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Letter to the Editor

An assay method for interferon alpha

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Dear Sir,

Naito et al. (1983) reported on a method for the interferon assay using trypsin (type IX No. T-0134 from porcine pancreas, Sigma). We also reported that the interferon alpha level in the body fluids of rabbits which had been administered with interferon alpha could be assayed using the trypsin method (Naito et al., 1984). During the development of the assay method for interferon alpha using trypsin, it was found that interferon alpha lost its sensitivity to the trypsin method. In order to solve this severe problem, various probabilities were examined. The interferon alpha sample used in the present study was obtained from the Kyoto Prefectal Red Cross Blood Center. The interferon alpha used in the previous reports (Naito et al., 1983 and Naito et al., 1984) were from Human Leucocyte Interferon (Hu-Inf. alpha), Lot 35-411, 1×10^6 U in a vial, and produced on March 18, 1981. A vial of the same lot was stored in a frozen state, but the interferon content was zero when assayed using trypsin in December, 1984. Interferon might be inactivated when stored for a long period of time in a frozen state. The slope of the calibration curve of the interferon Lot 424-2, 1 vial, 1×10^6, Hu-Inf alpha, produced on December 26, 1983, was approximately half of that obtained by Naito et al. (1983) when assayed in June, 1984. Then, the interferon level in the Hu-Inf alpha, Lot 35-428, 1 vial 3×10^6 U, produced on November 16, 1984, was assayed in December, 1984 using the trypsin method immediately after opening the vial, and it was found to be zero. In order to determine the cause of the decreasing content of interferon in the plasma, we asked the Red Cross to send us any information concerning interferon in the blood preserved at the Red Cross Blood Center, and we had the following reply: the number of molecular species in newer lots tended to decrease, and newer lots of plasma contained less species of interferon, but purer ones, as was found by bioassays. Thus, trypsin-sensitive interferon alpha molecular species might have been decreased during the purification procedures in the recent products. On the other hand, trypsin-sensitive interferon alpha might have contained contaminants in the preparation.

Hayashi et al. (1979) reported that anti-viral activity was found in the plasma of mice after the glycyrrhizin preparation was i.p. administered. Their procedure was repeated in the present study, and the interferon-like substances in the plasma of mice was assayed using the trypsin method (Fig. 1).

It was found that even when only a physiological saline solution was injected i.p. in mice, an interferon-like substance sensitive to the trypsin method was produced, though in a minute amount, and when glycyrrhizin was injected, the amount of

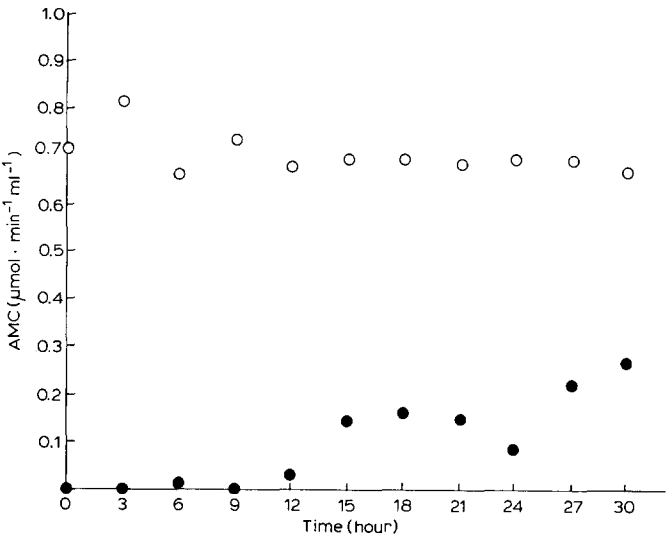

Fig. 1. Interferon-inducing activity of glycyrrhizin in mice. Key: ○, intraperitoneal administration of normal saline solution; ●, intraperitoneal administration of glycyrrhizin. The data corresponding to each sampling time after the administration of a normal saline solution were deducted from all observed values.

AMC at the corresponding time interval, obtained by subtracting the control (saline solution) from the glycyrrhizin group, showed a gradual two-peak phase pattern. Such a two-peak pattern did not agree with the pattern obtained by Hayashi et al. (1979), and it might be due to the different methods of the interferon assay. However, if interferon was induced by the administration of glycyrrhizin, the thus induced interferon can be assayed using the trypsin method. An accurate examination may enable us to ascertain why interferon-like substance assayed using the trypsin method appeared in the plasma of mice by the i.p. injection of a physiological saline.

It was found that the trypsin-sensitive interferon-like substance disappeared from the plasma in a frozen state within 4 days. Ebina et al. (1981) reported that when OK-432 was injected i.p. in mice, the interferon production was found in a two-peak pattern after 15 and 35 h after the administration. In the present study, their procedure was followed, and interferon-like substances induced in plasma by OK-432 were assayed using the trypsin method. Interferon-like substances did not appear in the plasma 15 h after the administration, and 0.123 nmol/min/ml AMC was produced 35 h after the administration. The difference might be due to the method of assay in both cases.

In conclusion it may be said that glycyrrhizin and OK-432, which are known to induce interferon alpha in animals, induced interferon-like substances which could be assayed using the trypsin method. Thus, it may be assumed that the trypsin method can be applied to interferon assay, and the method does not show any

impurity but only some molecular species of interferon can be assayed using the trypsin method.

Early interferon could be applied clinically in a wide spectrum of diseases probably because of its impure or multi-molecular compositions. However, as interferon has been purified recently, the scope of application has been narrowed, and some molecular species may not be effective to so far effective symptoms, pharmacologically. We are going to attempt to characterize trypsin-sensitive interferon molecular species in connection with their pharmacology.

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