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Discussion on Improvement of Toxicological Pathology Study

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Toxicological pathology plays a key role in drug safety assessment. To enhance the research level of toxicological pathology, the following studies should be carried out urgently: setting up a standard operation procedure (SOP) for toxicological pathology assessment; emphasizing on immunotoxicology evaluation; adopting a new experiment model of replacement, featuring high speed and reliability; introducing new techniques and new models in toxicological mechanism research; and establishing a new appraisal system to screen innovative drug and rapid and high precision methods for early security assessment, detection and measurement.

Key words drug safety assessment, toxicological pathology, immunotoxicology evaluation, short-term replacement model, highthroughput toxicology

1 The Status of Toxicological Pathology

Toxicology can be defined as the study of the damage, pathogenesis and systemic influence after the exposure of drug or chemicals. Toxicological pathology can provide much information that is important for toxicology. For animals are used in most toxicity tests, Toxicological pathology is essential and necessary to find out drug-induced lesions, confirm the target sites of drug, and explore the etiology in toxicity tests, such as acute toxicity tests, chronic toxicity tests, teratogenic tests, and carcinogenic tests. Furthermore, the longer the test is, the more important is toxicological pathology. Drug safety assessment involves in various contents such as the toxicity dosage, safety effect-toxicity ratio, target organs of agents, the persistence period of toxic effect, accumulated toxicity, the structure-toxicity relationship, etc. However, ultimately it is toxicological pathology reports that offer fundamental evidence and descriptions for all toxicity tests. Toxicopathology assessment can determine the target sites, degree, character and prognosis of drug-induced injures, and consequently afford main warrant for toxicity studies. Thus, toxicological pathology is a fundamental component in the whole toxicology studies and determines the level of drug safety evaluation.

The Good Laboratory Practice for Non-clinical Laboratory Studies (shortened as GLP) was first issued in 1994 in China for running-test, and then was modified and issued again in 1999. Therefore, certain experts point out that as the late beginning of GLP and the absence of specialist members, many problems are present in toxicological pathology in China. For instance, there is no identified standard in the clarification, description and qualitative evaluation on lesions at cellular and tissues level. There are no standardized pathological terms either. As a result, drug assessor of government cannot exactly understand what kind of disease has been induced after the exposure of drugs even they have the pathology reports in hand. Another case is that some toxicological pathology workers design experiment carelessly, so the results cannot combine with the characters of the test, and the results from different levels have no internal relations and eventually the conclusion cannot be taken. All the situations mentioned above reflect the absence of precisely and exactly standard evaluation systems in toxicological pathology in China.

Undoubtedly, these disadvantages can be overcome by setting up a standard operation procedure (SOP) for toxicological pathology assessment, complying with the rules strictly, and improving the skill of toxicopathology members. Moreover, toxicological pathology needed improving eagerly, so that it can keep pace with the rapid international development.

2 To Emphasize on the New Tendency in Immunotoxicology Evaluation

Toxicopathology study was considered as a traditional and classic science, but it still need improving

with the speedy development in sciences and technologies. For further analysis of target organs or tissues, fully understanding toxicity mechanism, acquiring general and reliable safety information and subsequently improving drug safety assessment, toxicopathology should combine with other associated subjects such as biochemistry, immunology, molecular biology and toxicological genomics, to introduce a variety of new methods and techniques.

Recently a new tendency, emphasizing on immunotoxicity evaluation, appeared in drug safety evaluation and toxicological pathology assessment. Especially in the chronic toxicity tests, noticing the abnormity of immunity organs and tissues, and subsequently basing on toxicological pathology results, it can be determined whether immunotoxicity tests need to be performed and evaluated. Immunity system is important for organism to resist infection and tumors. The immunity system response to drug is mainly shown as depression or stimulation of immunity function, anaphylactic reaction, and development of autoimmune diseases. The disorder of immunity function may result in numbers of serious diseases. When drug depresses immunity function, many kinds of life-threatening disease, including infection, tumors, AIDS, etc. would present.

Interest has been focused on immnotoxicology evaluation since the early of 1970's. However, the development of immunotoxicology evaluation system was prevented at that time for the complicated interaction between cells comprising immunity system, the diversity of methods used for immunotoxicology evaluation and the uncertainty of immunotoxicology results from different methods. It is in recent 10 years that the system has been gradually progressed with the development of science and technology and the co-operation of international organizations. Europe and America have advanced the guidance for immunotoxicology evaluation, and Japan has instituted a corresponding protocol[1]. It has been one of the most interested issues to be emphasized on and build up the examination index and technique plate in drug safety evaluation and toxicological pathology study. Therefore, corresponding guidance in immunotoxicology evaluation should be established as soon as possible in China, so that we can keep pace with the rapid international development.

3 To Establish Animal Models of Replacement for Rapid Toxicity Detection

Detecting the carcinogenic potential of drug at the early stage of drug research and development is very important in drug safety assessment and toxicological pathology study. In 1997, experts pointed out in the ICH conference on carcinogenic evaluation that using mice to examine the non-genomic carcinogen in long-term carcinogenic tests would make pseudo-positive and pseudo-negative results. Therefore rats, another rodent animals, and a rodent short-term replacement model should be used to evaluate the carcinogenic potential of drug. Drug carcinogenic evaluation system may be established in China by building up the rat medium-term hepatocarcinoma model (initiation/promotion model) and combining it with rat long-term carcinogenic tests. It is well known that most natural or synthesized chemicals are metabolized in the liver, especially the non-genomic ones. Liver is one of the most important target organs, so the hepatocarcinoma model system is undoubtedly significant to rapidly detect the carcinogenic potential of drug and other substances, and subsequently to define the genomic or non-genomic carcinogens. Such work has been preceded abroad, and it is reported that 291 chemicals have been evaluated with this model and the positive percent of hepatocarcinoma is 90% (57/63), genomic and non-genomic carcinogens are 97% and 84% respectively. The model can also detect the carcinogen, co-carcinogen, anti-carcinogen and other agents such as natural products, food additives and environmental pollutant, etc.[2]. Compared with other evaluation systems, the standard medium-term hepatocarcinoma model system can detect rapidly and exactly. Besides, the model can simultaneously study carcinogenesis mechanism of drug and chemicals at cellular and molecular levels, aided by advanced methods and techniques, especially on the suspected, potential carcinogens such as growth factors[3], and P450 isoenzyme[4]. Moreover, the model can also detect the changes at protein and gene level. Therefore, the establishment of this model system provides a technological plate for further studying carcinogenesis mechanism systemically and can be used widely.

4 To Introduce New Techniques into Carcinogenesis Mechanism Study

Confocal Laser Scanning Microscope (CLSM), Laser Capturing Microdissection (LCMD) and Flow Cytometry (FC) are the most effective biological tech-

niques that developed recently. CLSM, especially combined with immunofluorescence, can exactly detect pathological changes at cellular level and even at molecular level, consequently provide credible basis for fully understanding the progression and development of toxicological effects. LCMD is a new and advanced technology combining morphology with molecular biology, by which the doubtful single cell can be dissected and directly used for RNA, DNA and proteins analysis. Therefore, it may improve the veracity and the level of toxicopathology study, and consequently provide special, sensitive, reliable and rapid information for quantitative analysis. National experts have recognized the importance of these techniques mentioned above. However, these advanced methods were rarely used in drug safety assessment or toxicopathology study. We can study the toxicity mechanism of drug, chemicals at cellular and molecular levels, and detect the changes at protein and gene level, aided by these new equipments and devices. Meanwhile the research level of toxicopathology study may be improved from qualitative analysis to quantitative analysis.

5 To Establish a High Throughput Toxicology (HTP-Tox) for Novel Drug Safety Evaluation

Nowadays many methods for early, rapid, and sensitive safety assessment are emphasized in foreign countries. In addition,in recent 2 or 3 years, many researchers performed safety assessment during drug discovery progress, although it was used underwent in pre-clinical study during drug research and development. It is during the screening of lead candidate of chemicals and the discovery and selection for lead candidate that safety evaluation is performed. HTP-Tox is aimed to increase the opportunity of success during drug discovery progress and reduce the risk and costs of drug development, consequently make drug research and development succeed early. It arrested much concentration and needed resolving urgently. Of course, it is our goal to improve the level of toxicopathology study and safety evaluation for our nation.

6 To Catch up with the International Development of Toxicopathology Studies

Toxicopathology assessment is to observe clinical situations, biochemical reactions and changes in function, metabolism as well as morphology after the exposure of drug, so it is an integrated result. The creditability of toxicopathology study relies on the correctness of each progress in drug safety assessment and the reliability of result from each level as well as is ensured by the improvement and combination of technique plate of toxicological pathology. To catch up with the international development of toxicopathology study, large amount of work should be aimed to establish a scientific, strict, systemic and intact toxicopathology assessment system with a whole standard and operation. Just as the proverb said, long distance walking begins from beneath the foot. We should do fundamental work firstly, namely standardize experiment procedures, and institute the diagnostic standard for commonly used laboratory animals, important organs and disease as well as accumulate and conclude background information of all kinds of animals and pathological changes. Then there are regulations to be obeyed and cases to be consulted for in toxicopathology study. Consequently, the veracity and reliability of toxicopathology results were improved. On this basis, mobilize national pathologists scattered in pharmacy, medicine, zoology and bromatology, then set up a technique plate that both adapt to our national facts and keep pace with the international development in toxicopathology.

Toxicopathology study began in 1960s when chemical industries presented and developed fast. Subsequently many new chemical products entered into the social environment and produced many harmful effects never heard of before. As a result, these toxic substances were noticed and provision of performing pathology evaluation in toxicity tests was instituted. In 1970s, GLP protocol was issued by FDA in America, then corresponding standard operation procedures were gradually instituted in other nations according to the safety evaluation information of USA, and consequently toxicopathology evaluation tends to be strict and complete[8]. Some countries set down general standards for toxicological pathology diagnosis and classification for commonly spontaneous diseases in laboratory animals[9]. Thus, the regulations and methods for toxicopathology assessment were gradually standardized. In the middle of 1990s, IFSTP (International Federation of Societies of Toxicology Pathology) was established. Five conferences were held so far, which activated the academic discussions and technique exchanges in toxicopathology. A series of related regulations and stan-

dards for toxicopathology evaluation were instituted under the harmonization of multination. Besides holding of anniversary meetings and publishing of periodical journals in Japan, Seminar on experimental pathology histotechnology has been held, which particularly concentrates on resolving technique problems such as sample preparation, section staining and microscopy examination, in order to increase the research level of toxicological pathology[10].

Recently, toxicopathology attracts much attention and is considered as playing a key role either in the traditional and conventional toxicity assessment or in the modern toxicity mechanism studies with advanced techniques[11, 12]. To catch up with the international development as soon as possible, therefore, we toxicopathologists should realize the important task, be farseeing, surefooted and innovative, try to work together to improve our domestic drug safety evaluation to a new height.

References

[1] Ito N. et al. Early detection of carcinogenic substances and modifiers in rats. Mutation Res., 2000, 462: 209—217.

[2] Steinmetz K. L., Klaunig J. E. Transforming growth factor- α in carcinogen-induced F344 rat hepatic foci. Toxicol. and Appli. Pharmacol., 1996, 140: 131—145.

[3] Shoda T. et al. Liver tumor-promoting effect of β-naphthoflavone, a strong CYP 1A1/2 inducer, and the relationship between CYP1A1/2 induction and Cx32 decrease in its hepatocarcinogenesis in the rat.Toxicol. Pathol., 2000, 28: 540—547.

[4] Pitot H. C., Dragan Y. P. Chemical carcinogenesis. In "asarett and Doull's Toxicology The basic science of poisons" 6th ed. (C. D. Klaassen Ed.). USA McGraw-Hill companies, Inc. 2001: 241—320.

[5] Nakamura W. ICH meeting about immunotoxicology. The 29th Japanese Society of Toxicology, 2002: 177.

[6] Roman D. et al. Laser technologies in toxicopathology. Toxic. Pathol., 2002, 30 (1): 11—4.

[7] Drews J. Drug discovery today-tomorrow. Drug Discovery Today, 2000: 2—4.

[8] Paget G. E., Thomson R. Standard operation procedure in pathology.Inveresk Research International Ltd, 1979: 1—7.

[9] Greavers P. Histopathology of preclinical toxicity studies: Interpretation and relevance in drug safety evaluation, Elsevier Science Publishers B. V., The Netherland, 1990: 7—11.

[10] Boorman G. A. et al. Toxicogenomics, drug discovery, and the pathologist. Toxicol. Pathol., 2002, 30 (1): 15—27.

[11] Pilling A. M. The role of the toxicologic pathologist in the preclinical safety evalution of biotechnology-derived pharmaceuticals. Toxicol. Pathol., 1999, 27 (6): 678—88.

[12] Ito N. The new toxicopathology, Japan Nakayama Publisher, 2002, 175—180.

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[10] Shen J., Min Z. Q., Gu J. C. A new type of material test system-the true tension-compression triaxial facility. In: Strength Theory. New York, Beijing: Science Press, 1998, 551—556.

[11] Yu M. H. Twin Shear Theory and Its Application. Beijing: Science Press, 1998, (in Chinese) 834.

[12] Jiang J. J. Nonlinear Finite Element Analysis of Concrete Structures. Xi'an: Shaanxi Science and Technological Press, 1994.

[13] Shen Z. J. Summary on the failure criteria and yield functions (English Abstract), Chinese J. of Geotech. Eng., 1995, 17 (2): 1—9.

[14] Shen Z. J., Yu M. H. Summary on the failure criteria in deviatoric and meridian plane. In: Strength Theory, New York, Beijing: Science Press, 1998, 61—68.

[15] Yu M.H., He L. N., Song L.Y. Twin shear stress theory and its generalization, Scientia Sinica (Sciences in China), English Edition, Series A, 1985, 28 (11): 1174—1183.

[16] Shen Z. J. Theoretical Soil Mechanics (in Chinese). Beijing: China Hydraulic Power Press, 2000.

[17] Zhang X. Y. Plasticity for Geomaterials (in Chinese). Beijing: Transportation Press, 1993.

[18] Yu M. H., He L. N. A new model and theory on yield and failure of materials under complex stress state. In: Mechanical Behavior of Materials-VI, eds. M. Jono and T. Inoue, Vol, 3, Oxford: Pergamon Press, 1991, 841—-846.

[19] Yu M. H. New System of Strength Theories (in Chinese). Xi'an: Xi'an Jiaotong University Press, 1992.

[20] Yu M. H. Unified Strength Theory and Its Applications. Berlin: Springer-Verlag, 2003.

[21] Yu M. H. Concrete Strength Theories and Its Applications (in Chinese). Beijing: Higher Education Press, 2002.