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Reduced Amount of Japanese Encephalitis Viral RNA in the Infected Cells Treated with Human Interferon Beta

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Abstract: Reverse transcriptase-polymerase chain reaction (RT-PCR) revealed that the amount of Japanese encephalitis (JE) virus-specific positive sense RNA was found to be reduced in the infected Hep-2 cells treated with human interferon beta at 1,000 IU/ml in the medium compared with untreated specimens.

Key words: PCR, Interferon beta, Japanese encephalitis, Viral RNA

INTRODUCTION

Our previous experiment demonstrated that human interferon beta (IFN) significantly inhibited the growth of JE virus in human cell lines, as measured by the infective virus production in the infected culture fluids (Hu et al., 1992). In the present study, the efforts were made to see whether the IFN treatment can inhibit the virus growth at the level of viral RNA synthesis by using reverse transcriptase polymerase chain reaction (RT-PCR).

MATERIALS AND METHODS

Cells and virus: The human cell line, Hep-2, was used as the host cells to test IFN effect on JE virus replication, while BHK21 cells were used for infectivity assay of JE virus by plaque formation as described before (Hu et al., 1992). Both cell lines were grown in Eagle's medium in Earle's saline supplemented with 9% fetal calf serum and 0.2 mM each nonessential amino acids at 37°C. The seed of JE virus Nakayama strain was prepared in C6/36 cells at 28°C and infectivity was expressed by plaque forming units (PFU)/ml as described before (Hu et al., 1992).

IFN: Human fibroblast interferon beta (BM532) was provided by Toray Corporation as freeze-dried preparation. IFN-treatment of the cell and virus inoculation were previously described (Hu et al., 1992). The freeze-dried IFN preparation was reconstituted with physiological saline, and filtrated through 0.2 μm filter to prepare the stock solution of 10,000 IU/ml. The IFN was supplied to the cell culture medium before and after virus inoculation at
final concentration of 1,000 IU/ml. The infected culture fluid and cells were harvested Day 0 (just after 2 hr virus absorption), Day 1 and Day 2 after infection at 37°C, and were kept at -70°C. The infected fluid was used for the infectivity assay on BHK21 cells and the infected cell suspension was used to extract RNA.

**Extraction of RNA and reverse transcriptase-polymerase chain reaction (RT-PCR):** The whole RNA of infected cells and control cells was extracted according to the standard protocol (Sambrook *et al.*, 1989). RT-PCR was conducted in 2 steps: transcription of viral RNA to the first strand cDNA and cDNA amplification. For positive (+) strand RNA detection, 10 microliter of RNA sample was added with 40 microliters of RT-PCR mixture containing anti-sense primer in the reaction tube, which was incubated at 52°C for 10 min for transcription followed by 96°C 5 min to stop the reaction. To the reaction tube was added 50 microliters of RT-PCR mix containing sense primer and Tth DNA polymerase and 2 drops of mineral oil, and PCR amplification was repeated as described (Morita *et al.*, 1992). Five microliters of the PCR product was subjected to agarose gel electrophoresis, and amplified DNA fragments were visualized by ethidium bromide staining. The negative (−) strand viral RNA detection was performed by just using opposite sense primers in above steps. The nucleotide sequences of both primers were shown in Table 1.

| Table 1. Nucleotide sequence of sense and anti-sense primers to detect JE viral RNA by RT-PCR |
|---|---|---|
| Code | Polarity | Nucleotide sequence |
| JE-M1-S | sense | GGCGACACACGCCTGGGACTT |
| JE ER | antisense | AGCATGCATTGGTGCCTA |

**RESULTS**

The first experiment was performed to detect positive (+) sense RNA using 25 cycles of DNA amplification. No JE virus-specific band was detected on Day 0 (both IFN treated and untreated), while Day 1 and Day 2 specimens showed reduced amount of JE virus-specific bands in IFN-treated cells compared with untreated cells (Fig. 1). The results demonstrated the reduced amount of positive (+) sense viral RNA in the IFN treated infected cells and this (+) RNA increased from Day 0 to Day 1 or Day 2. These results corresponded with the virus infectivity titer in the infected fluids (untreated cell Day 0: 1.4×10², Day 1: 1.8×10⁷ and Day 2: 4.5×10⁷, while IFN-treated cell Day 0: 9.1×10¹, Day 1: 6.5×10⁴ and Day 2: 1.6×10⁵ PFU/ml).

Detection of (+) RNA was repeated to compare the 20 and 25 cycles of DNA amplification and the result was shown in Fig. 2. By 20 cycles of amplification, untreated Day 1 and Day 2 infected cell specimen showed stronger bands compared with less intense bands of IFN-treated Day 1 and Day 2 cell specimens. While 25 cycles of amplification did not show
Fig. 1. Etidium bromide-stained DNA bands amplified by 25 cycles RT-PCR using JE virus-specific primer for the RNA prepared from infected and uninfected Hep-2 cells with or without IFN-treatment.
Lane 1: negative control, lane 2: uninfected cells, lane 3: infected fluid (positive control), lane 4: untreated Day 0, lane 5: IFN-treated Day 0, lane 6: untreated Day 1, lane 7: INF-treated Day 1, lane 8: untreated Day 2, lane 9: IFN-treated Day 2. Figures on the right side of the gel represent the size of molecular weight marker in base pairs. The IFN-treatment was performed at final concentration of 1,000 IU/ml before and after virus infection.

Fig. 2. Etidium bromide-stained DNA bands amplified by 20 and 25 cycles RT-PCR using JE virus-specific primer for the RNA prepared from infected and uninfected Hep-2 cells with or without IFN-treatment.
Lanes 1-6: 20 cycles, lanes 7-12: 25 cycles of amplification. Lanes 1 and 7: negative control, lanes 2 and 8: infected fluid (positive control), lanes 3 and 9: IFN-treated Day 1, lanes 4 and 10: untreated Day 1, lanes 5 and 11: IFN-treated Day 2, lanes 6 and 12: untreated Day 2. Figures on the right side of the gel represent the size of molecular weight marker in base pairs. IFN-treatment was performed at final concentration of 1,000 IU/ml before and after virus infection.
clear-cut difference between the IFN-treated and untreated cells in terms of the intensity of the amplified bands. The virus infectivity in respective infected fluid corresponded with the intensity of the DNA band found by 20 cycles of amplification (Day 1: $3.6 \times 10^7$ and Day 2: $8.5 \times 10^7$ of untreated cells, compared with Day 1: $1.2 \times 10^4$ and Day 2: $3.8 \times 10^4$ PFU/ml of IFN treated cells).

The negative (−) strand detection did not provide positive bands so far tested (data not shown).

**DISCUSSION**

Although PCR method was difficult to quantitate, if suitable cycle was used, it could be a useful method to detect viral RNA semiquantitatively. Our results demonstrated that in human IFN treated infected cells, the JE virus-specific positive sense RNA was reduced in amount compared with the untreated control. The result would provide scientific base for use of IFN to the treatment of flavivirus infection by inhibiting viral RNA replication. The failure to detect any negative sense viral RNA may reflect its smaller amount resulting from the asymmetrical viral RNA replication, synthesizing larger amount of (+) strand on limited amount of (−) strand template.

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**REFERENCES**


