

Histological Comparison Study with Primo Node and Immature Liver Tissue on Liver Surface in Rat

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흰쥐의 간 장기 표면에서 관찰되는 프리모 노드와 미성숙 간조직과의 조직학적 비교연구

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Abstract

목적 : 프리모 노드와 미성숙 간조직에 대한 조직학적 특성을 비교하고자 하였다.

방법 : 흰쥐에서 간절제 후, 간장기 표면에서 관찰되는 프리모 노드와 그와 유사한 미성숙 간조직을 H&E, Oil red O, Masson trichrome and van Gieson 염색방법을 통해서 비교 연구하였다.

결과 : 상기 실험 연구를 진행한 결과, 다음과 같은 결론을 얻었다.

1. 조직의 일반적인 특징을 관찰할 수 있는 H&E 염색결과, 프리모 노드에서는 많은 수의 조그마한 동(sinus)이 관찰되었고 적혈구나 영양을 공급하는 혈관의 분포는 관찰되지 않았다. 이와 반대로 미성숙 간조직에서는 동이 관찰되지 않았으며 혈관의 분포와 적혈구가 관찰되었다.
2. 양 조직의 지방성분의 유무를 관찰하기 위한 Oil red O 염색결과, 프리모 노드에서는 지방성분이 관찰되지 않았으나 미성숙 간조직에서는 관찰되었다.
3. 양 조직의 콜라겐성분의 유무를 관찰하기 위한 Masson trichrome and van Gieson 염색결과, 프리모 노드에서는 약간의 콜라겐 성분이 관찰되었으나 elastic 성분은 관찰되지 않았으며, 미성숙 간조직에서는 콜라겐 성분과 elastic 성분이 관찰되지 않았다.

결론 : 상기 결과는 프리모 노드와 간조직과는 조직학적 성질이 다른 것으로 사료되며, 특히 콜라겐 성분의 적은 결과는 프리모 노드가 불규칙한 형태를 이루고 있는 이유에 대한 실마리를 제공하였다. 이러한 결과는 프리모 노드의 특성에 대한 기본 자료로 활용될 수 있을 것이다.

Key words : Primo node, immature liver tissue, Oil red O stain, Masson trichrom stain, van Gieson stain

I . Introduction

The first introduction of Bong-Han theory of primo vascular system different from the vascular, nervous, and lymphatic systems was announced by

Bong-Han Kim in the early of 1960's¹. Kim claimed that the primo vascular systems (PVS) consisting of the primo nodes (Bonghan corpuscles) and primo vessels (Bonghan ducts) were anatomical basic structures of traditional acupuncture systems (named *Kyungrak* theory or meridians). Because Kim found the primo nodes and vessels in where were being put on the acupoints lower part in the skin¹.

And Bong-Han Kim classified PVS to superficial primo nodes and vessels, organ-surface primo nodes and vessels, intravascular primo nodes and vessels, nervous primo nodes and vessels². However, Kim's reports have been neglected for the past 40 years because of the difficulties on reproducing his results^{3,4}.

In last several years, Bong-Han theory has been revived through the research to find the anatomical structure by Soh and his members of Biomedical Physics Laboratory of Seoul National University (SNU) using the staining with Janus Green B⁵, Alcian blue^{6,7}, Acridine Orange⁸, Trypan blue^{9,10} and nanoparticles¹¹⁻¹⁴.

In our previous studies on PVS, there were the results in about the morphology studies of organ-surface primo nodes and vessels¹⁵ and distinguish point internal PVS from torn mesentery tissue¹⁶. In this study, we found the primo nodes on liver surface after hepatectomy surgical procedure and distinguished it from the immature liver tissue for comparison study in rat. The present report will be useful to know the characteristic feature of primo node.

II. Materials and Method

1. Animals

Sprague Dawley rats weighting about 250-300 g (5-6 weeks) used in this study were obtained from Samtaco Laboratory Animal Company (Osan,

Korea). The animals were housed in a temperature-controlled environment (23°C) with 60% relative humidity, a 12-h light/dark cycle, and ad-libitum access to food and water. All the procedures involving the animals and their care conformed to institutional guidelines, which were in full compliance with current international laws and polices (Guide for the Care and Use of Laboratory Animals, National Academy Press, 1996). And the study was approved by the Institute of Laboratory Animal Resources in Wonkwang University.

2. Surgical procedures

The rats injected with urethane (1.5 g/kg), i.m. before the surgical operation. All surgical procedures were performed under general anesthesia. We incised the skin along the medial alba of the abdomen very carefully and opened the abdomen. Suprahepatic vena cava were clipped with forceps before hepatectomy. The liver Isolated from abdominal cavity were moved to PBS (pH 7.2) pool rapidly and washed out for 3-5 times vary carefully. We searched for primo-nodes on the liver lobe surfaces carefully by using small surgical instruments such as iris scissors, microforceps, and needles for manipulation. The search for the nodes and the vessels was carried out under a stereoscopic microscope (SZX10, Olympus, Japan).

3. Tissue preparation and staining

The samples were isolated washed in PBS slowly and gently and directly fixed in 10% neutral-buffered formalin (NBF) at 4°C overnight. And fixed samples were embedded in paraffin, sliced with 4-6 μm in thickness. The sliced samples were

stained with hematoxylin & eosin, oil red O (AMRESCO, USA)¹⁷, masson trichrome(Singma-Aldrich, USA)^{18,19}, van gieson(Singma-Aldrich, USA), periodic acid-methenamine silver(PAM, Singma-Aldrich, USA) after deparaffin process.

III. Results and Discussion

1. The primo nodes were found after hepatectomy surgical procedure

After hepatectomy surgical procedure, the immature liver tissue was discovered frequently at the region of hepatic portal vein and artery. And the primo nodes and vessels were found frequently too. If the liver tissue will be immature, on the exterior the shapes of immature liver tissues are similar to primo node. So we need the comparison study on the primo node with immature liver tissue.

The pictures of fig. 1 were the primo node and immature liver tissue in stereo microscope. Left picture (a) was the typical characteristic feature of primo node, milky color, semi-transparent. And the characteristic feature of immature liver tissue (b) was bright pink color, semi-transparent.

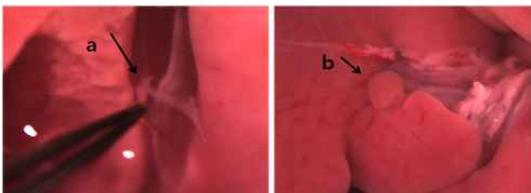


Fig. 1. Comparison picture of the primo node from the immature liver tissue in rat with light stereo-microscopy after hepatectomy.

a : the primo node on liver surface, b : immature liver tissue. Usually the primo nodes and immature liver tissues were found at the region of hepatic portal vein and artery.

immature liver tissue in H&E stain results

In the primo-node (fig. 2. left), small sinus of $10\sim 20\mu\text{m}$ sized were observed like Hong's²⁰, Lee's²¹ and our previous results in H&E stain. This picture on characteristic feature of primo node (fig. 2, left) was shown the typical characteristic feature of primo node, small sized of sinus, no or a few wall of body like sponge. And in side those sinus, a nucleus was observed frequently in a sinus, and no blood vessels for nutrition.

In immature liver tissue (fig. 2, right), a huge blood vessel of $80\mu\text{m}$ sized and small size of blood vessels were observed for nutrition in similar size of the primo-node, and also the RBC were observed inside the blood vessel. Various sinus was observed inside the primo-node and immature liver tissue, that of immature liver tissue's size was more irregular than that of primo-node. And there was no nucleus frequently in sinus of immature liver tissue.

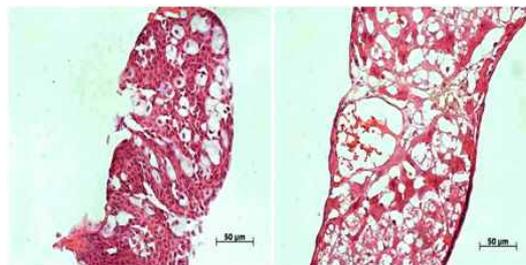


Fig. 2. Comparison results of Hematoxylin & eosin stain on primo node with immature liver tissue in rat.

Left ; the primo node, Right ; the immature liver tissue. In the primo-node, small sinus of $10\sim 20\mu\text{m}$ sized were observed and the each sinus have a nucleus in it. But in the immature liver tissue, huge sinus of about $80\mu\text{m}$ sized were observed and the each sinus have RBC for its nutrition.

2. The primo nodes were different from

3. The primo nodes were different from

immature liver tissue in oil red O stain results

Oil red O stain is the useful method for finding the lipid factor in experimental stain method¹⁷⁾. We used Oil red O stain for finding that the primo node and immature liver tissue observed on liver surface had lipid factor or not. Because the primo node and adipocyte were observed in similar part frequently on liver surface so we want to know the observed primo node on liver surface have a kind of adipocyte or not.

The lipid factor was colored with red in Oil red O stain. In the primo node, there were negative results (fig. 3, left) but in the immature liver tissue, there were positive results (fig. 3, right). These results suggested strongly that the primo nodes had no lipid factor, fat tissue or adipocyte. This characteristic feature of primo node will be distinguishing point different from the immature liver tissue.

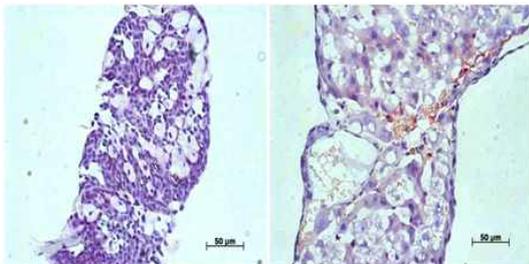


Fig. 3. Comparison results of Oil red O stain on primo node with immature liver tissue in rat

Left ; the primo node, Right ; the immature liver tissue. The lipid factor was colored with red in Oil red O stain. In the primo node, there were negative results but in the immature liver tissue, there were positive results.

4. The primo nodes were different from

immature liver tissue in Masson trichrome stain and van Gieson stain results

Collagen fiber is the extra cellular protein to have important role to maintain the tension for matrix of tissue. And the Masson trichrome stain^{18,19)} and van Gieson²²⁾ stain is staining for collagen fiber and elastic collagen fiber. From these results, we could know the primo node's organized binding force.

In Masson trichrome stain, collagen factor were stained with blue color, and there were a little negative stain in primo node, and massive positive stain in immature liver tissue. This results were similar with lee et al.'s results of primo node inside lymphatic vessels in rabbit⁶⁾. In van Gieson stain, elastic factor were stained with pink color, and there were negative stain in primo node but positive stain results in immature liver tissue.

These results suggested that the organized binding force of primo node was very weak and the reason of irregular form of primo node except but the explanation of primo vessel's elastic nature.

IV. Conclusion

From the above experiment results for the characteristic feature comparison study about the primo node and the immature liver tissue using H&E, Oil red O, Masson trichrome, van Gieson stain, the next results were produced. 1st, in H&E stain, there are so many small sinus with nucleus inside in primo node, but many blood vessels with red blood cell in immature liver tissue. 2nd, in lipid factor results from Oil red O stain, there are negative results in primo node but positive results in immature liver tissue. 3rd, in collagen factor

results from Masson trichrome and van Gieson stain, there are a little collagen factor and no elastic factor in primo node but a lot of collagen factor and elastic factor in immature liver tissue.

These results would be useful to know the characteristic feature of primo node.

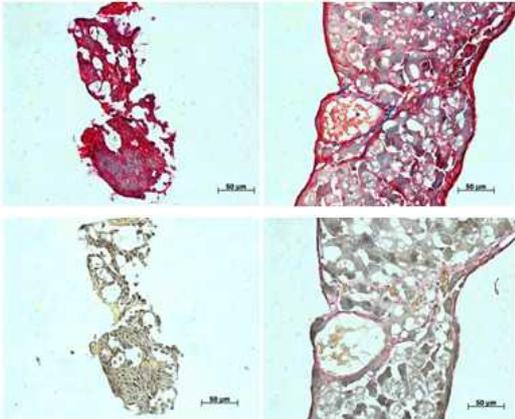


Fig. 4. Comparison results of Masson trichrom and van Gieson stain on the primo node with immature liver tissue in rat

Upper and left ; the primo node with masson trichrom stain, upper and right ; the immature liver tissue with masson trichrom stain, lower and left ; the primo node with van Gieson stain, lower and right ; the immature liver tissue with van Gieson stain. In Masson trichrome stain, collagen factor were stained with blue color, and there were a little negative stain in primo node, and massive positive stain in immature liver tissue. In van Gieson stain, elastic factor were stained with pink color, and there were negative stain in primo node but positive stain results in immature liver tissue.

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