Convenient Biological Assay for Polyethylene Glycol-Interferons in Patients with Hepatitis C

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The vesicular stomatitis virus cytopathic effect reduction assay is suitable to quantify polyethylene glycol-alpha interferon 2a (PEG–IFN-α2a) and PEG–IFN-α2b. Human serum and ribavirin did not interfere with the assay. This bioassay was successfully used for assaying PEG-IFN-α2a and PEG-IFN-α2b in serum samples from patients undergoing combination therapy.

The success of any antiviral treatment depends on three groups of factors, those of the host, virus, and drugs (4, 8). However, patients chronically infected with hepatitis C virus (HCV) with similar viral and host factors and who received the same treatment schedule often exhibit discrepancies in terms of virus eradication (7). A relationship between variability in drug concentrations in the blood and treatment efficacy has been suggested for ribavirin and alpha interferon (IFN-α) (1, 5), but this has not yet been investigated for polyethylene glycol (PEG–IFN-α). Such studies require a convenient assay for measuring PEG–IFN-α in patient sera.

Detection of IFN-α levels in serum can be performed by immunoassay or bioassay (12). The immunoassay measures the physical quantity of material but does not differentiate between active and inactive molecules. Bioassays for IFN-α are based on the protection of cultured cells against the cytopathic effect of a challenge virus (6, 10, 11).

In this study, we tested the suitability of the vesicular stomatitis virus (VSV) cytopathic effect reduction assay for PEG–IFN-α quantification.

The entire procedure of the bioassay was performed with a 96-well microtitration plate. Samples were placed in the first row of wells. Twelve serial twofold dilutions were carried out with Eagle minimal essential medium (MEM) supplemented with 10% fetal bovine serum. An aliquot (0.1 ml) of a suspension of Madin-Darby bovine kidney cells (150,000/ml) was placed in each well. After incubation for 18 h at 37°C, a confluent cell monolayer was obtained. The culture supernatant was drawn off and replaced with a suspension of virus in MEM without fetal bovine serum; 100 50% tissue culture infective doses of VSV was placed in each well in a volume of 0.1 ml. After an additional 24 h of incubation, the cytopathic effects were estimated by visual examination of the monolayers under a microscope. The greatest dilution of sample that still protected the cells was evaluated by comparison with the standard dilution range. The following controls were included in each test: two cell controls, five virus controls (100 50% tissue culture infective doses and dilutions of this viral suspension of 10−1, 10−2, 10−3, and 10−4). Solutions of PEG–IFN-α2a (Roche, Neuilly sur Seine, France) or PEG–IFN-α2b (Schering-Plough, Levallois-Perret, France) in MEM ranging from 0 to 500 IU/ml were assayed as described above. The measured titer correlated well with the solution titer for both PEG–IFN-α2a (r2 = 0.99) and PEG–IFN-α2b (r2 = 0.99). The dose-response line (Fig. 1) was used as the working standard for the subsequent experiments.

The bioassay detected 5 IU of PEG–IFN-α per ml in 95% of the assays. No false positive was observed when assaying a concentration of 0 IU/ml, indicating the high specificity of the test. The reproducibility of the result was verified by repetition of six determinations of five titers (Table 1).

Human serum from naive patients containing concentrations of PEG–IFN-α2a or PEG–IFN-α2b ranging from 0 to 500 IU/ml was assayed. Measured titers and solution titers were highly correlated (PEG–IFN-α2a, r2 = 0.97; PEG–IFN-α2b, r2 = 0.99). The slope of the dose-response line in human serum was not different from that of the working standard (PEG–IFN-α2a, P = 0.44; PEG–IFN-α2b, P = 1). Serum samples from 10 normal volunteers and 18 chronic HCV carriers who were not on treatment were assayed; the titer was 0 IU/ml in all cases.

Concentrations of ribavirin (Schering-Plough, Levallois-Perret, France) ranging from 1,000 to 5,000 ng/ml were assayed as were solutions of PEG–IFN-α. The cell monolayers were al-

| TABLE 1. Reproducibility of the VSV cytopathic effect reduction assay |
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| PEG-IFN solution titer (IU/ml) | Measured titer (IU/ml) | Coefficient of variation (%) |
| 12 | 16.1 ± 4.9 | 30 |
| 25 | 32.2 ± 9.8 | 30 |
| 50 | 57.3 ± 17.4 | 30 |
| 100 | 89.6 ± 20 | 22 |
| 250 | 175 ± 0 | 0 |

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Values are means ± standard deviations of six determinations.
ways completely destroyed. Those concentrations of ribavirin were not cytotoxic.

Human serum containing 3,000 ng of ribavirin per ml and PEG–IFN-α titers ranging from 0 to 500 IU/ml were assayed. The measured titers correlated well with solution titers (PEG–IFN-α2α r² = 0.98; PEG–IFN-α2β r² = 0.95). The slopes of the dose-response line in human serum plus ribavirin were similar to those of the working standard (PEG–IFN-α2α, P = 0.77; PEG–IFN-α2β, P = 0.31).

Serum samples from 25 patients treated with PEG–IFN-α2b plus ribavirin and from 9 patients treated with PEG–IFN-α2a plus ribavirin were assayed. Estimated concentrations in serum ranged from 0 to 125 IU/ml for PEG–IFN-α2b and from 0 to 190 IU/ml for PEG–IFN-α2a (Fig. 2).

The VSV cytopathic effect reduction assay appears to be suitable for quantifying PEG–IFN-α2a and PEG–IFN-α2b. Variations can be kept to a minimum by strict standardization of the assay. Controls for cell viability and for the total destruction of the cell monolayer by VSV in the absence of IFN-α are included in each plate. Every sample is assayed twice under the same conditions. Hence, our data show good reproducibility of the assay.

Human serum was reported to protect Madin-Darby bovine kidney cells against the cytopathic effect of VSV even without...
IFN-α in the sample (11). The similarity of the slope of the dose-response line in serum to that of the working standard confirmed that the inhibition observed was truly due to PEG–IFN-α. Comparison of the slope of the dose-response line in samples containing ribavirin to that of the working standard also excluded distortion of the test results by ribavirin.

HCV-induced endogenous IFN-α, which would lead to over-estimation of the sample titer (9), was not found in the blood of chronic HCV carriers who were not on treatment (1, 2). Growth or cytotoxic factors in serum were observed in a limited number of cases, but they did not hamper interpretation of the assay.

The VSV cytopathic effect reduction assay was used to quantify the antiviral activities of PEG–IFN-α2a and PEG–IFN-α2b in serum samples from patients on combination therapy. The data variability was great for both types of PEG–IFN-α. The elapsed time from the last dose of PEG–IFN-α could be a factor in titer variability, but postdose levels of PEG–IFN-α in serum were reported to be essentially flat for most subjects (3). Discrepancies in PEG–IFN-α titers in serum could also be attributed to inter-individual variability in PEG–IFN-α pharmacokinetics.

This study shows that the VSV cytopathic effect reduction assay is suitable for assaying PEG–IFN-α2a and PEG–IFN-α2b. The concentration of PEG–IFN-α in serum samples from patients on combination therapy can be measured with this bioassay.

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