

Assessment of Rabbit Spleen Size Using Ultrasonography

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(Received 7 December 2006/Accepted 30 March 2007)

ABSTRACT. Assessment of spleen size using the ultrasonography has become a standard practice in human. However, the assessment is not established method in experimental animals. To establish the index to assess the spleen size using ultrasonography, we measured the cross-section image of rabbit spleen during endotoxin shock. The image of the cross-section was appeared as triangle, and the height of the triangular image was defined as the spleen index. This spleen index showed strong correlation with the spleen weight. In conclusion, this method is suitable for observation of changes in rabbit spleen size and may reduce the number of rabbit in the longitudinal studies.

KEY WORDS: endotoxin shock, rabbit spleen, ultrasonography.

J. Vet. Med. Sci. 69(8): 841-842, 2007

Longitudinal studies are important in the research for appropriate experimental animal models, but most currently available models sacrifice a lot of animals to analyze the time course of these models. To reduce the number of animals, application of medical imaging systems should be valuable. In line with this thinking, ultrasonography appears to be a very suitable technique for organ imaging in animals.

Previous study shows that the spleens are increased in both size and weight in the rabbits with experimentally induced chronic serum sickness [1]. In this model, the splenomegaly is observed in the responsive rabbit, but not in the non-responder. Further, it has been reported that the autoimmune reactions in Brown Norway rats and MRL/lpr mice are related with the spleen weight [8, 9]. These reports allow us to speculate that the real-time observation of spleen size may be useful for the selection of the responder animals and the monitoring of immunological response in the various experimental models. Thus, the observation of spleen using ultrasonography is intriguing for the survey of immunological state before the onset of the disease and the sacrifice in the animal models.

Rabbit has become the preferred experimental model for investigation of human diseases such as infection [5, 6] and immune disorders [2, 3]. In this study, female white Japanese rabbits (kbl:JW, specific pathogens free) were obtained from Oriental Yeast, Kitayama Labes (Ina, Japan). The rabbits were kept in the animal center at JIMRO until they were 13 to 21 weeks old (2.5 to 3.3 kg). This study was admitted by the experimental animal committee in our institute and was with strict adherence to the guidelines of the science council of Japan applied to the experimental animals. Before ultrasound imaging, the hair on to left ventral area from the 12th rib to the front of femur was carefully shaved. Ultrasound images were obtained using SUD-1200 X-Plus

with 8-MHz 11R-convex (scope of 190°) probe (Shimadzu Medical, Osaka, Japan). This instrument was suitable size for rabbit. During imaging, the rabbit was held in the prone position by a trained animal keeper without anesthesia.

For detection of the spleen image, the probe was positioned between the end of eleventh rib and twelfth rib on the left side, and was approximately perpendicular to body surface. The spleen in the ultrasound imaging was seen as a dark (hypoechoic) structure on the stomach (Fig. 1). When the ultrasound-beam orientation was in the cephalo-caudal axis, the spleen appeared between the stomach and the left kidney, and then the cross-sectional image of spleen was observed as a triangle (Fig. 1).

At first, we had tried to evaluate the rabbit spleen size using the human practice method, which uses the splenic vein or tip for decide the measurement position [7]. However, we did not found any landmark, such as the splenic vein or tip, in the ultrasonographic image, because the rabbit splenic vein

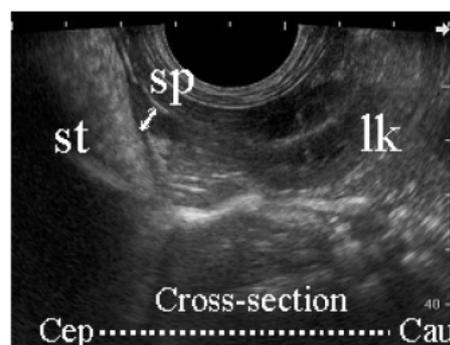


Fig. 1. Ultrasound image of the spleen in the rabbit at cephalic-caudal axis. Ultrasound image at cephalic-caudal axis was obtained in an untreated rabbit. The spleen index is shown as arrow line. The orientation is shown as dotted bars in these photographs. Cep, Cephalic; Cau, Caudal; St, Stomach; lk, left kidney; sp, spleen.

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Table 1. The spleen index in each rabbit

Rabbit ID No. ^{a)}				
No. 1	No. 2	No. 3	No. 4	No. 5
4.57 ± 0.40	2.77 ± 0.06	4.52 ± 0.16	8.46 ± 0.14	3.28 ± 0.11

Data are mean ± standard deviation from three repeat measurements.

a) Each rabbit was prepared in a variety of body weight and age.

was too smaller than human and the tips of the spleen were seen covered by the stomach or rib. Next, in the cross-sectional image, we measured “the spleen index”, which was seen as a height of the triangle image (Fig. 1). The positions of the stomach and the left kidney were considered as suitable landmarks instead of the spleen vein or the spleen tip, because these positions determined the probe location.

We show the reproducibility of spleen index (the height of the triangular image) in Table 1. The measurements of spleen index were recorded three times in the same rabbits, and the mean value was taken for statistical analysis. In this method, it was possible to complete within 15 min and to obtain the index with standard deviation less than 0.5. Further, the other operators confirmed the reproducibility of repeated measures (data not shown).

We investigated the correlation between the spleen index and the spleen weight. After ultrasound imaging, the rabbits were sacrificed by exsanguination under complete anesthesia. Immediately after sacrifice, the spleen was removed and its net weight was measured. As a consequence of the assessments, the spleen index showed a clear correlation with the spleen weight (Fig. 2).

Finally, we analyzed the rabbit spleen size during endotoxin shock. It has been known that lipopolysaccharide (LPS) injection induced enlargement of spleen in mice [4]. LPS from *E. coli* 0127:B8, purified by trichloroacetic acid extraction was obtained from Sigma-Aldrich (St. Louis, MO, U.S.A.) and was dissolved in sterile pyrogen-free saline (Otsuka, Tokyo). LPS was adjusted to 1.0 mg/kg in 0.6 ml saline and was administered intravenously through the ear vein. This study was conducted as a blind study. LPS injection caused spleen enlargement in rabbits by 1.5 to 3-fold increase in spleen weight after 24 to 48 hr (n=3, data not shown). The spleen index did not significantly change in the rabbit injected with saline, while the ultrasonographic operator clearly detected significant increase in the index in the rabbit injected with LPS after 24 hr, compared to 0 hr (Fig. 3).

In conclusion, this ultrasonographic method is suitable for real-time observation of changes in rabbit spleen size. It is expected that this method will be useful to study the experimental models and to reduce the number of rabbit in the longitudinal studies.

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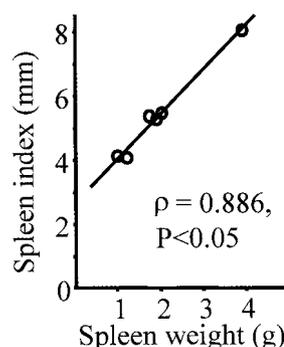


Fig. 2. Correlation between spleen index and spleen weight. Data from both spleen index in ultrasound measurement and weight of extirpated spleen after ultrasound measurement in the same rabbits were used for the scatter-plot (n=6). The correlation coefficients were analyzed by using the spearman's rank test. Data are represent the mean values derived from 3 separate observations.

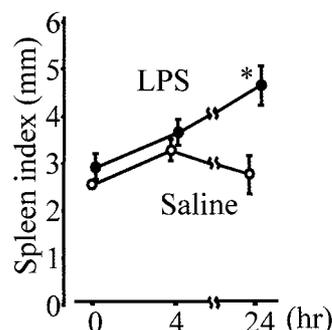


Fig. 3. The follow-up spleen enlargement induced by LPS injection. These results are from a single rabbit followed up for 24 hr. The result of LPS treatment is representative from three rabbits. Data are the mean ± standard deviation derived from three measurements. * P<0.05 for 24 hr vs 0 hr by student's *t*-test.

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