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A pathological observation on the experimental rabbit viral hepatitis

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실험적 토끼 바이러스성 간염의 병리학적 관찰

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초록 : 국내에서 발생된 토끼 virus성간염의 병리학적 소견을 명확히 규명하기 위하여 2~14개월령의 재래토끼에 간염에 이환된 토끼의 간조직 유제를 접종한후 임상 및 병리학적으로 관찰하였던 바 다음과 같은 결과를 얻었다.

접종한 30마리중 90%인 27마리가 감염되어 접종후 96시간이내에 폐사되었고 2~3월령 3두는 감염이 일어나지 않았다. 임상증상으로는 식욕부진, 체온상승, 연변 등이 나타났고 3두에서는 비강내로부터 혈액성 포말이 관찰되었다. 부검소견으로는 장기의 충출혈, 간장의 변성이 전예에서 관찰되었고 소장의 카타르성 장염과 방광내 혼탁한 뇨충만도 관찰되었다.

광학현미경적 소견으로는 간괴사가 정도의 차이가 있기는 하나 폐사된 27두 전예에서 관찰되었고, 출혈빈도는 폐가 가장 많았고 심장, 간장, 비장, 신장 및 흉선 순으로 나타났다. 한편 출혈이 관찰되지 않은 예에서도 간의 괴사소견은 현저하였다. 소장의 카타르성 장염과 뇌의 혈관주위 임파구 침윤도 관찰되었다.

이상의 결과를 종합해 볼 때 본 질병에서 출혈소견은 개체에 따라 변화가 심하였으나 중요한 소견은 간염으로 나타났고 원인 virus는 일차적으로 간에 친화성을 가지며 동시에 viremia를 일으켜 전신장기에 출혈을 야기하는 것으로 추정된다.

Key words : rabbit, hepatitis, virus, haemorrhagic disease.

Introduction

Since 1984, a new acute fatal viral disease of rabbits has been described in China[1~3], Korea[4~7], Mexico[8] and European countries.[9~12] The disease is characterized by high morbidity in adult rabbits. The characteristic pathologic findings are hemorrhages in the lungs, liver, spleen, kidneys and heart of naturally infected rabbits.[1,3,5,7] Because of its haemorrhagic feature in natural cases, the disease is named as viral haemorrhagic pneumonia[1,13] or viral haemorrhagic disease[3], X disease[12], picornavirus haemorrhagic fever[1], although the disease reported from many countries is essentially identical to those described by Liu et al[1] in clinical and pathological findings.

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However, the frequency of pathologic lesions in those organs of experimental cases was not mentioned until now.

In this paper, pathological findings in rabbit viral hepatitis, namely rabbit haemorrhagic disease are described in experimentally infected rabbits.

Materials and Methods

Experimental animals : Thirty healthy rabbits, from 2 to 14 month-old, were used in this study.

Inoculum : Liver of rabbit with viral hepatitis was homogenized to be 20% in phosphate buffer. After centrifugation of liver suspension, the supernatant was clarified through 0.45 ㎛ membrane filter(Gelman) and used for inoculum as shown previously.[5,6]

Experimental methods : Rabbits were intramuscularly inoculated with 0.2㎖ of inoculum. Body temperature of all rabbits were measured at 18 hours before and after inoculation. Clinical signs were observed frequently during daytime. All experimental rabbits were necropsied after death. At necropsy, gross lesions at each organ were examined. Tissues of liver, spleen, lungs, kidneys and brains were fixed in buffered formalin, embedded in paraffin, sectioned and stained with H-E.

Results

Clinical signs : The increase of body temperature ranging from 0.1℃ to 20℃ was recognized at 18 hours postinoculation(PI) in twenty four cases of experimentally infected thirty rabbits. There was a decrease of body temperature in five cases died within 24 hours PI.

Anorexia, mild diarrhea and nasal bleeding with bloody foam were recognized in 25(83%), 5(17%) and 3 rabbits(10%), respectively. Ninety percent of experimental rabbits died within 96 hrs PI(Table 1). Three rabbits with 2~3 months old recovered from infection.

Necropsy findings : At necropsy, haemorrhage and hyperemia were the conspicuous findings in many organs of experimental rabbit viral hepatitis. The affected liver was pale, swollen and hyperemic with occasional haemorrhages. Haemorrhages were found in lungs, spleen, liver, kidneys, trachea, thymus and heart, in order. In two cases, haemorrhagic feature was not recognized in any organs. In three cases, there was bloody foam from nostrils. There were also catarrhal enteritis in small intestine and retention of turbid urine in urinary bladder in some cases.

Histopathologic observation : Major histopathologic findings of rabbit viral hepatitis are peripheral necrosis of

Table 1. Clinical signs and mortality in experimental rabbit viral hepatitis

Age	No.	Necropsy PI(Hrs)					Anorexia	Nasal bleeding	Diarrhea
(Month)		24	48	72	96	120<			
2~3	8		2/8	2/8	1/8	3/8	3/8	0/8	2/8
4	2		2/2				2/2	0/2	0/2
6	10	4/10	6/10				10/10	1/10	3/10
10	2	1/2	1/2				2/2	1/2	0/2
12	1		1/1				1/1	0/1	0/1
14	7	5/7	2/7				7/7	1/7	0/7
	30	10	14	2	1	3	25	3	5
	(100%)	(33%)	(47%)	(7%)	(3%)	(10%)	(83%)	(10%)	(17%)

Table 2. Histopathologic findings in experimental rabbit viral hepatitis

Age	No.	Liver	Haemorrhage						Catarrhal	Cuffing
(Month)		necrosis	liver	spleen	kidneys	lungs	heart	thymus	enteritis	in CNS
2~3	8	5/8	1/8	1/8	0/8	2/8	0/8	1/8	4/8	4/8
4	2	2/2	0/2	1/2	0/2	2/2	2/2	0/2	2/2	2/2
6	10	10/10	3/10	2/10	3/10	6/10	7/10	0/10	8/10	6/10
10	2	2/2	2/2	1/2	1/2	2/2	1/2	2/2	1/2	1/2
12	1	1/1	1/1	0/1	1/1	0/1	0/1	0/1	1/1	1/1
14	7	7/7	2/7	3/7	2/7	5/7	3/7	3/7	6/7	4/7
	30	27	9	8	7	17	13	6	22	18
	(100%)	(90%)	(30%)	(27%)	(23%)	(57%)	(43%)	(20%)	(73%)	(60%)

liver and haemorrhagic lesions in kidneys, lungs, heart and some other organs.

In liver, necrotic hepatitis was found in all of 27 rabbits which were necropsied after death. There were severe and moderate necroses in 25 cases and mild peripheral necroses only in 2 rabbits. The frequency of microscopic haemorrhagic feature was recognized in lungs, heart, liver and other organs, in order(Table 2).

Seventy seven percent of experimental rabbits(23/30) suffered from catarrhal enteritis which might be associated with soft feces. Perivascular lymphocytic cuffing in brain was found in 47% (14/30) of rabbits, where the number of inflammatory cells increased with time course.

Discussion

Rabbit viral hepatitis, namely rabbit haemorrhagic disease is a new viral disease in rabbits.[1,3,5] The nature of the causative agent of this disease has been disputed into picornaviruses[3,4,5], caliciviruses[9,10] and parvoviruses.[8] Recently, the causative virus of this disease has been classified into a subgroup of the family caliciviridae.[9,10] The potential pathogenicity of this virus has already been reported by many authors[3~5,9~11] in both experimental and natural cases.

This disease is an acute, highly infectious disease with a short incubation period and a high mortality.[2,3,7,8] Common clinical signs are increase in body temperature, anorexia and depression. Nasal bleeding from nostrils are also recognized in some cases. In this report using Korean isolate of rabbit hepatitis virus, clinical signs are basically similar to those of previous workers.[4~7] In rare cases, the decrease of body temperature may be due to the check time around death. These rabbits were supposed to be suffered from the increase of body temperature after virus challange.

The gross and histopathological findings in this disease are haemorrhage and necrosis in liver, necrosis and swelling in spleen, congestion and hemorrhage in kidneys, pulmonary edema and hemorrhage in lungs, hemorrhage and hyperemia in trachea, hemorrhage in heart, encephalitis and catarrhal enteritis. Liver has been regarded as one of target organs according to histopathologic examinations.[5,8]

In this paper, there were necrosis of liver and haemorrhages in many organs of all rabbits died of viral hepatitis. Even in some rabbits which had no haemorrhage, conspicuous peripheral necrosis in liver was consistent findings. The haemorrhagic frequency in organs was too variable to induce conclusion depending on its severity among organs.

Young rabbits, which were 2~3 months old, recovered from infection. Less susceptibility in young rabbits than that in old rabbits was also confirmed previously[2,5] and it might be caused by maternal immunity and/or relatively different immune mechanisms aganist infections.

On the central nervous system, there were non-suppurative meningitis and demyelination[3] and degeneration of neurons in brains.[14] In this report, there were perivascular cuffing in the central nervous system similar to the natural cases reported previously.[5]

On the mechanism of hemorrhages in this disease, damage of vascular endothelial cells may be occurred after viremia. Haemorrhages in lungs and heart may shorten the incubation time leading to sudden death. Tissue distribution of viral antigens in relation to haemorrhage is under the progress using immunohistochemistry.

Summary

This paper dealt with the clinical and pathological observations on the experimental rabbit viral hepatitis. Rabbits with 2-14 months of ages were intramuscularly inoculated with virus suspension. The results were summarized as follows.

Ninety percents of experimental rabbits inoculated with virus died within 96 hours postinoculation. Common clinical signs were inappetence, increase in body temperature, depression, mild diarrhea and in three cases, bloody foam from nostrils was recognized.

At necropsy, in most of the experimental cases, there were hyperemia or haemorrhages in many organs and pale liver. Intestinal catarrh and retention of turbid urine in urinary bladder were seen in some cases.

Histopathologically, liver necrosis was found in all the cases died of this disease. However, there was a difference in the severity of hepatic necrosis. Haemorrhages were recognized in lungs, heart, liver, spleen, kidneys and thymus, in order. Liver necrosis was marked even in the cases with no haemorrhage. Perivascular cuffing in brain and catarrhal enteritis in small intestine were seen in many cases.

From these results, consistent and primary lesion in this viral disease is hepatitis in susceptible rabbits. It was concluded that rabbit hepatitis virus might have the properties of hepatotropism and consequently induce peripheral necrosis in the liver leading to acute viremia with haemorrhages.

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