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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

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