Short Communication

COMPARISON OF TWO GUIDELINES ON IMMUNOTOXICITY TESTING OF MEDICINAL PRODUCTS*

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Despite the lack of formal immunotoxicity testing guidelines, the assessment of immune function has been a routine component of toxicity testing for over twenty years. The European Agency for the Evaluation of Medicinal Products (EMEA) and The US Food and Drug Administration (FDA) have recently adopted new guidelines for immunotoxicity testing of new medicinal products. These two guidelines are compared in this article.

KEY WORDS: *drugs, immunotoxicology, regulatory guidance, tiered testing approach*

Abbreviations: CDER - Center for Drug Evaluation and Research; CPMP - Committee For Proprietary Medicinal Products; EMEA - European Agency for the Evaluation of Medicinal Products; FDA - Food and Drug Administration; ICH - International Conference of Harmonization.

The use of immunologic endpoints in toxicologic assessment of drugs and chemicals has led to the development of immunotoxicology as a scientific discipline (1). Immunotoxicology is defined as the study of the adverse effects of occupational or therapeutic exposure to chemical or biological material on the immune system. Immunotoxicology was born in the late 1960s and early 1970s, and in the mid to late 1980s it was already in full bloom (2, 3, 4). The First International Conference on Immunotoxicology was held in 1983. In the 1990s, regulatory agencies started to include immunotoxicology data in their risk assessment procedures (2, 5).

EMEA AND FDA IMMUNOTOXICITY GUIDELINES

The European Agency for the Evaluation of Medicinal Products (EMEA) and The US Food and Drug Administration (FDA) recently published guidelines addressing the investigation of immunotoxicity of new medicinal products.

In July 2000, the CPMP (Committee for Proprietary Medicinal Products) within EMEA adopted a new version of Note for Guidance on Repeated Dose Toxicity (6). This Note for Guidance was first adopted in October 1983 and has been updated with Guidance on Immunotoxicity.

In October 2002, the Center for Drug Evaluation and Research (CDER), Food and Drug Administration published the Guidance for Immunotoxicology Evaluation of Investigational New Drugs (7).

Both guidelines define that all new medicinal products should be screened for immunotoxic potential within the preclinical evaluation of drug toxicity (in at least one repeated dose toxicity study, generally incorporated within standard rat 28 day toxicity study). They also emphasise a weight-of-evidence approach to immunotoxicity evaluation as opposed to implementing a standard set of tests to be conducted with every investigational drug. Both guidelines recommend tiered testing approach as the optimal strategy for the assessment of medicinal

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products. Figure 1 shows the algorithm of Tiered testing and neither applies to either biotechnology-derived medicinal products or vaccines.

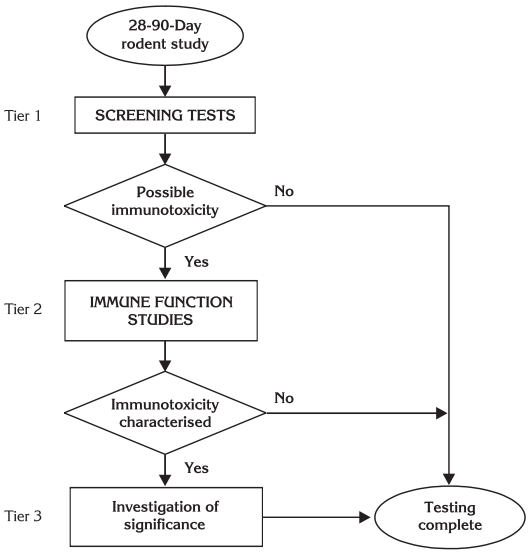

Figure 1 *Algorithm of tiered testing approach in immunotoxicity testing of medicinal products*

There are some differences in recommended parameters that should be evaluated in the initial screening phase (Tier 1, Figure 1) between the EMEA and FDA guidelines. The FDA guidelines recommend determining serum biochemical markers such as globulin levels in the initial screening phase. However, decreased basal serum globulin level is a relatively insensitive indicator, because under normal circumstances the immune system should be challenged with antigen and a particular antibody response evaluated to detect immunosupression. On the other hand, EMEA recommends additional functional parameters such as the distribution of lymphocyte subsets and NK-cell activity as a part of the screening phase. If these two are unavailable, the initial screening phase should be completed with the primary antibody response to a T-cell-dependent antigen (e.g. sheep red blood cells). Phenotyping and the NK-cell activity assay can be incorporated in the existing 28-day animal study but T-cell-dependent antibody response assay requires an additional subset of animals.

The T-cell-dependent antibody response assay is described in the FDA guideline as the best general-purpose functional assay, and it is recommended

Table 1 *List of parameters recommended for observation in the screening phase according to EMEA and FDA guidelines on immunotoxicity*

	EMEA	FDA
INITIAL SCREENING PHASE	Haematology Lymphoid organ weights (thymus, spleen, draining and distant lymph nodes) Microscopy of lymphoid tissues (thymus, spleen, draining and distant lymph nodes, Peyers patches) Bone marrow cellularity Distribution of lymphocyte subsets NK-cell activity If later two are unavailable: primary antibody response to a T-cell dependent antigen (e.g. sheep red blood cells) should be done	Haematology Lymphoid organ weights (thymus, spleen, draining and distant lymph nodes) Microscopy of lymphoid tissues (thymus, spleen, draining and distant lymph nodes, Peyers patches) Bone marrow cellularity Globulin levels

Table 2 *List of specialized assays defined in FDA guideline on immunotoxicity*

MEDICINAL PRODUCT	ADDITIONAL ASSAYS REQUIRED
TOPICAL APPLICATION	Sensitising potential (Guinea pig maximization test (GPMT), Buehler assay (BA), The murine Local lymph node assay (LLNA))
INHALANT	Respiratory sensitising potential (IgE response in mice following dermal exposure, serum cytokine patterns induced by topical exposure, LLNA)
INDICATED FOR HIV INFECTION	Additional immune function studies as a part of the standard nonclinical assessment
FOR USE IN PREGNANT WOMEN	Effect of drug on immune system in F1 offspring in reproductive toxicology (Developmental immunotoxicity)
ACCUMULATED IN IMMUNE SYSTEM TISSUES	Additional immune function studies as a part of the standard nonclinical assessment

as additional study (Tier 2, Figure 1). It should be performed if the results from screening phase demand further studies.

Table 1 compares the lists of parameters that should be observed in the screening phase according to the EMEA and FDA guidelines.

Additionally, the FDA guidelines define that, in certain cases, medicinal products may require specialised assays to assess their immunotoxic potential. These assays are listed in Table 2. This is something that is not specified in the EMEA guideline.

CONCLUSION

There is a great debate going on between toxicologists as to what is necessary to be compliant with both EMEA and FDA regulations as well as what studies are required (5). It seems that there is a need for the European Community, the USA, and Japan to harmonize approaches in immunotoxicity testing, either within the context of the International Conference of Harmonization (ICH) or outside this process.

However, guidelines are not mandatory, they are a starting point rather than an end point. Immunotoxicity testing requires scientific flexibility. No guideline can provide sufficient information to cover all possible cases; all persons involved should be willing to discuss and consider variations in testing strategy according to the state of art and ethical standards in animal experimentation. Emphasis on the objectives of the study rather than on adherence to a rigid protocol allows the design of a test programme which will make it appropriate for the tested compound.

The objectives of immunotoxicity testing should be the identification of potential target organs, characterisation of toxic effects, identification of dose response relationship, potential reversibility, and identification of parameters for clinical monitoring.

At the end of immunotoxicity testing, toxicologists should be able to answer several "simple" questions: What is happening in animals? Why is it happening? When (in what dose range)? How does it apply to humans?

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Sažetak

USPOREDBA SMJERNICA O ISPITIVANJU IMUNOTOKSIČNOSTI LIJEKOVA

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KLJUČNE RIJEČI: *imunotoksikologija, lijekovi, pristup ispitivanju u koracima, smjernice*

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