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SHORT COMMUNICATION

INTERFERON-α IN SERUM AND CARCINOMATOUS PLEURAL EFFUSION AFTER REPEATED INTRAPLEURAL INJECTIONS OF ANTITUMOR AGENTS

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Pleural effusions and sera of two patients with lung cancer were tested after intrapleural injection of OK-432 as an anticancer drug for IFN-α activity by biological assay and for IFN-α as an antigen by radioimmunoassay. The titers by radioimmunoassay were fairly consistent with those by biological assay, but were usually higher. In Case 1, IFN-α was observed fairly early after administration of OK-432 and only in pleural effusions. In Case 2, induction of IFN-α at low level was observed late after the first administration of OK-432 both in the pleural effusion and serum and was detected only by radioimmunoassay.

A streptococcal preparation, OK-432 (Chugai Pharmaceutical Co., Tokyo, Japan), has been found to have potent antitumor activity in experimental animals and humans. Clinical trials of OK-432 have shown its immunotherapeutic value in patients with cancer, but although it is widely recognized that intrapleural injection of OK-432 is often very effective in reducing carcinomatous effusion, the mechanism of its action is not fully understood. OK-432 is reported to induce interferon (IFN) in experimental animals (Saito et al., 1982) and augment NK activity of patients with cancer (Uchida et al., 1982). Recent studies have shown that IFN and NK activity play important roles in the antitumor activity of OK-432. We report here that IFN-α appeared in sera and in carcinomatous pleural effusions of patients with lung cancer during and after repeated intrapleural injections of OK-432.

Two patients with lung cancer with carcinomatous pleural effusion were given 1, 3, 5 and 5 KE of OK-432 twice a week by the intrapleural route. Dosage of OK-432 is expressed by mg dry weight of *Streptococcus haemolyticus* Su strain, and its 0.1 mg corresponds to 1 KE (Klinische Einheit). Sera and pleural effusions were obtained just before administation of OK-432 and stored at −85C. IFN-α levels in the materials were measured with an IFN-α RIA KIT using monoclonal anti-human IFN-α antibody (Dainabot Radioisotope Laboratories) and were expressed as international reference units (IU)/ml. Radioimmunoassay is reported to be an easier method for measurement of low levels of human IFN-α in serum, and to be more reproducible and quicker than biological assay (Secher and Burke, 1980; Secher, 1981; Walker et al., 1982). IFN-α titers in clinical specimens measured by radioimmunoassay were fairly consistent with those measured by biological assay and the radioimmunoassay was more sensitive than the biological assay. Another advantage of the radioimmunoassay over bioassay is that it is not affected by the presence of IFN inducers in the specimens. The biological activity of IFN-α was measured by the dye-uptake method (Finter et al., 1969; Pidot, 1971) using a bovine cell line (MDBK) which is much more sensitive to human IFN-α than to human IFN-β and not sensitive to human IFN-γ (Gray et al., 1982; Gresser et al., 1974). At same time, antiviral activity which may include the activities of IFN-α, β and γ was measured by the dye-uptake method using a human amnion cell line (FL). In Case 1, very high titers of antiviral activity were found (approximately 445–625 IU/ml) in pleural effusions. Their detailed investigation is now in progress.

Case 1: A 61-year-old female (T.K.) with lung cancer (adenocarcinoma) was given OK-432 together with 6 mg of mitomycin C (MMC) by intrapleural injection. Within two weeks after the final intrapleural injection of antitumor agents, the carcinomatous pleural

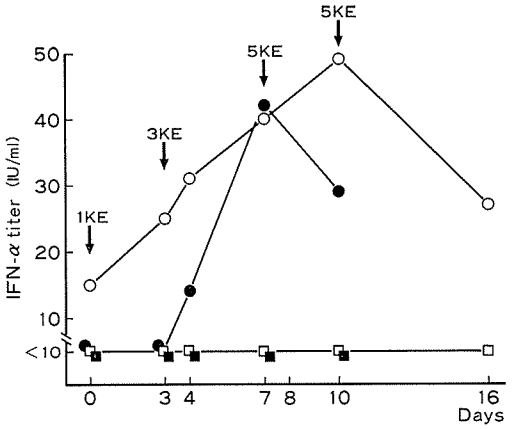

FIGURE 1. IFN-α levels in pleural effusions and sera from Case 1. IFN-α titers in the specimens were measured by radioimmunoassay (open symbols) and biological assay (closed symbols). Circles and squares indicate IFN-α titers in pleural effusions and sera, respectively. Arrows indicate the times of intrapleural injections of OK-432 and MMC.

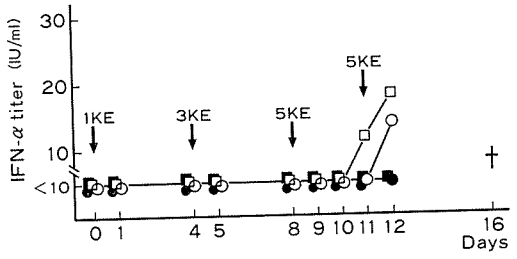

FIGURE 2. IFN-α levels in pleural effusions and sera from Case 2. IFN-α titers in the specimens were measured by radioimmunoassay (open symbols) and biological assay (closed symbols). Circles and squares indicate IFN-α titers in pleural effusions and sera, respectively, Arrows indicate the times of intrapleural injection of OK-432. The cross indicates the time of death.

effusion decreased. The IFN-α levels in the serum and pleural effusion of Case 1 are shown in Fig. 1. During repeated intrapleural injections of antitumor agents, IFN-α appeared in the pleural effusion but was not detectable in the serum. The highest IFN-α titer was 49 IU/ml and the titer dropped to 27 IU/ml on day 6 after the last injection. The patient has

since survived for 8 months.

Case 2: A 76-year-old man (T.H.) with lung cancer (small cell carcinoma) and bladder cancer was given OK-432 alone by intrapleural injection. During treatment no improvement was observed and he died after 16 days. The IFN-α levels of Case 2 are shown in Fig. 2. After repeated intrapleural injections of OK-432, the IFN-α levels rose slightly reaching 18 IU/ml in the serum and 13.5 IU/ml in the pleural effusion.

In this work, IFN-α was estimated as a biologically active substance by biological assay and as an antigenic substance by radioimmunoassay. The titers of IFN-α in pleural effusions were usually higher when measured by radioimmunoassay than by biological assay. This suggests that the biological activity of IFN-α is usually less stable than its antigenic activity (Case 1).

The late appearance of IFN-α in the pleural effusion of Case 2 may reflect the absence, or small amount, of cells producing IFN-α in the pleural effusion. The effect of IFN-α induced by OK-432 on the clinical course of the patients remains to be elucidated. But it is noteworthy that in Case 1, despite of the presence of a high level of IFN-α in the pleural effusion, no IFN-α was detectable in the serum and that in Case 2, although the level of IFN-α was low, a higher level of IFN-α appeared in the serum than in the pleural effusion after intrapleural injection of OK-432. Findings in Case 2 may indicate leakage of OK-432 or the transport of IFN-α from the pleural cavity to the blood stream. As already stated, Case 1 had an interferon level of 445 IU/ml in the pleural effusion as measured with the biological assay system, i.e. FL vs. VSV system, and this activity increased to 625 IU/ml after administration of OK-432. This activity was much higher than the IFN-α level estimated with an IFN-α RIA kit. The discrepancy in the titers may have been caused by the presense of IFN-β or IFN-γ in the pleural effusion, but because no international reference human IFN-γ or antibody against human IFN-γ is available, detailed analysis of the compositions of IFN in the pleural effusion was not possible. We are now trying to analyse the types of IFN using anti-human IFN-α and anti-human IFN-β.

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