Effect of Enzymes on the Growth of Human and Animal Rotaviruses

Kunihiko SATO, Shuichi TOKUHISA¹⁾, and Yuji INABA²⁾

The Kyushu Branch Laboratory, National Institute of Animal Health, Chuzan, Kagoshima 891-01, ¹⁾National Institute of Animal Health, Tsukuba, Ibaraki 305, and ²⁾Department of Veterinary Epizootiology, College of Agriculture and Veterinary Medicine, Nihon University, Fujisawa, Kanagawa 252, Japan

(Received 11 November 1994/Accepted 2 February 1995)

ABSTRACT. The growth of group A human, bovine, equine and porcine rotaviruses were enhanced by pretreatment of virus with pancreatin, trypsin, protease, alkaline phosphatase or pepsin and incorporation of these enzymes in maintenance medium. In contrast, α -amylase or lipase inhibited the growth of equine and porcine rotaviruses. The other enzymes, adenosin deaminase, lactase, lysozyme, ribonuclease or triose-phosphate isomerase gave little or no change in the growth of all four rotaviruses.—**KEY WORDS:** enzyme, growth, rotavirus.

J. Vet. Med. Sci. 57(3): 569–570, 1995

A major impediment to rotavirus investigations was eliminated by using proteolytic enzymes or roller tube culture technique or both, and most group A rotavirus isolates can be routinely propagated in cultures of MA104 cells, a stable cell line derived from embryonic rhesus monkey kidney [1–4, 7]. It was of interest, therefore, to test the effect of enzyme on the growth of rotavirus. In the present paper, we attempted to find enzymes, which are effective for rotavirus infectivity other than proteolytic enzymes.

For comparison of rotavirus growth, group A rotaviruses used were the Hochi strain of human rotavirus (HRV) at the 5th passage level in MA104 cells [4], the Lincoln strain of bovine rotavirus at the 9th passage level in primary calf kidney cells after receiving from Dr. C. A. Mebus, University of Nebraska, Nebraska, U.S.A. [3], the HI-23 strain of equine rotavirus at the 2nd passage level in MA104 cells after receiving from Dr. H. Imagawa, Equine Research Institute, Japan Racing Association, Tochigi, Japan [2], and the OSU strain of porcine rotavirus at the 2nd passage level in MA104 cells after receiving from Dr. E. H. Bohl, Ohio Agricultural Research and Development Center, Ohio, U.S.A. [6].

Enzymes used were adenosine deaminase (Sigma, A-9626), alkaline phosphatase (P-L, 544-00971), α -amylase (Wako, 014-03731), lactase (Sigma, L-7004), lipase (Sigma, T-8003), lysozyme (P-L, 544-01071), pancreatin (Difco, 521-00521), pepsin (Sigma, P-7012), Protease (Sigma, P-4630), ribonuclease (P-L, 545-00641), triose-phosphate isomerase (Sigma, T-2507) and trypsin (Sigma, Type III). Controls, using PBS (0.15 M NaCl, 0.01 M phosphate buffer PBS, pH 7.2) instead of enzymes, were set up, treated, and assayed in similar manner.

Confluent tube (110 × 10 mm) cultures of MA104 cells were washed three times with Eagle's minimum essential medium (MEM). The growth medium used was MEM containing 10 per cent tryptose phosphate broth (TPB, Difco), 10 per cent calf serum, 10 units/ml penicillin, 100 μ g/ml streptomycin, and 1 μ g/ml fungizone, and the maintenance medium (MM) was MEM containing 10 per cent TPB, 0.5 per cent sodium glutamate. Zero point one per cent yeast extract, anti-biotics and each enzyme with various concentrations, and was adjusted to pH 7.4 with

Table 1. Concentrations of various enzymes used in pretreatment of virus and incorporation in the maintenance medium

Enzyme	Concentration (μ g/ml)	
	Pretreatment	Incorporation
Adenosin deaminase	1.0	0.1
Alkaline phosphatase	25.00	25.0
α -amylase	75.0	7.5
Lactase	5.0 I.U. ^{b)}	0.5 I.U.
Lipase	7500.0	750.0
Lysozyme	7500.0	750.0
Pancreatin ^{a)}	250.0	25.0
Pepsin	7500.0	750.0
Protease	10.0	1.0
Ribonuclease	250.0	25.0
Triose-phosphate isomerase	5.0	0.5
Trypsin	10.0	1.0

a) Pancreatin contains many enzymes, including amylase, trypsin, lipase, ribonuclease and protease.

b) I.U.: International units.

Table 2. Effect of various enzymes on rotavirus growth

Enzyme	Rotavirus (Strain)			
	HRV ^{a)} (Hochi)	BRV (Lincoln)	ERV (HI-23)	PRV (OSU)
Adenosin deaminase	1.5 ^{b)}	3.5	2.5	3.5
Alkaline phosphatase	3.2	5.8	4.5	6.2
α -amylase	2.5	3.8	≤ 0.5	≤ 0.5
Lactase	2.5	3.5	ND ^{c)}	ND
Lipase	2.5	3.5	≤ 0.5	3.2
Lysozyme	2.5	3.5	2.5	3.5
Pancreatin	5.5	6.5	7.5	6.5
Pepsin	3.0	3.5	3.5	6.5
Protease	3.2	4.5	4.5	6.5
Ribonuclease	1.5	3.5	1.8	3.5
Triose-phosphate isomerase	2.5	3.5	2.5	3.5
Trypsin	4.2	4.8	6.5	6.5
Control PBS	1.5	3.5	1.8	3.5

a) HRV=Human rotavirus; BRV=Bovine rotavirus; ERV=Equine rotavirus; PRV=Porcine rotavirus.

b) Log TCID₅₀/0.1 ml

c) Not done.

7.5 per cent NaHCO_3 solution.

The optimal concentration of each enzyme was determined under the condition of confluent monolayer which enhanced rotavirus growth, and was not affected by a dose used. The concentration of each pretreatment enzyme was 10 times strength of the optimal concentration used in MM (Table 1).

Assays were followed by the method described by Sato *et al.* [4]. Briefly, pretreatment of virus with each enzyme was done at 37°C for 30 min. Serial 10-fold dilutions of the treated material were made with MM and each dilution was inoculated in 0.1 ml volume into 3 tube cultures of MA104 cells. After incubation at 37°C for 60 min, the cultures were washed three times with MM containing no enzyme, fed with 0.5 ml of MM incorporated with the determined concentration of enzyme, and incubated in a roller drum at 37°C for 5 days. Cytopathic effect (CPE) was observed, and fifty per cent tissue culture infectious dose ($\text{TCID}_{50}/0.1 \text{ ml}$) was calculated.

As shown in Table 2, the growth of human, bovine, equine and porcine rotaviruses were enhanced by the treatment not only with the proteolytic enzymes, such as pancreatin, protease and trypsin but also with the other enzymes, alkaline phosphatase and pepsin. In contrast, the treatment with α -amylase or lipase inhibited the growth of equine and porcine rotaviruses. The treatment with the other enzymes tested, adenosin deaminase, lactase, lysozyme, ribonuclease or triose-phosphate isomerase gave little or no change in the growth of human, bovine, equine and porcine rotaviruses tested.

As mentioned above, the method with pancreatin treatment used in this study may be helpful in adapting the non-group A rotaviruses, which are also fastidious viruses, since the pancreatin treatment produced the highest infective titers of all group A rotaviruses tested. This

method has been successfully applied to propagate a group C porcine rotavirus, one of the extremely fastidious rotaviruses, in primary porcine kidney cell cultures [5]. Furthermore, it is worthy to note that the treatment with alkaline phosphatase enhanced the growth of all group A human and animal rotaviruses, although the mechanism of the enzyme to the rotaviruses is not known. Therefore pancreatin and alkaline phosphatase seem to be helpful in establishing new isolates and in preparing rotavirus antigens in these cell cultures. Further detailed studies are necessary to elucidate the mechanism of enhancement-effect of alkaline phosphatase on the growth of group A rotaviruses and to investigate the effect of this enzyme on non-group A rotaviruses.

ACKNOWLEDGEMENT. We are grateful to Dr. Tatsuhiko Shinozaki, Taketani Hospital, Kiyose, Saitama, Japan, for supporting this study.

REFERENCES

1. Fukusho, A., Shimizu, Y., and Ito, Y. 1981. *Arch. Virol.* 69: 49–60.
2. Imagawa, H., Wada, R., Hirasawa, K., Akiyama, Y., and Oda, I. 1984. *Jpn. J. Vet. Sci.* 46: 1–9.
3. Mebus, C. A., Kono, M., Underdahl, N. R., and Twiehaus, M. J. 1971. *Can. Vet. J.* 12: 69–72.
4. Sato, K., Inaba, Y., Shinozaki, T., Fujii, R., and Matumoto, M. 1981. *Arch. Virol.* 69: 155–160.
5. Terrett, L. A. and Saif, L. 1987. *J. Clin. Microbiol.* 25: 1316–1319.
6. Theil, K. W., Bohl, E. H., and Agnes, A. G. 1977. *Am. J. Vet. Res.* 38: 1765–1768.
7. Theil, K. W. 1990. pp. 35–72. *Viral Diarrheas of Man and Animals*, chap. 3 (Saif, L. J. and Theil K. W. eds.), CRC Press, Florida, U.S.A.