thickening and proliferation of subpleural fibroblasts were found. (original magnifications ×42)

DISCUSSION

We performed a preliminary study of percutaneous absolute ethanol injection into the lung to assess the feasibility of clinical application as another modality for local treatment of human lung cancers. Our results confirmed the feasibility and safety of absolute ethanol injection into the lung.

Percutaneous ethanol injection is widely used for the treatment of small hepatic tumors. On the other hand, percutaneous ethanol injection has never been tried for lung tumors, probably because other local therapies such as surgery and radiation are effective, and/or serious side effects are anticipated if ethanol spills into the pleural cavity. However, our present results show clearly that no serious systemic side effects accompany the injection of absolute ethanol into the lung. Although we experienced a few pneumothoraces or effusions in our pilot study, such complications were thereafter avoided by injecting the ethanol slowly. Slow injection prevented the reflux of ethanol even in continuously breathing rabbits. If alcohol leakage into the thoracic cavity is avoided, the injected ethanol will tend to localize, minimizing lung damage. The site of injection was the peripheral lung field where arterioles, venules, capillary, and bronchioles existed histologically. Subsequent reactions in the lung included pulmonary edema and in-

flammatory changes, seen on CT images as significant increases in nodule size from day 1 to day 3. These severe inflammatory reactions disappeared, however, within 8 days. Subsequently, the main pathological features were those of chronic granulomatous inflammation including calcification. Chronic histopathological findings, such as septal fibrosis and intrabronchial granuloma, were commonly observed in patients with diffuse lung disease. Thus it may be necessary to estimate the saturation of blood oxygen to assess the safety of this therapy when this technique is applied to humans. In a pilot study, in which we used relatively large amounts of ethanol, lobar necrosis was observed in one rabbit. In fact, the rabbit lung is too small to allow the injection of ethanol into the actual peripheral lung field. Thus, we considered this extensive necrotic reaction to be due to damage sustained by the central pulmonary vessels, rather than a direct effect of the ethanol. This finding suggested that ethanol injection is contraindicated for centrally located pulmonary nodules.

Necrosis was successfully induced by the injected ethanol. The anti-tumor effect of the injected ethanol is of clinical interest, but unfortunately no appropriate model of lung tumor was available. Therefore only the effect on the normal lung tissue was investigated in this study. The histology of peripheral lung tumor is various, such as metastatic cancer to lung, undifferentiated carcinoma, localized bronchioloalveolar carcinoma, and so on. Some could be resistant to injected ethanol, and others be sensitive. Therefore, anti-tumor effects for each lung histology would need to be investigated in the future.

An unexpected side effect was also noted. Multiple focal necrotic areas, possibly produced by transbronchial spread of injected ethanol, had not been anticipated. Once injected ethanol enters the endobronchial space, it can potentially reach relatively distant regions and might produce necrosis. Accompanying exfoliation of the bronchial epithelium, subsequent endobronchial granulation formation and re-epithelialization would then ensue. These findings might limit the clinical use of this technique.

Although there have been no reports on percutaneous ethanol injection for lung cancer, some authors[9, 10] have reported on other modes of ethanol injection for lung cancer, e.g., transbronchial ethanol injection of tracheobronchial lesions and intraoperative ethanol injection of operable lung cancer. They reported that this method was safe and useful. However, their clinical experience is very limited and the results are preliminary. Masaki *et al*.[10] reported that they injected up to 10 ml of ethanol under intraoperative direct observation. Based on our results, it could be quite dangerous to inject such a large volume of ethanol into the lung because of the risk of transbronchial spread. Moreover, the intraoperative position of the patients was decubitus, which would allow ethanol to reach the proximal bronchial tree once it entered the endobronchial space. Basic experiments have been limited in this field and more research is obviously needed before clinical application.

Percutaneous needle insertion into the lung is frequently applied to the diagnosis of lung tumors.[11] The use of CT guidance in performing transthoracic needle biopsy is well established. Diagnosis has been reported to be highly accurate regardless of lesion size or location.[12] Complications with these techniques were reported to occur in 24–40% of cases, mostly mild pneumothorax.[11, 13, 14] Considering these advantages, the transthoracic mode of injection is anticipated to gain acceptance in the future.

In conclusion, we performed a preliminary study of percutaneous absolute ethanol injection into the lung to assess the feasibility of this technique, and found it to be a possible alternative to conventional surgery or radiation therapies for the treatment of lung cancer.

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