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Establishment of Immunotoxicology Evaluation Procedures for Pharmaceuticals

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ABSTRACT *: The Japan Pharmaceutical Manufacturers Association, with the cooperation of the Japan Association of Contract Laboratories for Safety Evaluation, launched a collaborative study with 38 companies aimed at elucidating the correlation between histopathological/hematological findings and immune function. Seven substances were individually administered to Crj : CD (SD)IGS rats for 14 or 28 days. Their immunotoxicity was assessed by histopathology, hematology, plaque-forming cell assay, enzyme-linked immunosorbent assay of serum antibody to sheep red blood cells, and flow cytometry. Appropriate procedures for immunotoxicology evaluation of pharmaceuticals were considered.*

Key Words *: Immunotoxicology, Evaluation procedures, Pharmaceuticals, Histopathology, Hematology, Immune function*

I. INTRODUCTION

Due to the complexity of the immune system, immunotoxicity must be evaluated from several aspects (Luster *et al.*, 1988; Vos and Van Loveren, 1994). In the course of safety evaluation of pharmaceuticals, histopathological and hematological data are obtained through repeated dose toxicity studies and offer some information concerning immunotoxicity. These data have been used for immunotoxicology evaluation, but many methods to test for immune function have become available. Therefore, the relationships between histopathological/hematological findings and immune function need to be re-examined.

II. METHODS

1. Substances

Many paradigms of correlation between histopathological/hematological findings and immune function are possible. We therefore used as many substances as possible in the present study, including diphenylhydantoin, promethazine hydrochloride, nortriptyline hydrochloride, indomethacin, haloperidol, propranolol hydrochloride and fluorouracil.

2. Animals

Crj:CD(SD)IGS rats, which are used in about 60% of the participating laboratories of pharmaceutical companies for repeated dose toxicity studies, were chosen for the present study. Some of them were kindly offered to each laboratory by Charles River Japan Inc. Male rats were used in this study because they do not have the estrus cycle and their belligerence can be averted by keeping one or two rats in a cage under good laboratory practice standards.

3. Study Design

The vehicle or three doses of each test substance were administered to Crj:CD(SD)IGS rats for 14 or 28 days. Each dose group consisted of eight animals. As a positive control, cyclophosphamide was administered to five or eight animals. One day after the last administration, the animals were anesthetized to collect lymphoid tissues for histopathology, plaque-forming cell (PFC) assay and flow cytometry, and blood samples for hematology, and serum sample for

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enzyme-linked immunosorbent assay (ELISA). Some animals were immunized with sheep red blood cells (SRBC) 4 day before removing the spleens for PFC assay, and 6 days before collecting serum samples for ELISA.

4. Histopathology and Hematology

The thymus, spleen, lymph node and bone marrow were observed by microscopy using slides stained with hematoxylin and eosin, and diagnoses were made using standardized criteria for histopathology. Red blood cell count, white blood cell count, differential white blood cell ratio, hematocrit and hemoglobin were included in the hematological examination.

5. Immune Function Tests

From a wealth of immune function tests, we chose assays of the production of antibodies to a T-cell dependent antigen such as PFC assay and ELISA of serum antibody to SRBC. Several subsets of immune competent cells, i.e. antigen-presenting cells, helper T cells and B cells are involved in antibody production to a T-cell dependent antigen. Even if only one of these subsets is functionally damaged, the specific antibody may not be produced. Therefore, we set these assays as a first priority for the immune function test. In addition, flow cytometric analysis of lymphocyte subsets in the thymus and spleen were performed with cells stained with fluorescein isothiocyanate (FITC)-labeled anti-rat CD3 for mature T cells (G4.18; Pharmingen), FITC-labeled anti-rat CD4 for helper T cells (W3/25; Serotec), R-phycoerythrin-labeled anti-rat CD8 for cytotoxic/suppresser T cells (OX-8; Serotec), FITC-labeled anti-rat CD45RA for B cells (OX-33; Serotec), or FITC-anti-rat NKR-P1A for NK cells (10/78; Pharmingen). NK cell activity could not be determined by assays of the production of serum antibody to T-cell dependent antigens. With the antibody recognizing NK cell surface antigen, flow cytometric analysis could serve as a quantitative assay of NK cells. Although flow cytometry is not a function test *per se*, it can impart information on the immune function when activation markers are defined.

III. ARISING ISSUES

Several data have been obtained and are now under analysis.

The present collaborative study has highlighted various issues, such as the strain and diet of rats and the duration of administration. The strain we chose is not an inbred stain of rats. When the serum antibody titers to SRBC between Crj:CD(SD)IGS rats and F344:DuCrj rats were compared by ELISA, the standard deviation was higher in the former than the latter. A higher standard deviation may affect the sensitivity of the assay. However, administration of cyclophosphamide used as a positive control significantly suppressed the antibody production. Some extent of immune suppression was detected using an outbred strain of rats. Another issue was the fact that the participating laboratories use many types of diet for Crj:CD(SD)IGS rats. The effects of the ingredients of animal diet on immune function need to be considered. Finally, during the course of the present study, we decided to attempt immunotoxicology evaluations for administration periods shorter than 28 days.

IV. CONCLUSIONS

The present study has revealed several types of correlation between histopathological/hematological findings and immune function. Another result has been the adaptation of several new methods for immunotoxicology by the various laboratories. The present study should provide insights for the establishment of immunotoxicology evaluation procedures for pharmaceuticals, which is the final goal of this collaborative study. Finally, immunotoxicolgy evaluation procedures for pharmaceuticals are being discussed and developed in different parts of the world (Hastings, 1998; Kim *et al.*, 2000; CPMP/EMEA, 2000), and we hope that an international approach can be realized on this issue in the near future.

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