



Workshop 3.6 Establishing the 3Rs principle in Japan (JSAAE-Workshop)

Lecture

Optimisation of the h-CLAT (human Cell Line Activation Test) protocol and inter-laboratory validation study

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There have been a number of attempts to develop non-animal alternative methods for skin sensitisation testing, and one of the major approaches is to evaluate phenotypic and functional changes of dendritic cells (DCs) derived from human peripheral blood or cord blood. However, the effects of chemicals on the surface phenotype of DCs differ depending on the source of the cells, which is undesirable for a routine test. To overcome this problem, we have evaluated several human cell lines (e.g. THP-1; monocytic leukemia cell line). The aims of this study are as follows: 1. to optimise each step of the test, including culture time, antibody selection and effect of FcR blocking, and 2. to

conduct an inter-laboratory validation study in order to confirm the robustness of the test. Based on the findings, the protocol for this assay was optimised. We used the optimised protocol to evaluate nine chemicals in an inter-laboratory validation study. Expression of CD86 and CD54 on the cells was measured after 24 h and 48 h exposure to six known allergens (e.g. DNCB, pPD, NiSO₄) and three non-allergens (e.g. SLS, Tween 80). The results indicate that a battery test involving measurement of CD86 and CD54 expression on THP-1 cells treated with test chemical for 24 h would be a useful *in vitro* skin sensitisation model.



Lecture

Skin irritation: Validation of 3-dimensional skin model

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Validation committee in the Japanese Society for Alternatives to Animal Experiments (JSAAE) has validated skin toxicity studies using a 3-dimensional cultured human skin model (skin model) commercially available in Japan. We used this material to screen the skin toxicity of various agents to anticipate the result of human patch testing, although this use differs from hazard identification of chemicals in the ECVAM validation study.

Since 2000, we have performed these pre-tests using 3 models (TESTSKIN™: TOYOBO Co. Ltd. and Vitrolife-Skin™: Gunze Co. Ltd. and EPI-200 (EpiDerm™): Kurabo Industries Ltd.). In this pre-validation study, 19 laboratories participated excluding the kit suppliers. Three chemicals were selected, coded and supplied to each laboratory. These results were presented at WC4 in New Orleans and contributed to the Alternative to Animal Testing and Experimentation (AATEX).

Furthermore, we performed a validation study using skin model. At first, we validated an alternative test to corrosivity with the ECVAM protocol using Vitrolife-Skin™ and EPI-200. In this validation, 6 laboratories participated and tested 12 chemicals. As the next step, we validated an alternative to skin irritation test with ET₅₀ protocol using TESTSKIN™ and with ET₅₀ and PI (Post-Incubation) protocol using Vitrolife-Skin™. In these validation studies, each of 9 laboratories participated and tested 41 and 14 chemicals. These models have been evaluated in JaCVAM in order.

These results show that the skin model is adequate for evaluating corrosivity and may be useful for evaluating skin irritation, the reliability of which was similar to that of animal testing.

Lecture

Educational issues of 3Rs in Japan

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The scientist in Japan begins also to recognise the importance of 3Rs in the biomedical education as a social concern for laboratory animals welfare rises.

Science Council of Japan proposes that it is necessary to educate the 3Rs about the animal experiment as part of the bioethics education at the university involved in the biomedical education and research. Moreover, to strengthen the animal welfare further, it was proposed to take 3Rs to the law though the guideline for animal experiment was established at each univer-

sity, and an independent restriction by the institutional animal care and use committee was done.

Therefore, 3Rs will be taken to animal protection act of Japan, Law For The Humane Treatment and Management of Animals, which the revision will shortly be scheduled.

In this presentation current state of education about animal experiment involved in 3Rs will be introduced based on questionnaires from medical school in Japan.



Lecture

Establishment of JaCVAM and welcome to WC6 in 2007/Tokyo

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Japanese Society for Alternatives and Animal Experiments (JSAAE) was established in 1982 by Tsutomu Sugawara et al. and has been contributing to the research on alternative methods (AM), validation of AM, education about 3R, and communication with the public. By co-operation with research group supported by MHLW and Japanese Cosmetic Industry Association (JCIA), JSAAE conducted validations of *in vitro* cytotoxicity tests, eye irritation tests, skin irritation tests, skin corrosivity tests, and phototoxicity tests. Based on these efforts, Japanese government approved the establishment of new section in National Institute of Health Sciences for the management and evaluation of AM and for the international co-operation in this field. This section is small. However, we consider that it is a good start. We named the section, Japanese Center for the

Validation of Alternative Methods (JaCVAM). It will open in this Autumn.

We are very pleased to host the 6th World Congress on Alternatives and Animal Use in the Life Sciences (WC6) in 21-25 August, 2007 in Tokyo. WC6 is also sponsored by ACT and Japanese Science Council and co-sponsored by Japanese Society of Toxicology, Japanese Society of Laboratory Animal Sciences, The Japanese Environmental Mutagen Society, Japanese Society for Laboratory Animal and Environment, and International Society of Toxicology and International Society on Toxicology. We are expecting the meeting will contribute to the developments and acceptance of new AM and activate further research on AM.

Lecture

Phototoxicity: 3T3 NRU PT and proposing a new battery system

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Several *in vitro* phototoxicity methods have been developed to assess the phototoxic potentials of substances. These can be classified into two groups: the methods for screening purposes and tests focusing on the specific mechanisms of phototoxic reactions. Among these methods 3T3 Neutral Red Uptake Phototoxicity Test (3T3 NRU PT) was accepted as an established alternative method by ECVAM. In the year 2004, 3T3 NRU PT was adopted as OECD guideline for chemicals (OECD Test Guideline TG432). In Japan, Evaluation Committee of Japanese Society of Alternatives to Animal Experimentation in co-operation with NIHS carried out the peer review of 3T3 NRU PT in order to verify the propriety as a method for regulatory acceptance. We confirmed that the 3T3 NRU PT is a good screening method to predict phototoxicity potentials. However

there are some limitations to adopt the substances in this assay. It is difficult to evaluate water insoluble substances clearly because of the cell culture system. In order to improve this problem, a new battery system with the yeast growth inhibition phototoxicity assay (Yeast assay) and the red blood cell photohemolysis assay (RBC assay) was proposed from a Japanese industry. The inter-laboratory validation study of this assay was performed using nine test substances. The correlation with *in vivo* data of the battery system was better than those of the single use of each assay. The other results and the future investigation of this assay and our recent studies using 3T3 NRU PT will be discussed.



Lecture

Skin sensitisation: Human cell line activation test (h-CLAT) using THP-1 cell. The relationship between CD86/CD54 expression and THP-1 cell viability

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Several *in vitro* skin sensitisation methods using human cell lines have been reported. In our previous study, we optimised our human cell line activation test (h-CLAT) using THP-1 cells (monocytic leukaemia cell line) and conducted an inter-laboratory study. We found that measuring CD86/CD54 expression may be useful for predicting skin sensitisation at the concentration with a certain level of cytotoxicity. The aim of this study was to confirm the relationship between CD86/CD54 expression and viability of THP-1 cells in the h-CLAT. In this study, twenty allergens (e.g. DNCB) and nine non-allergens (e.g. SLS) were evaluated. For each chemical, more than 10 concentrations that gave a predicted cell viability range of 20-95% were used. The data showed that:

1. Expression patterns of CD86/CD54 differed depending on chemical. For the most allergens, some cytotoxicity

(70-90% cell viability) was needed for enhancement of CD86/CD54 expression.

2. The criteria "CD86 \geq 150 or CD54 \geq 200" resulted in an accuracy of 93% which confirms appropriate cut-off criteria for h-CLAT.
3. A dose setting of serial 1.2-fold dilutions based on CV75 (estimated dose of 75% cell viability) may provide a good prediction of allergens.
4. A good correlation was observed between EC3 of LLNA and EC150 (CD86) or EC200 (CD54) of h-CLAT. So EC150 or EC200 may be used as an estimate of allergic potency (EC150/200: Estimated Concentration required to induce 150% or 200% CD86/CD54 expression).

These findings suggested that h-CLAT would be a more robust *in vitro* skin sensitisation test.

Lecture

The activities of JSAAE – past, present and future

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The Japanese Society of Alternatives to Animal Experiments (JSAAE) was established in 1982 as an academic society, it now has more than 300 memberships and 13 supporting organisations. The main objective of the JSAAE is to promote alternative research and development, and education in alternatives to animal experimentation based on the Three Rs concept. The outcome of such activities is presented in annual meeting and official journal of Alternatives to Animal Experimentation (AATEX) in the JSAAE. In the last two years, we have performed and planned several validation studies such as skin irrita-

tion assay using three-dimensional skin model, battery system of phototoxicity assay using erythrocytes and yeast, and an alternative of the LLNA assay without radioactive compound. The JSAAE encourages young scientists by presentation of awards in annual meeting and excellent articles contributed in journal of AATEX. As part of JSAAE activities, we are responsible for promoting a global view of the Three Rs concept to other Asian partners and we are trying to exchange information and technical transfer to South Korea and China.