WHOLE BLOOD TNF-α PRODUCTION AS A SENSITIVE MEASURE FOR IMMUNOTOXICITY OF ANTICANCER DRUGS

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ABSTRACT — The immunotoxicity of anticancer drugs has been measured with whole blood TNF-α production induced by LPS-stimulation. Cis-platinum (CDDP) or 5-fluorouracil (5-FU) was given intravenously to mice once a day for three days. The amount of produced-TNF-α lowered significantly in CDDP 2 mg/kg and 5 mg/kg, while the number of leukocytes scarcely changed even when a significant decrease in body weight was observed at the dose of 5 mg/kg. On the other hand, 5-FU 20 mg/kg also caused a significant decline in the amount of produced TNF-α but it showed no apparent effects on the number of leukocytes and the body weight. These results indicate that LPS-induced TNF-α production is a more sensitive measure than leukocyte count used conventionally to detect the immunotoxicity of two anticancer drugs, CDDP and 5-FU, and suggest that it might be a more practical index for hazard identification in clinical treatment with anticancer drugs.

KEY WORDS: Whole blood TNF-α production, Measure of immunotoxicity, Anticancer drugs, CDDP, 5-FU, More sensitive measurement

INTRODUCTION

The immunotoxicity of anticancer drugs in human use is increasingly recognized as a potential hazard, as the drugs cause infection easily (Buchheidt et al., 2004), interstitial pneumonia (Kurokawa et al., 2001) and reduced-anticancer immunity (Gudewicz, 1988)

Thus, the drugs are generally accepted to show severe immunotoxicity (Graziano et al., 1999) as an undesirable effect which reduces their clinical efficacy. Therefore, for better medical treatment effect in anticancer therapy, it is important to detect drug-induced immunotoxicity early with a more practical assay. However, leukocyte count, a conventional clinical measurement for immunotoxicity, has a disadvantage in that sensitivity for hazard identification is low (Denecker et al., 1997), (Lokich et al., 2003), (Kawaguchi et al., 2005). In this study, the immunotoxicity of two anticancer drugs, cis-platinum and 5-fluorouracil, has been measured in mice with whole blood TNF-α production to discuss its significance for hazard identification in treatment with anticancer drugs.

MATERIALS AND METHODS

Animals

ICR male mice were purchased from Nihon SLC (Hamamatsu, Japan). The animals were housed for at least 7 days in this laboratory after their arrival. Constant temperature and humidity (22±1°C, 55±10%) were maintained with a fixed 12-hr light-dark cycle and free access to food and water. Guiding principles for the care and use of laboratory animals approved by The Japanese Pharmacological Society were followed in this study.

Compounds

Anticancer drugs cis-platinum (II) diamine dichloride (CDDP, Sigma Chemical Co. St. Louis, U.S.A.) and 5-fluorouracil (5-FU, Kyowa Hakko Kogyo Co. Ltd., Tokyo, Japan) were dissolved in saline at appropriate concentrations.

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Blood samples
CDDP (2 mg/kg, 5 mg/kg) or 5-FU (5 mg/kg, 20 mg/kg) was administered intravenously once a day for three days to ICR mice (10 weeks age) weighing 35-45 g at the volume of 5 ml/kg. Saline was administered for the control mice. Blood sample containing sodium heparin (15 IU/ml), (Mochida Pharmaceutical Co., Ltd., Tokyo, Japan) was taken 22 hrs after final dosing using a heparinized syringe.

TNF-α production induced by lipopolysaccharide (LPS)
Blood sample (0.3 ml) was added to a tube (LPS content < 0.03 EU/tube, Sekisui Chemical Co. Ltd., Osaka, Japan) for blood collection in which LPS (derived from E. coli055B5, List Biological Laboratories, Campbell, CA) saline (0.3 ml) is contained beforehand. The tube was incubated at 37°C mixing gently. Four hrs later the tube was centrifuged at 1,600×g for 10 min. The TNF-α content in the supernatant was measured with ELISA kit (Factor-Test-X Mouse TNF-α ELISA KIT, Genzyme Co. Ltd., Massachusetts, USA). The amount of TNF-α produced by LPS stimulation was determined by the difference from the TNF-α content of the sample without LPS addition.

Leukocyte count
The number of leukocytes in a blood sample was determined by a method of automated cell counting (Sysmex K-1000, Sysmex Co. Ltd., Kobe, Japan).

Statistical analysis
Statistical analysis was performed with Welch’s t-test (Ichihara, 1990) and p value <0.05 was considered to be significant.

RESULTS AND DISCUSSION
The time course change of TNF-α production induced by LPS-stimulation was examined first. TNF-α production was already detected 1 hr after incubation of LPS-additive whole blood. The amount of produced-TNF-α increased with incubate time, showing plateau level 4-6 hrs after incubation as well as results reported in a human blood model (Suzuki et al., 2002).

Fig.1 shows a dose-dependent curve of LPS-induced TNF-α production. LPS-additive whole blood was incubated for 4 hrs. The amount of produced-TNF-α increased in a dose-dependent manner in concentrations of 0.5-500 ng/ml, showing an exponential increase between concentrations of 5 to 50 ng/ml. Hence, in a follow-up experiment to study the immunotoxicity of anticancer drugs, whole blood was incubated for 4 hrs with LPS (20 ng/ml) and then the level of TNF-α in separated plasma measured.

Table 1 shows the amount of produced-TNF-α, the number of leukocytes and the body weight of when CDDP or 5-FU was given intravenously to mice once a day for three days. In CDDP 2 mg/kg and 5 mg/kg, the amount of produced-TNF-α was lowered significantly in comparison to the saline, while the number of leukocytes scarcely changed even when a significant decrease in body weight was observed at the dose of 5 mg/kg. On the other hand, 5-FU 20 mg/kg caused a significant decline in the amount of produced TNF-α, although it showed no apparent effects on the number of leukocytes and the body weight. At a dose of 5 mg/kg, the drug had no significant effects on the TNF-α production.

Most anticancer drugs prevent tumor growth by blocking the multiplication of tumor cells. They also act suppressively on the cell of the normal marrow system with a rapid cell multiplication cycle like tumor cells, and as a consequence damage immunity function (Gudewicz, 1988) to cause a decline of cancer immunity preventing tumor growth (Multhoff et al., 1996). Thus, the drugs originally have a double-edged sword in terms of preventing tumor growth and causing reduced-anticancer immunity. In addition, if the immunological host defense declines, it will become easy to concur with infection (Buchheidt et al., 2004). Therefore, controlling the immunotoxicity of anticancer drugs is very important for cancer therapy and development of a more sensitive test for hazard identification is considered to contribute greatly to better medical treatment with anticancer drugs.

In this study, TNF-α production was shown to be more sensitive than leukocyte count for detecting the immunotoxicity of two anticancer drugs, CDDP and 5-FU. The leukocyte count used widely as a clinical test appears to be a low-sensitive measure for detection of immunotoxicity induced by anticancer drugs. Indeed, the number of leukocytes scarcely changed even when CDDP 5 mg/kg caused a significant decrease in body weight. These results indicate that TNF-α production might be a practical index for detecting an immunotoxicity in clinical treatment with anticancer drugs. This postulation is supported by a few reports (Denecker et al., 1997), (Langezaal et al., 2001) citing the advantages of TNF-α production for detection of immunotoxicity. In addition, further experiments would dem-
TNF-α production as a measure for immunotoxicity of anticancer drugs.

Demonstrate the practicability of this index in long-term dosage of anticancer drugs and in the recovery phase. Although unclear in detail, it might be an explanation for detection sensitivity being better that TNF-α production probably from macrophages in whole blood cells is a primary response in the immune system followed by activation of humoral and cellular immunity (Jansky et al., 2003). In a subsequent experiment for

![Graph showing dose-dependent curve of TNF-α production induced in mice by LPS.](image)

**Fig. 1.** Dose-dependent curve of TNF-α production induced in mice by LPS.

**Table 1.** Immunotoxicity of CDDP and 5-FU in mice.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Amount of TNF-α produced (ng/ml)</th>
<th>No. of leukocytes (×100/μl)</th>
<th>Change in body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>1.31 ± 0.36</td>
<td>40.2 ± 10.6</td>
<td>0.1 ± 0.5</td>
</tr>
<tr>
<td>CDDP (2 mg/kg)</td>
<td>0.63 ± 0.26**</td>
<td>38.3 ± 24.4</td>
<td>0.5 ± 1.3</td>
</tr>
<tr>
<td>CDDP (5 mg/kg)</td>
<td>0.58 ± 0.27**</td>
<td>43.7 ± 18.2</td>
<td>-1.9 ± 0.7**</td>
</tr>
<tr>
<td>5-FU (5 mg/kg)</td>
<td>1.54 ± 1.02</td>
<td>67.0 ± 25.7</td>
<td>0.8 ± 0.6</td>
</tr>
<tr>
<td>5-FU (20 mg/kg)</td>
<td>0.89 ± 0.22*</td>
<td>42.2 ± 25.9</td>
<td>-0.6 ± 0.7</td>
</tr>
</tbody>
</table>

Each figure indicates the mean ± S.D. of six animals (three animals for TNF-α response in 5-FU 20mg/kg). * p<0.05 : significant relative to the saline, ** p<0.01 : significant relative to the saline.
more understanding, it would be important to determine if the decline in the amount of TNF-α produced depends on a numerical fall of leukocytes or not. As reported previously, we have developed a more validated, simple human in vitro test for whole blood TNF-α production (SEK-5001 test) (Kobayashi et al., 2002). The SEK-5001 test might be an useful tool for early detection of immunotoxicity induced by anticancer agents. As reported, an anticancer drug, irinotecan, caused a decrease of LPS-induced TNF-α production in this system prior to leukopenia in patients with malignant tumor (Kawaguchi et al., 2005).

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REFERENCES


