大韓獸醫學會誌(1992) 第32卷 第2號
Korean J Vet Res (1992) 32(2) : 207~210

Ultrastructure of virus particles in the liver of piglets infected with porcine enterovirus serotype 3

Tae-kyun Shin, Cha-soo Lee*, Min-do Huh**

Department of Veterinary Medicine, Cheju National University, Cheju
*College of Veterinary Medicine, Kyungpook National University, Taegu,**
*Department of Fish Pathology, National Fisheries University of Pusan, Pusan, Korea***

(Received Oct 16, 1991)

Enterovirus감염 돼지의 간에서 virus의 형태학적 관찰

신태균 · 이차수* · 허민도**
제주대학교 수의학과
경북대학교 수의과대학*
부산수산대학교 어병학과**

(1991. 10. 16 접수)

초록 : Enterovirus감염 돼지의 감염초기 virus의 동태를 규명하고자 초유를 섭취하지 않는 1~2일령의 돼지에 돼지 enterovirus 3형을 경구감염시킨후 간조직을 전자현미경으로 관찰하였던 바 다음과 같은 결과를 얻었다.

감염후 3일째 부검한 돼지의 간에서 간세포의 심한 공포변성이 인정되었고 간 동양혈관 내피세포 및 Kupffer세포에서 picornavirus의 특징적인 virus결정이 출현하였으며 확장된 ER은 virus 결정과 밀접하게 관련되어 있었다. 또한 동양혈관 세포와 간세포에서는 미숙한 virus모양의 입자들로 구성된 세포질내 봉입체가 관찰되기도 하였다. 이상의 소견으로 보아 돼지 enterovirus 3형은 신경친화성 뿐만 아니라 감염초기 간에 친화성이 큰 것으로 생각된다.

Key words : porcine enterovirus, liver, picornavirus, ultrastructure.

Introduction

Porcine enteroviruses have been associated with various clinical syndromes including polioencephalomyelitis, female reproductive disorders, diarrhea, pneumonia, pericarditis and myocarditis.[1] Pathogenesis of porcine enterovirus-induced polioencephalomyelitis has been well evaluated by electron microscope.[2~4] Crystalline arrays of virus particles characteristic of the family Picornaviridae[5] have been recognized as a helpful marker for locating viral antigens in the affected cells of central nervous system.[2,3] Although porcine enterovirus has been occasionally isolated from the liver as well as brain of affected pigs in both the experimental and spontaneous porcine enterovirus infections[6~8], the role of porcine enterovirus in the causation of hepatic changes in swine has not been yet assessed. There are also few studies on hepatic lesions on porcine enterovirus infection in pigs, while liver is one of the frequently affected organs on enterovirus infections in some vertebrates.[9,10]

In the course of studying porcine enterovirus infec-

tion in pigs[2,11,12], hepatic changes were recognized in neonatal piglets which died suddenly within 3 days postinfection. The present paper is concerned with ultrastructural changes associated with virus particles in the liver of piglets infected with porcine enterovirus serotype 3.

Materials and Methods

Inoculum : The porcine enterovirus used in this experiment was originally isolated from the brain of a pig with polioencephalomyelitis. It was identified as porcine enterovirus serotype 3.[12]

Experimental Animals : Two litters of colostrum-deprived, neonatal piglets were orally inoculated with 1ml of tissue culture suspension containing $10^{7.5}$ $TCID_{50}$/0.1mℓ. Two control piglets were given 1mℓ of virus-free tissue culture suspension. Each group of piglets was maintained in a separate isolator.

Preparation of Tissues : The experimental piglets showing severe tremor were necropsied. The liver specimens from the affected piglets either died or be killed were processed for electron microscopic examination.

The liver specimens were minced and fixed in 2% paraformaldehyde-2.5% glutaraldehyde(0.075M cacodylate buffer), postfixed in 1% osmic acid, dehydrated and embedded in Epon 812. Ultrathin sections of the specimens were stained with uranyl acetate and lead citrate and examined by Hitachi 800 electron microscope.

Results

Affected piglets showed mild tremor on the 2 days postinoculation and ataxia leading to sudden death within 3 days postinoculation. None of the controls had any clinical signs.

Histopathologic findings of the liver were acute swelling of hepatocytes, vacuolar degeneration, condensation of nuclear chromatin and pyknotic nuclei in the sinusoid endothelial cells.

Ultrastructurally, crystalline arrays of virus particles were recognized in the damaged sinusoid endothelial cells(Fig 1). Kupffer cells also contained crystalline arrays of virus particles in the cytoplasm(Fig 2). The approximate diameter of virus particles ranged from 27nm to 30nm. Each particle was polygonal.

Virus crystals were closely associated with dilated rough ER of Kupffer cells. In the affected hepatocytes, there were polyribosomes, dilation of rough ER and cytoplasmic aggregates of electron dense, virus-like particles in the cytoplasm.

Discussion

Porcine enteroviruses have been known as an important causative agent associated with embryonic and early neonatal death.[6,7] After oral infection of germfree pigs with the ECPO-6 strain of porcine enterovirus, viremia was detected as early as the 48 hours postinoculation and for the next 3 days.[8,13] In the present study, colostrum-deprived, neonatal piglets inoculated with porcine enterovirus showed mild tremor as early as the 2nd day postinoculation, leading to sudden death within 3 days postinoculation. These findings might be caused by acute viremia. When specific pathogen free pigs, 4 and 6 weeks old, were intranasally infected with the ECPO-6 strain of porcine enterovirus, there were hepatic discoloration, thickening of the lobular septa and bile duct hyperplasia in the liver.[14] In this study, histological changes of the liver were similar to those of acute hepatitis.[15,16]

Hepatic lesions were produced regardless of the route of porcine enterovirus infection either intranasally[14] or orally in this study.

On the pathogenesis of viral disease, it's important to identify viral antigens. Fortunately , it's possible to identify viral antigens by routine electron microscope as porcine enterovirus compartmentalize in the form of crystalline arrays within the cytoplasm. Crystalline arrays of virus particles, characteristic of the family Picornaviridae[5], were recognized in the sinusoid cells of the liver in this study and vascular endothelial cells of the central nervous system in our previous publication.[2]

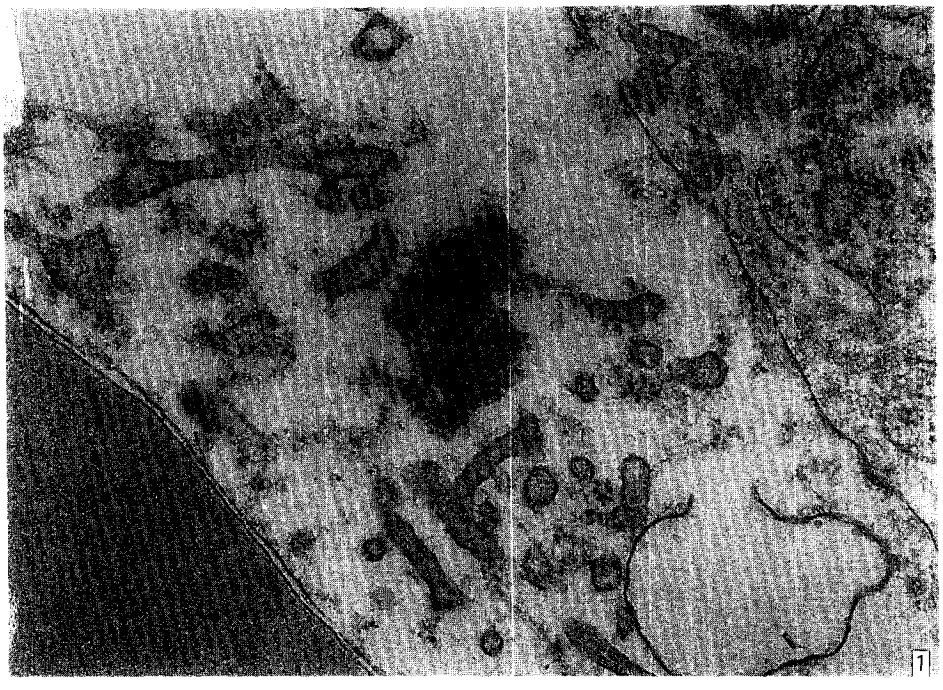
These findings ascertain the possibilities that porcine enterovirus serotype 3 has hepatotropism as well as neurotropism and cardiotropism.[1]

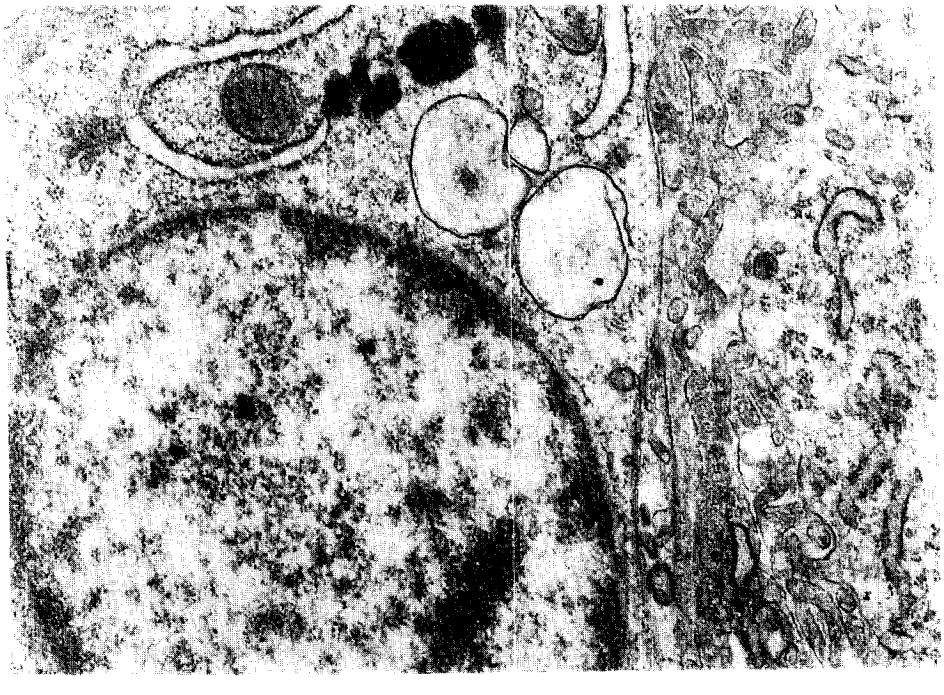
Legends for figures

Electron micrographs were obtained from the livers of the infected piglets which were necropsied at 3 days postinfection.

Fig 1. Crystalline arrays of virus particles in the hepatic sinusoid cell. × 76,000.

Fig 2. Virus crystals are found in a Kupffer cell. × 38,000.





Summary

Oral infection of colostrum-deprived, neonatal piglets with porcine enterovirus serotype 3 can result in hepatic lesions with a short incubation period. In the thin section of the affected liver, crystalline arrays of virus particles characteristic of picornavirus were identified in the sinusoid endothelial cells and Kupffer cells. There were also cytoplasmic aggregates of electron-dense, virus-like particles in the hepatocytes. These findings suggest that porcine enterovirus serotype 3 has hepatotropism as well as neurotropism.

References

1. Derbyshire JB. Porcine enterovirus infections. In : Leman AD, Straw B, Glock RD, et al., ed. Diseases of Swine. 6th ed. Iowa : *Iowa State University Press* 1986 ; 325~330.
2. Shin TK, Lee CS. Electron microscopic observations of the vascular endothelial cells in the central nervous system of piglets infected with porcine enterovirus serotype 3. *Korean J Vet Res* 1988 ; 28 : 137~143.
3. Koestner A, Kasza L, Holman JE. Electron microscopic evaluation of the pathogenesis of porcine polioencephalomyelitis. *Am J Pathol* 1966 ; 49 : 325~337.
4. Long JF. Pathogenesis of porcine polioencephalomyelitis. In : Olsen RG, Krakowka S, Blakeslee JR, ed. Comparative pathobiology of viral disease, Vol. 1. *Boca Raton, Florida : CRC Press* 1985 ; 179~197.
5. Godman GC. Picornaviruses. An atlas. In : Dalton AJ, Haguenau F. ed. Ultrastructure of animal viruses and bacteriophages. *New York, London : Academic Press* 1973 ; 133~153.
6. Fujisaki Y, Ikeo T, Hayashi S, et al. Isolation of porcine enterovirus from a newborn piglet with nervous symptoms(Japanese). *Bull Natl Inst Anim Health* 1981 ; 82 : 1~18.
7. Huang J, Gentry RF, Zarkower A. Experimental infection of pregnant sows with porcine enteroviruses. *Am J Vet Res* 1980 ; 41 : 471~473.
8. Baba SP, Bohl EH, Meyer RC. Infection of germfree pigs with a porcine enterovirus. *Cornell Vet* 1966 ; 56 : 386~394.
9. Howard CR. Viral hepatitis. In : Brown F, Wilson G, ed. Virology. In : Wilson G, Miles SA, Parker MT, ed. Topley's and Wilson's principles of bacteriology, virology and immunology , 7th ed. *London : Edward Arnold* 1984 ; 451~471.
10. Anderson AA. Picornaviruses of animals : Clinical observations and diagnosis. In : Kurstak E and Kurstak C, ed. Comparative diagnosis of viral disease. Vol. IV. Vertebrate animal and related viruses. Part B-RNA viruses. *New York, London, Toronto, Sydney, San Francisco : Academic Press* 1981 ; 1~63.
11. Shin TK, Lee CS. Polioencephalomyelitis in pigs experimentally infected with porcine enterovirus isolated in Korea. I. Histopathological observation. *Korean J Vet Res* 1985 ; 25 : 103~112.
12. Shin TK, Lee CS, Kwon HJ, et al. Serological classification of porcine enteroviruses isolated in Korea. *Korean J Vet Res* 1987 ; 27 : 223~226.
13. Kasza L, Holman J, Koestner A. Swine polioencephalomyelitis virus in germfree pigs : Viral isolation, immunoreaction and serum electrophoresis. *Am J Vet Res* 1967 ; 28 : 461~467.
14. Meyer RC, Woods GT, Simon J. Pneumonitis in an enterovirus infection in swine. *J Comp Pathol* 1966 ; 76 : 397~405.
15. Okuno T, Sano A, Deguchi T, et al. Pathology of acute hepatitis A in humans. Comparison with acute hepatitis B. *Am J Clin Pathol* 1984 ; 81 : 162~169.
16. Dienstag JL, Popper H, Purcell RH. The pathology of viral hepatitis types A and B in chimpanzees. A comparison. *Am J Pathol* 1976 ; 83 : 131~148.