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Experimental intraperitoneal injection of alcohol in rats: Peritoneal findings and histopathology

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ABSTRACT

Purpose: This study aimed to evaluate the macroscopic and microscopic peritoneal findings after intraperitoneal injection of alcohol in rats.
Methods: From January to February 2012, 20 male rats were used in this study: 15 rats received intraperitoneal injection of 0.1 mL 99.9% alcohol (group 1: experiment group) and 5 rats received intraperitoneal injection of 0.1 mL normal saline (group 2: control group). Animals from each group were sacrificed the day after alcohol injection and each week thereafter. Macroscopic and microscopic examinations of the peritonea and abdominal cavity were performed in each rat.
Results: There was no significant peritoneal abnormality on macroscopic view, except for a whitish-colored parietal peritoneum around the injection site in 3 animals from group 1. In all but 1 of the animals in group 1, mild to moderate peritoneal inflammation or fibrosis was observed 1 and 2 weeks after alcohol injection. However, the peritoneal abnormality of alcohol injection had dissipated by week 3. Peritoneal abnormalities were not observed in group 2.
Conclusion: An intraperitoneal injection of alcohol in rats caused peritoneal inflammation or fibrosis during the first 2 weeks. However, these peritoneal abnormalities were short-lived and had completely disappeared after 3 weeks.
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1. Introduction

Alcohol (i.e., ethanol) is a widely used sclerosing agent in clinical practice because of its effectiveness, ease of use, long shelf-life, and low cost [1–6]. Alcohol sclerotherapy is the first-choice treatment for benign cystic lesions of various organs and has been used to treat benign solid thyroid nodules or venous malformations [2–6]. A concentration of 95–100% is most commonly used because it rapidly coagulates the cells lining the cyst [7]. However, because of the coagulative power of alcohol, concerns have been raised about potential complications from alcohol leakage during or after the procedure.

The experimental assessment of intraperitoneal ethanol leakage may be helpful to the doctors who perform alcohol sclerotherapy for benign intraabdominal lesions by predicting potential complications. To the best of our knowledge, no animal study regarding the histopathology or related findings following intraperitoneal injection of alcohol was found. The purpose of this study was to evaluate the macroscopic and microscopic findings following intraperitoneal injection of alcohol in rats, as well as the related findings.

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2. Materials and methods

All experiments were approved by our Institutional Animal Review Board and were conducted in accordance with the Korean Physiological Society's Guiding Principles on the Care and Use of Animals. From January to February 2012, this study employed 20 male Sprague-Dawley rats (Samtaco, Osan, South Korea), each weighing approximately 200 g (7 weeks old). All rats were maintained in an air-conditioned area, and were regularly provided with water and laboratory chow ad libitum. The dosage of alcohol was determined on the basis of the recommendation to keep alcohol doses below 1 mL alcohol/kg in humans [8]: the maximum dose of alcohol for a rat weighing 200 g can be calculated as 0.2 mL, and a half of the maximum dose of alcohol was used in this study.

The rats were divided into 2 groups: 15 animals were injected intraperitoneally with 0.1 mL of 99.9% alcohol (Merck, Darmstadt, Germany) and were classified as the experiment group (group 1). The remaining 5 rats were injected intraperitoneally with 0.1 mL of normal saline and were classified as the control group (group 2). The dosage of alcohol was 0.1 mL in each rat, and was arbitrarily determined by considering the fact that the maximum amount of alcohol per sclerotherapy session in humans is 1 mL alcohol/kg of body weight. An alcohol concentrations of 99.9% was selected based on prior reports of alcohol sclerotherapy in peritoneal cysts [3]. The dosage of alcohol and normal saline was the same.

Injection of alcohol and normal saline was carried out by an experienced member of the research team. Each rat was anesthetized with a 5% enflurane–oxygen mixture (1 L/min). The animal's respiratory patterns and extremity color were closely monitored. Prior to injection, each rat was placed in a supine position and the skin was scrubbed with povidone-iodine (polyvinylpyrrolidone iodine). A 23-gauge needle attached to a 1 mL syringe was inserted on the upper side of the navel and 0.1 mL alcohol was injected into the peritoneal cavity. For the accurate intraperitoneal injection of alcohol, the beveled portion of the needle tip was removed by repeatedly folding it using the forcep, and the position of the needle in the intraperitoneal cavity was confirmed prior to injection of alcohol by administering 1 mL room air. Following alcohol injection, the puncture site was marked by Gentian violet to identify injection site on the sacrificial day. The entire procedure was performed under general anesthesia in each rat.

On the day after alcohol injection, 3 rats in group 1 and 1 rat in group 2 were sacrificed and abdominal cavity in each rat was macroscopically examined by the same investigator, and then he collected tissue samples for microscopic examination. This was repeated each week until 4 weeks after alcohol injection. The macroscopic appearance of the abdominal cavity was subsequently evaluated in all 20 rats and microscopic analyses were performed on tissue samples obtained from 3 sites: (1) the parietal peritoneum, including the injection site and a further site remote from the site of injection; (2) the visceral peritoneum, including the small bowel with mesentery and large bowel with mesocolon; and (3) a free-margined portion of liver. All specimens were fixed in 10% neutralized formalin and processed with paraffin-embedding. After making paraffin-blocks, specimens were sectioned by 5 μm thickness by microtome and stained with hematoxylin and eosin stain. There is no standardized grading system for inflammation in histology, but the evaluation of inflammation of peritoneum and liver was performed by single pathologist as follows: none; no infiltration of inflammatory cell, mild; a

Table 1
Macroscopic findings and microscopic results of the abdomen after injection of alcohol (group 1) and normal saline (group 2) in rats.

Group	Day sacrificed	Macroscopic finding	Microscopic finding of the peritonea	
			Parietal	Visceral
1	1st	Normal	Moderate inflammation	Mild inflammation
	1st	Normal	Mild inflammation	Mild inflammation
	1st	Normal	Mild inflammation	Mild inflammation
	7th	Whitish change of parietal peritoneum, Visible injection site	Mild inflammation Fibrosis	Mild inflammation
	7th	Whitish change of parietal peritoneum, Visible injection site	Mild inflammation Fibrosis Foreign body reaction	Moderate inflammation Foreign body reaction
	7th	Normal	Normal	Normal
	14th	Normal	Mild inflammation Foreign body reaction	Mild inflammation
	14th	Normal	Mild inflammation	Normal
	14th	Whitish change of parietal peritoneum	Mild inflammation	Normal
	21th	Normal	Normal	Normal
	21th	Normal	Normal	Normal
	21th	Normal	Normal	Normal
	28th	Normal	Normal	Normal
	28th	Normal	Normal	Normal
	28th	Normal	Normal	Normal
2	1st	Normal	Normal	Normal
	7th	Normal	Normal	Normal
	14th	Normal	Normal	Normal
	21th	Normal	Normal	Normal
	28th	Normal	Normal	Normal

few inflammatory cells including lymphocytes or plasma cells (less than 10/HPF [400×]), moderate; some dozens of lymphocytes or plasma cells infiltrates in 1HPF (400×), and severe; intense infiltration of inflammatory cells.

3. Results

Of the 20 rats, no infection or other complications in the abdominal wall around injection site were found. Macroscopic and microscopic findings after intraperitoneal injection of alcohol in rats are summarized in Table 1. In group 1, the abdomen of all 3 rats sacrificed 1 day after alcohol injection showed no color change of the peritoneum or other macroscopic abnormality, but they microscopically revealed mild to moderate inflammation of the parietal or visceral peritoneum (Fig. 1). And, 2 animals from group 1 displayed a patchy or round shaped, lighter-colored parietal peritoneum around the injection site on macroscopic view, 1 week after alcohol injection. This finding was present in only 1 animal by 2 weeks post-injection. These animals also had mild peritoneal inflammation, and

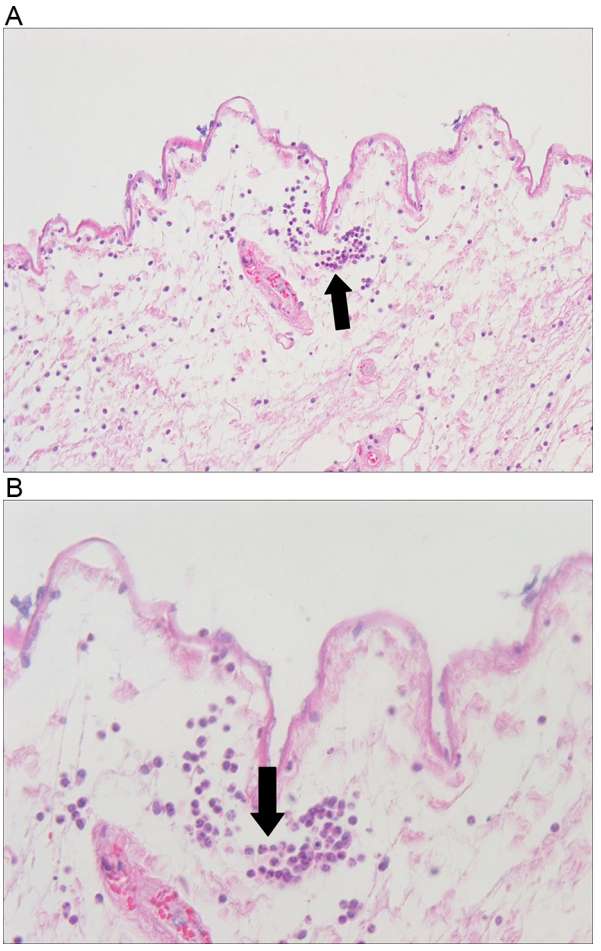

Fig. 1. Microscopic findings of the peritoneum in a rat sacrificed 1 day after intraperitoneal alcohol injection. Moderate inflammation with neutrophils (arrow) is observed in the parietal peritoneum (H&E, 200× (A) and 400× (B)).

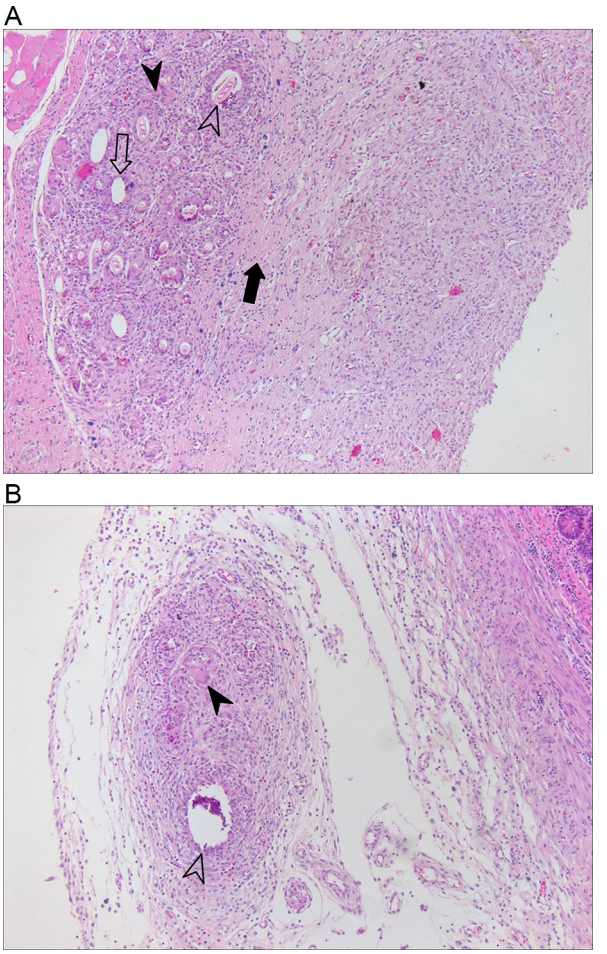

Fig. 2. Microscopic findings of the peritoneum in a rat sacrificed 1 week after intraperitoneal alcohol injection. Mild to moderate inflammation is observed in addition to fibrotic change (black arrow) and foreign body reaction (open arrow) in both the parietal (A) and visceral (B) peritonea. Foreign body reaction, comprising histiocytes and foreign body-type multinucleated giant cells (black arrowhead) with foreign material (open arrowhead), is noted in the epimysium (H&E, 100×).

fibrosis or foreign body reaction on microscopic inspection, which was more pronounced in 1 week than in 2 week post-injection (Fig. 2).

By 3 and 4 weeks, all animals in group 1 had a normal peritoneal anatomy on both macroscopic and microscopic examinations. All animals in group 2 showed a normal peritoneum on both macroscopic and microscopic examinations at all time-points examined. Hepatic abnormalities or ascites were not observed on macroscopic or microscopic examination in any of the animals from either group, at any time-point examined in this study.

4. Discussion

Alcohol is a widely used sclerosant in the treatment of benign cystic lesions in humans. Alcohol has the advantages of being inexpensive, efficacious, and easy to use [2–6,9]. Alcohol concentrations ranging from 95% to 100%

have been used because, at these concentrations, alcohol rapidly (1–3 min) coagulates the cells lining the cyst [7]. Sufficient amount of alcohol, which can replace 10–90% of the aspirated fluid in the cystic lesions, and multiple alcohol injections for large cystic lesions (>400 mL in volume) have been proposed [10,11]. In general, it is recommended that the amount of alcohol injected should not exceed 1 mL alcohol/kg of body weight, and that a single dose should not exceed 20 mL [8,10,11].

Alcohol exerts its therapeutic effect by inducing an intense inflammatory reaction when it comes into contact with the epithelial lining of benign cysts, or transected lymphatic vessels. The cells lining the cyst cavity are fixed, thereby disabling their ability to secrete fluid and promote cyst enlargement [1]. In tissues, ethanol induces cellular dehydration and protein denaturation, followed by coagulation necrosis, small vessel thrombosis, hemorrhagic infarction, and reactive fibrosis [5].

Alcohol leakage can result in necrosis of the surrounding tissue. Several reports indicate that alcohol sclerotherapy can cause major complications, including skin necrosis, peripheral nerve palsies, and hypotensive crisis, as well as fatalities from cardiac arrest and pulmonary emboli [8,12–16]. These complications are closely related to intravascular injection of alcohol for sclerotherapy of vascular malformation, and absolute alcohol can be used as a thromboembolic agent by direct intravascular injection [8,14–16]. A greater hemodynamic changes can arise from a single bolus injection of absolute alcohol rather than as a result of the total amount of absolute alcohol used during alcohol embolotherapy of arteriovenous malformations of the extremities [17]. One study has demonstrated that alcohol can cause a sclerosing cholangitis-like abnormality in the bile ducts or significant neurotoxicity [7]. However, these complications may be attributed to procedural errors, such as incorrect positioning of the needle or catheter drainage [12–14]. Therefore, alcohol leakage is a serious concern particularly in cases of alcohol sclerotherapy for intraabdominal or retroperitoneal cystic lesions.

To the best of our knowledge, only 1 study has examined experimental intraperitoneal injection of alcohol [18]. This study showed intraabdominal complications related to peritoneal leakage of significant amounts of alcohol in rats. However, the study focused on the peritoneal finding of picibanil by using intraperitoneal alcohol injection in a limited number of rats as control group [18]. They injected a 0.5 mL alcohol, which caused various degrees of peritoneal adhesion and inflammation. In contrast, we used 0.1 mL alcohol in an attempt to reduce the likelihood of severe peritoneal inflammation or intraabdominal complications, which we suggest is proportional to the amount of alcohol injected. Considering the recommendation to keep alcohol doses below 1 mL alcohol/kg in humans [8], the maximum dose of alcohol for a rat weighing 200 g can be calculated as 0.2 mL. The results of the present study show that 0.1 mL intraperitoneal injection of alcohol caused mild to moderate peritoneal inflammation and fibrosis in rats up to 2 weeks post-injection. Thereafter, the adverse effects of alcohol therapy slowly resolved and were no longer evident by 3 week post-injection. The reason for the complete resolution of peritoneal inflammation or fibrosis is not clear, but may be related to the hypothesis that a scattering alcohol in the injection happened because of a blunting of the needle caliber in cutting the needle tip when the alcohol becomes inert after rapid dilution with blood flow or fluid. Patchy or round-shaped, peritoneal discoloration around injection site supports this hypothesis. However, we cannot explain the exact cause of foreign body reaction, but it may be the unexpected intraperitoneal ingression of dust particles attached to rat's hairs because of no shaving.

This study has several limitations. The major limitation is the use of single dose of alcohol, and thus, comparison study using varying alcohol dose may be required. Second, this study focused on peritoneal findings occurring after alcohol injection. We did not assess the histopathology of all intraabdominal organs, or other systemic findings, such as fever or heart rate. Third, blood count analysis and other laboratory evaluations were not performed. Finally, long-term follow-up over 1 month was not conducted.

In conclusion, an intraperitoneal alcohol injection in rats promoted short-term peritoneal inflammation or fibrosis, which resolved 3 weeks post-injection.

Conflict of interest statement

All the authors have no conflict of interest.

Declaration of interest

No competing financial interests exist. The authors alone are responsible for the content and writing of the paper.

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