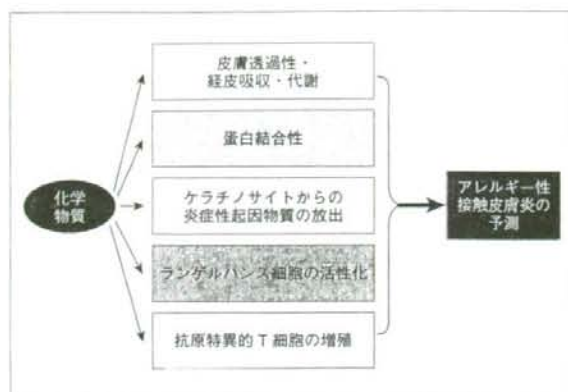

図2 アレルギー性接触皮膚炎の感作誘導における重要な作用機構

ならば，これらの試験を組み合わせたスキームの構築が重要であると考えている[16,17]．

① *in silico*

感作性物質の構造活性部位はほぼ明らかになってきており，専門家であれば，構造をみればある程度の感作の有無を予測できよう．Aptulaらはこれを統計学的な手法などによって検討を行い[18]，Ensleinらもデータベースを活かして感作性強度を判断してきた[19]．これらの文献を数値化したソフト(DEREK[20]やTOPKAT[21]など)が多数販売されており，かなりの精度で感作性の強度予測が可能である．ただし，データベース数(ヒトやモルモットの結果が揃っているデータ)がこれ以上増える可能性が低いこと，すべての化学物質が純品でなく，不純物が感作性を示す場合もあり，ここで陰性の結果を得たからといって以下の試験を省略できるわけではない．あくまでも有用な第一次スクリーニングである．

② ペプチド結合試験

感作性物質がハプテンに結合すること，特定のアミノ酸構造に結合することを利用して，ペプチド，とくにシステインをもつグルタチオンや合成ペプチドとの結合による挙動の変化をHPLC（高速液体クロマトグラフィ）で測定するものである．Katoら[22]，Gerberickら[23]，中村[24]の検討がなされている．Gerberickらの検討によれば[25]，システインやリジンペプチドとの反応性で得られた結果とLLNAの一致率は89%であり，4段階の強度分類も可能とされており，スクリーニング法として有用であるとされている．

③ 培養細胞を用いる試験

皮膚のなかで最初に感作性物質に曝露させる細胞はケラチノサイトである．皮膚に浸透された感作性物質はハプテンとしてケラチノサイトに結合し，サイトカインやケモカインを産生して感作源となる．ケラチノサイトを用いた感作性のスクリーニングはVandebrielらの検討があるが，あまり多くない[26,27]．

むしろ，アレルギーといえばランゲルハンス細胞の挙動であろう．以前から，ランゲルハンス細胞の活性に着目した検討がなされたが[28,29]，試験法を開発するうえで十分な細胞を確保できないという課題があった[30]．そこで，ランゲルハンス細胞起源であるヒト末梢血や臍帯血の細胞を樹状細胞に分化させる方法が検討されてきた．バイオマーカーとしては，MHC Class Ⅱ，CD86などの表面抗原[31-33]，TNF-α[34]，IL-1β[35]，IL-8[36]などのサイトカイン，CXCR4[37]，CCR7[38]，CCL2[39]などのケモカインおよびケモカインレセプターなどが報告されている．

しかし，細胞誘導に時間を要することや経費の問題などに加え，同一ドナーから入手できる細胞数に限界があり，かつドナー間の反応性に相違があることから，試験法の構築には支障が大きかった[31,35,40]．そこで，安定供給や個体差のない単球系の細胞株(CD14陽性のTHP-1細胞[33,41]，U937細胞[42,43]，CD34陽性のKG-1細胞[44]，MUTZ-3細胞[45]など)を用いた検討が主流となっている．

これらのなかで，資生堂と花王のグループがTHP-1細胞に着目し，その表面抗原のなかで，CD86およびCD54の変化を指標とした試験法を確立した[46,47]．この試験法は96 wellプレートを用いてフローサイトメトリーで解析を行う簡便な方法であり，ヒト細胞株活性化試験(human Cell Line Activation Test：h-CLAT)と命名されている．2施設間の比較試験では，高い一致性が報告されており，LLNAとの一致率も62物質で90%ときわめて高い．そこで，厚生労働科学研究の支援を受けた化粧品企業や欧州化粧品工業会との間で共同研究が実施され，技術移転の容易性や施設間再現性が検討されている状況である．本試験方法はバリデーションに移行すべき段階に来ていると考えられる．

④ 培養皮膚モデルを用いる試験

Coquetteらは，3次元培養表皮モデルに感作性物質を適用して，ケラチノサイトの放出するIL-1αやIL-8

を調べ，感作性物質とIL-8の関係を報告している[48]．

また，樹状細胞を導入した3次元培養表皮モデルによる感作性物質の挙動の報告もある[49, 50]．ランゲルハンス細胞の培養がむずかしく，免疫担当細胞を導入したモデルの開発は進んでいなかったが，幹細胞研究やヒト細胞の利用が進む昨今，このモデルの研究がより進むと考えられる．h-CLATなどのヒト樹状細胞株を用いたこれまでの成果が本モデルの構築に反映されれば，より精度の高いモデルの開発が期待される．本モデルは，種々の濃度や製剤の形態で感作性物質を適用し，その後のサイトカインやケモカインの挙動を検索できるという点で用量反応性や曝露評価に利用できる可能性が高く，リスク評価モデルとして期待されている．

おわりに

JaCVAMの立場は欧米との国際協調を重視して新規の動物実験代替法を確立することである．これはグローバリゼーションが進む昨今，避けては通れない課題である．だからといって，化粧品や化学物質の安全性評価の質を下げるべきではない．安全性の担保が第一優先である．昔のような化粧品による黒皮症を再燃させるわけにはいかない．黒皮症は原因究明に当たられた皮膚科医の努力と[51]，その後行政や業界が導入したモルモットを用いた試験法により激減したわけであり[52]，この安全性の質を担保しなければならい．安全性を担保しながら代替法の国際協調を進める．"出口があって実はないかもしれない"この問題を解く鍵は，「これまでにない技術」による新試験法の開発であると信じている．そのためには，基礎研究の充実が欠かせない．専門家の研究から素晴しい成果が得られることを期待してやまない．

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P&G ACTIVITIES RISK COMMUNICATION

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Perspectives on Validation and Regulatory Acceptance of Animal Alternative Testings in Japan

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From the Editor

Recent scientific advancement in understanding of toxic mechanisms enables risk assessors to develop new and revised toxicological test methods that better predict potential toxic effects of chemicals. In this issue, we update current status of research activities in the development of animal alternative methods that may refine, reduce, or replace animal use for toxicity testing. Contributors to this issue include Dr. Kojima at NIHS, Japan, and Dr. Scott Belanger, environmental toxicologist and Dr. Pauline McNamee, human safety toxicologist at P&G.

Seok (Soga) Kwon, Ph.D.

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In November 2005, Japanese Center for the Validation of Alternative Methods (JaCVAM) was established in the Division of Pharmacology at the National Center for Biological Safety and Research affiliated to National Institute of Health Sciences (NIHS) in Japan. JaCVAM facilitates the validation, peer-review, and international harmonization of alternative methods. Key objectives of JaCVAM are: 1) to facilitate 3R's* with Reduction and Replacement prioritized and 2) to ensure new test methods are validated, peer reviewed, officially accepted by the regulatory agencies, and internationally harmonized. JaCVAM has its own steering committee which is in charge of JaCVAM activities. JaCVAM facilitates validation studies and peer-review process for new methods. JaCVAM also proposes a validated method to the regulatory agencies for their acceptance.

JaCVAM's current activities and future directions on the validation and peer review of alternative to animal testings will be discussed in this paper. JaCVAM plays an important role in evaluating alternative test methods to be used in the safety assessment of cosmetic products. A new EU directive prohibiting *in vivo* tests on cosmetic ingredients (the 7th Amendment, Council Directive 2003/15/EC) [1] also engaged JaCVAM to actively support the validation of test methods.

The major activities of JaCVAM include:

1) Coordinate the technical evaluation and regulatory acceptance of new and revised test methods
2) Delegate validation work for new and revised test methods
3) Promote 3R's and facilitate international harmonization of alternative to animal testings

JaCVAM is currently coordinating validation studies and peer-review for several test methods (shown in Table 1). Most of the test methods are to be used in the safety assessment of cosmetic products. In Japan, there is a unique cosmetic product category called "Quasi-Drug (QD)" which requires pre-market registration approved by Japan government prior to their introduction to markets. Thus, JaCVAM is also involved in the

*3R's : Refinement, Reduction, Replacement

technical review of the overall safety evaluation process for QD products, which utilizes alternative test methods. In 2007, a new committee called "Review committee on the safety assessment for QD products" was established with participation of representatives including dermatologists, experts from cosmetic industries and Japanese Society for Alternatives to Animal Experiments (JSAAE). This committee is conducting a 3-year research project funded by Ministry of Health, Labor, and Welfare (MHLW) to provide technical guidance on the safety evaluation of QD products. The project progress is to be updated in 2009 when the 7th Amendment in EU becomes effective. Specific activities on the validation of alternative test methods on major toxicity endpoints related to cosmetic products in Japan will be discussed.

1) Skin Irritation

In April 2007, the European Center for the Validation of Alternative Methods (ECVAM) Scientific Advisory Committee [ESAC] endorsed the EPSKIN method employing cultured epidermis models (EPISKIN™)[2]. This endorsement triggered Japanese researchers to accelerate their validation activities. Japanese researchers have been using commercially available cultured skin/epidermis models such as TESTSKIN™ (Toyobo), Vitrolife-Skin™ (Gunze), and EpiDerm™ (MatTek and Kurabo). Pre-validation studies have been completed for these models with favorable results[3]. TESTSKIN™ model[4] and Vitrolife-Skin™ model[5-6] were further assessed for validation studies to be considered as alternative test methods for predicting skin irritation of cosmetic ingredients at their usage levels. These two skin models successfully completed validation studies. The results showed that their predictive capability of skin irritation is equivalent to those of Human Patch Test (HPT) and *in vivo* primary skin irritation test. However, these methods have not completed the peer-review process in Japan. Japanese manufacturers are planning to validate the Japanese skin models in the spring of 2008 by employing reference substances used by EU for the EPISKIN™ and currently seeking technical collaboration with JSAAE.

2) Eye Irritation

In 1998, the guidance for alternative methods on eye irritation utilizing a cytotoxicity method[7] was established[8]. This work was funded by MHLW. However, this guidance has not been widely utilized in Japan. For example, this guidance was not referred in "Questions and Answers (Q&A) attached to the QD manufacturing and marketing approval application and cosmetics criteria amendment request" issued in 2006[9]. This suggests that the cytotoxicity method has not been recognized as a validated alternative test method by the regulatory agencies. Therefore, JaCVAM is to conduct the technical evaluation of a cytotoxicity method by reviewing validation study results generated by

Toxicological endpoint	Test methods	Current activities
Phototoxicity	Testing Battery (Yeast-RBC): Yeast Growth Inhibition Phototoxicity Assay Red Blood Cell Photochemolysis Assay	Peer review in progress
Skin sensitization	Local Lymph Node Assay using ATP contact measurement (LLNA-DA)	Peer review in progress
	Local Lymph Node Assay using BrdU uptake measurement (LLNA-BrdU)	Validation in progress
	Human Cell Line Activation Test (h-CLAT)	Planning
Corrosiveness	Culture model	Regulatory acceptance in progress
Skin irritation	Culture model	Planning on peer review
Endocrine disrupter	Lumi-cell, CER-estrogen reporter assay	Validation in progress
Mutagenicity	Comet assay (*in vivo* or *in vitro*)	Validation in progress

Table 1. Current status of validation and peer review for animal alternative methods coordinated by JaCVAM

JSAAE, MHLW Scientific Research, EU and USA. In EU and USA, however, the review panels have completed the peer-review process for Isolated Eye Test methods, Isolated Corneal Test method, and Hen's Egg Test[10]. And the panels concluded that these methods could be used for screening purpose. JaCVAM is also planning to conduct the peer-review of these test methods.

3) Phototoxicity

In vitro 3T3 Neutral Red Uptake (NRU) phototoxicity test has been peer reviewed and accepted as a reliable alternative method in Japan[11]. This method had been previously adopted by OECD[12]. On the other hand, Shiseido developed a test battery including Yeast Membrane Disruption assay and Red Blood Cell Hemolysis test[13-15]. At Shiseido's request, a review committee of MHLW Scientific Research assessed the validity of these test methods. The test methods were further refined in response to results of the initial validation, and they have undergone the 2nd validation process. The peer-review of these methods is in progress and their regulatory acceptance will be assessed in 2008.

4) Skin Sensitization

The Local Lymph Node Assay (LLNA) can be used as an alternative method because it has been already adopted by OECD[16]. However, the use of radioisotopes (RI) limits the practical application of LLNA by Japanese laboratories. Thus, other methods involving no RI are currently under the validation process. They include LLNA-DA method (ATP content index)[17] and LLNA-BrdU method (BrdU uptake index)[18]. The LLNA-DA method showed favorable results of validation studies and was further progressed to the peer review process. The LLNA-BrdU method is in the process of 2nd validation with protocol modification in response to the 1st validation. In future, these test methods are expected to be widely utilized in Japan. New test methods are also continuously being developed for the safety assessment of cosmetic products because LLNA still requires animal use. Therefore, Shiseido and Kao developed h-CLAT (human Cell Line Activation Test) method[19,20] in collaboration with industry associations such as Japan Cosmetic Industry Association (JCIA) and the European Cosmetic Toiletry and Perfumery Association (COLIPA). The scientific validation for this test method is expected to occur. The Structural Activity Relationships (SAR) analysis could be used for predicting skin sensitization in future considering significant technical advancement in computer modeling areas. In addition to h-CLAT method, other new test methods are also being developed. They include peptide binding assay and cell culture methods. JaCVAM will coordinate validation studies and peer-review of these new test methods.

This paper described current status and future directions of the validation and regulatory acceptance of alternative to animal testings focusing on major toxicity endpoints related to cosmetic products in Japan. JaCVAM also coordinates technical evaluation of alternative methods for other toxicological endpoints such as acute toxicity and mutagenicity. Detailed information on these endpoints will be discussed in future. In collaboration with international organizations, significant progress has been made in the validation of alternative methods. JaCVAM's mission is to facilitate the validation of alternative methods while domestically developed test methods are prioritized.

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JaCVAM: An organization supporting the validation and peer review of new alternatives to animal testing

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Abstract

In November 2005, the Japanese Center for the Validation of Alternative Methods (JaCVAM) was established as part of the Division of Pharmacology at the National Center for Biological Safety and Research, affiliated with the National Institute of Health Sciences (NIHS) in Japan. The key objectives of JaCVAM are: 1) to ensure that new or revised test methods are validated, peer reviewed, and officially accepted by regulatory agencies, and 2) to expand international cooperation on alternatives to animal testing. This paper describes in further detail JaCVAM's current activities and future direction.

Keywords: validation, peer review, alternative, JaCVAM

1. Introduction

In November 2005, the Japanese Center for the Validation of Alternative Methods (JaCVAM) was established as part of the Division of Pharmacology at the National Center for Biological Safety and Research, affiliated with the National Institute of Health Sciences (NIHS) in Japan. One mission of JaCVAM is to promote practice of the 3Rs (Reduction, Refinement and Replacement) in the area of animal testing, with Reduction and Replacement prioritized in Japan. Key objectives of JaCVAM are: 1) to ensure that new or revised test methods are validated, peer reviewed, and officially accepted by the regulatory agencies, and 2) to expand international cooperation on alternatives to animal testing. The main activities of JaCVAM will focus on the following missions and objectives: 1) coordination of peer review and regulatory acceptance of new and revised test methods; 2) support of validation work for new and revised test methods; and 3) promotion of the 3Rs and 4) working to promote international partnership surrounding the issue of alternative methods.

2. Organization of JaCVAM and supporting groups

JaCVAM has five members: a director, Dr. Hajime Kojima; a visiting researcher, Dr. Mitsuteru Masuda; a researcher, Miss Shoko Arai; and two secretaries. Though JaCVAM is a small organization, it is supported by several groups, as shown in Fig. 1. Firstly, JaCVAM has its own steering committee, which is charge of JaCVAM activities. This committee is comprised of six members: Dr. Toru Inoue (Chair: Director of the National Center for Biological Safety and Research in NIHS), Dr. Yasuo Ohno (Vice President of NIHS, and a founder of JaCVAM), Dr. Kenichi Nakazawa (Head of the Division of Pharmacology), Dr. Mitsuteru Masuda (JaCVAM), Dr. Hiroshi Itagaki (Shiseido Co., Ltd., President of Japanese Society for Alternative to Animal Experiments: JSAAE) and Dr. Hajime Kojima (JaCVAM).

The roles of the JaCVAM steering committee are:

- ◆ To prepare the validation or peer review process of new or revised test methods.
- ◆ To evaluate international cooperation.
- ◆ To select advisory board members.
- ◆ To select peer review panel members.
- ◆ To select regulatory acceptance board members.
- ◆ To consult with NIHS support members.
- ◆ To check JaCVAM plans and reports.

Secondly, JaCVAM (and its steering committee) have an advisory board which monitors its activities. This board is comprised of eight members, including one dermatologist and other individuals belonging to the Japanese Society of Toxicology (JST), the Japanese Association for Laboratory Animal Science, the Japanese Society for Alternative Animal Experiments (JSAAE), the Japanese Pharmaceutical Manufacturers Association (JPMA), the Japanese Cosmetic Industry Association (JCIA), the Ministry of Health, Labour and Welfare (MHLW), and the Animal Welfare Network. These members are reelected every two years. The meeting of the advisory board is held at NIHS twice per year. JaCVAM reports on its activities

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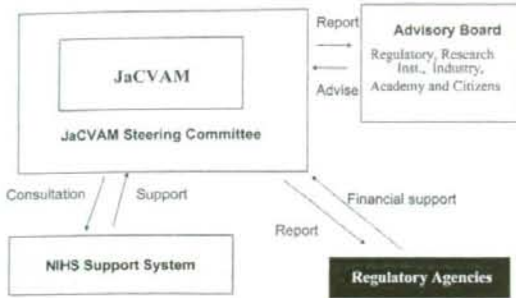

Fig. 1. Organization of JaCVAM

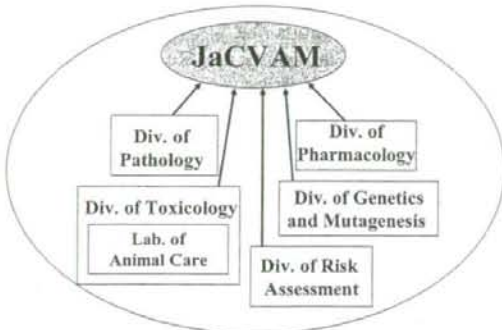

Fig. 2. NIHS Support System

and future programs to board members, and in return receives advice on JaCVAM's methods of operation.

Thirdly, JaCVAM is supported financially by the MHLW. The specific source of funding for JaCVAM's development and validation of alternative methods is the MHLW's Government Pension Investment Fund.

Finally, many laboratories at the NIHS, as shown in Fig. 2, assist the JaCVAM with scientific consultations. NIHS conducts research on the quality, safety, and efficacy of pharmaceutical products, foods, and chemicals in the environment. JaCVAM benefits from the large number of specialists working at the NIHS.

3. Organization for validation studies

In Japan, new or revised test methods that are recommended by domestic researchers or developers are validated by an applied science society like the JSAAE, the Japanese Environmental Mutagen Society (JEMS), or the Japanese Society for Dermatoallergology and Contact Dermatitis (JSDCD). A Validation Management Team, which consists of researcher of a particular toxicity, a specialist on validation studies, a biostatistician, and a study manager from each participating laboratory, validates the repeatability and reliability of the new or revised

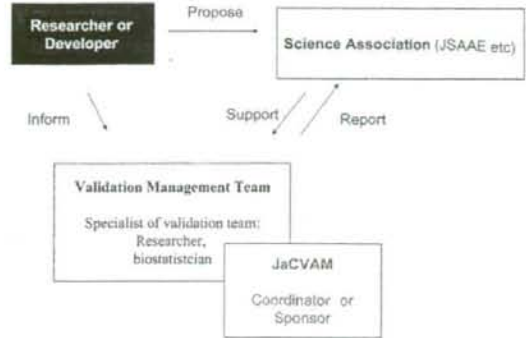

Fig. 3. Framework for Validating Alternative Methods

method according to OECD Guidance No.34 (OECD,2005) and the National Institute Health (NIH) report (ICCVAM, 1997). JaCVAM participates in this process as a specialist in validation studies, and is responsible for selecting participating laboratories, managing test materials (initial selection, blinding of test substances, and distribution to each laboratory), managing data (data sheet preparation and data collection) and providing financial support. The above details are illustrated graphically in Fig. 3.

4. Organization for peer review and regulatory acceptance

As shown in Fig. 4, JaCVAM has a framework for peer review and regulatory acceptance of alternative methods. After JaCVAM has received a request for peer review from a researcher or developer, the JaCVAM steering committee meets to deliberate on the proposal methods; this should take no longer than three months. Upon the receipt of permission for peer review, JaCVAM organizes the oversight committee in order to evaluate a new test method. The roles of this ad hoc committee are to collect references on the toxicity involved in a new test method, to prepare a draft report, to propose a new validation study, and to provide advice on further research related to the proposed test method. Based on the report and

Fig. 4. Framework for Peer Review and Regulatory Acceptance of Alternative Methods

references prepared by the oversight committee, a peer review panel evaluates a new or revised test method. The roles of this ad hoc panel consist of the principles and criteria as laid out in OECD guideline No. 34. The members of the oversight committee and the peer review panel assigned to evaluate a new test method are selected by the JaCVAM steering committee. They include five or six specialists selected from academia, industry, the JaCVAM regulatory acceptance board, and the NIHS supporting system.

JaCVAM and its steering committee have a regulatory acceptance board for new or revised methods. This board is comprised of ten members who are either biostatisticians or dermatologists or who are delegates of JST, JSAAE, JPMA, JCIA, the Pharmaceuticals and Medical Devices Agency (PMDA), or the NIHS. These members are reelected every two years. The meeting of this board is held at the NIHS on a case-by-case basis if necessary. This board reviews new or revised test methods based on the reports of the peer review panel and prepares a report and statement on the test method for regulatory agency.

5. International coordination

Significant progress has been made in validating alternatives to animal testing in collaboration with international organizations. Particularly important is the JaCVAM cooperation with the European Center for the Validation of Alternative Methods (ECVAM), and NICEATM (NTP Interagency Center for the Evaluation of Alternative Toxicological Methods)/ICCVAM (Interagency Coordinating Committee on the Validation of Alternative Methods). These three centers are closely united and this may increase international cooperation. Together, our three organizations validated and peer reviewed a new test method which has been approved by OECD guidelines.

Table 1. Current validation and peer review conducted by JaCVAM

Test method	Material	Current activities
Photoxicity	Yeast-RBC	Peer Review in progress
Skin sensitization	LLNA-DA	Peer Review in progress
	LLNA-BrdU	Validation in progress
	h-CLAT	Planning
Corrosivity	Culture model	Regulatory acceptance in progress
Skin irritation	Culture model	Planning on Peer Review
Endocrine disrupter	Lumi-cell, CER-estrogen reporter assay	Validation in progress
Mutagenicity	Comet assay (in vivo or in vitro)	Validation in progress

6. Conclusion

JaCVAM is currently coordinating validation studies and peer review for several test methods (shown in Table 1). Most of the test methods are to be used in the safety assessment of cosmetic products. JaCVAM's goals are to facilitate the validation of alternative methods with domestically developed test methods and to conduct peer review of these and internationally certified test methods, and promote practice of the 3Rs in the area of animal testing to accomplish our mission.

References

1. OECD (2005) OECD Series on testing and assessment Number 34, Guidance document on the validation and international acceptance of new or updated test methods for hazard assessment, ENJ/JM/MONO 14.
2. ICCVAM (1997) Validation and regulatory acceptance of toxicological test methods: a report of the ad hoc Interagency Coordinating Committee on the Validation of Alternative Methods. NIH Publication No: 97-3981, National Institute of Environmental Health Sciences (NIEHS).

ORIGINAL ARTICLE

Validation of human skin models for skin corrosivity tests in Japan

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Abstract

As shown in OECD test guidelines 430 and 431, the human skin epidermal assay and Transcutaneous Electrical Resistance Test (TER) were validated and peer reviewed as an alternative method to corrosivity testing; however, these methods have not been used widely in Japan. The problems related to techniques and evaluation are not clear. Therefore, we performed a validation study of EPI-200 (EpiDerm™), a 3-dimensional cultured epidermal model and Vitrolife-Skin™, a 3-dimensional cultured skin model made in Japan as a catch-up validation trial of alternatives for skin corrosivity testing using 13 chemicals including a positive control: 10% potassium hydroxide solution in Japan. From the obtained data, we identified the potential of utilizing these models to evaluate the corrosivity of a chemical.

***Key words:** Skin corrosivity, cultured epidermal model, cultured skin model, validation*

Introduction

Over the last decade, the European Centre for the Validation of Alternative Methods (ECVAM) has supported formal validation studies using *in vitro* tests as a replacement for the *in vivo* rabbit test for predicting skin corrosivity (Botham, et al.,1995, Barratt, et al., 1998, Fentem et al., 1998, Liebsch et al., 2000). As a result, two new test methods for skin corrosion, which incorporates a rat skin transcutaneous electrical resistance assay (TER) and two human skin epidermal assays, were included in Annex V of Directive 67/548/EEC in mid-2000, thereby making the use of *in vitro* alternatives for skin corrosivity testing of chemicals mandatory in the European Union (EC, 2000). As human epidermal model assays, two methods based on commercial human epidermal models, EPISKIN™ (EPISKIN, Chaponost, France) and

EpiDerm™ (MatTek, Ashford, MA, USA), were also endorsed.

Meanwhile, the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) in the USA prepared final recommendations on these methods for their consideration and acceptance where appropriate (NIH Publication No.02-4502; ICCVAM, 2002). As a result, these assays were published as an alternative method to corrosivity testing as shown in the OECD test guidelines 430(OECD 430; 2004), and 431(OECD 431; 2004).

In Japan, these methods have not been widely used. The problems related to techniques and evaluation are not clear. In the present study, therefore we performed a catch-up validation trial to evaluate skin corrosivity using the human epidermal and skin models, that is, evaluations were made based on the ECVAM experimental protocol.

We performed a validation study of EPI-200 (EpiDerm™), a 3-dimensional cultured epidermal model and Vitrolife-Skin™, a 3-dimensional cultured skin model as validation trials of alternative for skin corrosivity testing in Japan. From the obtained data, we investigated the possibility of utilizing these models to evaluate the corrosivity of a chemical. We may suggest using these models to the ad hoc. committee of toxicology at MHLW in Japan.

Materials and Methods

Study management and organization

The study was performed according to the Japanese Society for the Alternative to Animal Testing Experiments (JSAAE) validation scheme as shown in Fig.1. The chairman was Dr. I Yoshimura at the Fac. Eng. Tokyo Univ. Science, who is head of the validation committee in JSAAE. Dr. Ohno at the National Institute of Health Sciences (NIHS) prepared the protocol and supported this validation with a grant from MHLW. Six Laboratories joined the study as shown in Table 1, and a blind trial with 13 chemicals including a positive control (10% potassium hydroxide solution) was performed using the protocol. In addition, Dr. Y. Ohno, the chemical distributor, coded and distributed the test chemicals to be used in the blind trial. After submission of all coded data to biostaticians, an independent biostatistical analysis of the blind trial was performed at the Fac. Med. Kyoto Univ. and Fac. Eng. Tokyo Univ. Science. The study director at each laboratory, a chemical distributor, biostaticians and kit suppliers were organized into study management teams in this validation assay as shown in Fig. 1. Finally, the chairman reported the outcome of this validation and forwarded this report to JSAAE.

Technical transfer and preliminary tests

The management team performed the technical transfer by kit suppliers at NIHS, Tokyo on January 28, 2004. After that, technicians performed the preliminary test using 10% potassium hydroxide solution and benzalkonium chloride 10 % solution. A qualified technician from each laboratory participated in the technical transfer and the preliminary

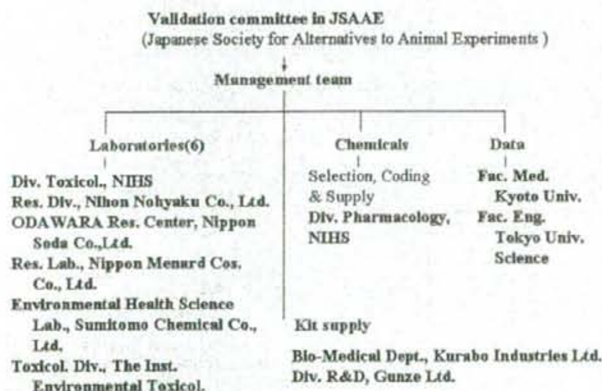

Fig.1 Organization of the validation

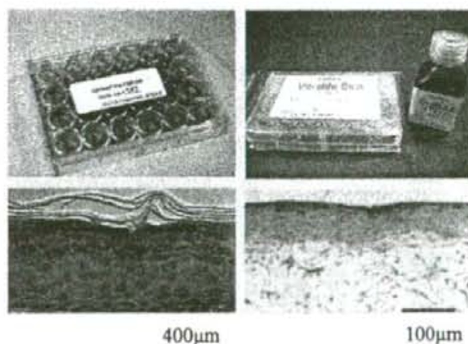

Fig.2 EpiDerm™ **Fig.3** Vitrolife-Skin™

test. All technicians obtained good results in this test.

Cultured epidermal and skin models

EpiDerm™ (EPI-200) models were purchased from KURABO Corporation (Osaka, Japan) as kits containing 24 models as shown in Fig.2, with sufficient amounts of Dulbecco's modified Eagle's medium (DMEM)-based assay medium, and phosphate-buffered saline (PBS) solution. These kits are made by MatTek Corporation (Ashland, MA, USA). The human epidermal model consisting of an epidermis with cornified layers was prepared as previously described (Liebsch et al., 2000).

Vitrolife-Skin™ models were supplied from Gunze Corporation Ltd. (Kyoto, Japan) as kits containing 24 models, collagen sponges without cells and sufficient amounts of DMEM-based assay medium, as shown in Fig.3. The human skin model consisting of a dermis and epidermis with cornified layers was prepared as previously described (Morikawa et al., 2002; Morota et al., 1998; Morota et al., 1999).

Materials

A total of 13 test chemicals including a positive control (10 % potassium hydroxide solution) were selected from the chemicals tested in the ECVAM skin corrosive validation study (Fentem et al., 1998, Liebsch et al., 2000). The chemical distributors selected test chemicals considering a balanced representation of the chemical classes, rate of corrosion or non-corrosion, solubility etc. from the total 60 chemicals tested in the ECVAM validation study. Test chemicals included six of which are known to be corrosive *in vivo*, six which are non-corrosive, six liquids, four solids and two powders, excluding the positive control. Each laboratory was sent the rotated 11 chemicals, including the positive control, in 13 test chemicals as shown in Table 2. Therefore, five data items from each laboratory for each chemical were obtained. All blinded test chemicals were treated as powerful drugs or poisons in each laboratory. The management team considered the minimum appropriate number of chemicals for catch up validation.

All test chemicals used were from the same batch and were purchased from Sigma Aldrich(Milwaukee, USA) and Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and were supplied to each laboratory by the chemical distributors. Phosphate-buffered saline (PBS) and isopropanol were obtained from Wako Pure Chemical Industries,

Table 1 List of members in skin corrosivity validation assay

Japanese Society for Alternative to Animal Experiments Validation Executive Committee

	Organization	Name
Chairman	Tokyo University of Science, Faculty of Engineering, Dept. Management Science	Isao Yoshimura
	National Institute of Health Science, Biological Safety Research Center, Division of Pharmacology	Yasuo Ohno

Study Director

	Organization	Name
	National Institute of Health Sciences, Biological Safety Research Center, Division of Toxicology	Tomoko Ando
	Nihon Nohyaku Co., Ltd., Research Division, Toxicological & Pharmaceutical Research Center	Katsuhiro Inagaki
	Nippon Soda Co., Ltd., Odawara Research Center, Toxicological Research Department	Mami Kuboki
	Nippon Menard Cosmetic Co., Ltd., Research Laboratories	Hajime Kojima
	Sumitomo Chemical Co., Ltd., Environmental Health Science Laboratory, Biochemistry Group	Yosuke Nakamura
	The Institute of Environmental Toxicology, Toxicology Division II, Laboratory of Immunotoxicology	Tadashi Kosaka

Kit supplier

	Organization	Name
	Kurabo Industries Ltd., Bio-medical Department	Hisashi Torishima
	Kurabo Industries Ltd., Biomedical Department	Michiru Genno
	Gunze Limited, Division of Research & Development	Noriyuki Morikawa

Coordinator

	Organization	Name
	Sumitomo Chemical Co., Ltd., Environmental Health Science Laboratory, Biochemistry Group	Naohiko Isobe
	Nippon Soda Co., Ltd., Agro Product Division, Regulatory Affairs Group	Yukihiro Kanaguchi
	National Institute of Health Sciences, Biological Safety Research Center, Division of Toxicology	Jun Kanno
	The Institute of Environmental Toxicology, Toxicology Division II	Takanori Harada
	Nihon Nohyaku Co., Ltd., Research Division, Toxicological & Pharmaceutical Research Center	Masaru Nogata
	Nippon Soda Co., Ltd., Agro Product Division, Regulatory Affairs Group	Mitsuo Hattori
	Nippon Soda Co., Ltd., Odawara Research Center, Toxicological Research Department	Yoshinobu Fujii
	The Institute of Environmental Toxicology, Toxicology Division II, Laboratory of Neurotoxicology	Sayaka Ishimine
	Sumitomo Chemical Co., Ltd., Environmental Health Science Laboratory, Biochemistry Group	Takashi Morimoto

Ltd. and 3-(4,5-dimethylthiazol-2-yl)- 2,5- diphenyltetrazolium bromide (MTT) and MTT formazan were obtained from Sigma Aldrich. They were supplied by the management team.

Methods

Chemical application procedure according to the ECVAM validation study.

The experimental steps of the method were performed according to the protocol used in phase III of the EpiDerm™ skin corrosivity test (Liebsch et al., 2000) with slight modifications. The EpiDerm™ models were equilibrated at 37°C and 5% CO_2 within one hour after receiving a kit and placed in 1 mL of DMEM-based assay medium in 6-well plates before use. If kept for a few days, it was preserved in a refrigerator. The Vitrolife-Skin™ models were placed in 250 μL of DMEM-based assay medium in 24-well plates and equilibrated for several hours'incubation (37°C, 5% CO_2) within a few days after receiving a kit. One hour before dosing, the models were transferred in 1 mL of DMEM-based assay medium to 6-well plates. Test chemicals were applied directly to the stratum corneum of two replicate models per chemical. Liquids (50 μL) were applied using a positive displacement pipette. Solids were crushed to a powder, if necessary, and 25 mg was applied using a spatula with the addition of 25μL of distilled water to ensure good contact with the surface. Two models were dosed with 100 μL distilled water as a negative control. After exposure for three or 60 min. at room temperature (15-25°C), two replicate models for each exposure time were rinsed thoroughly with PBS to remove the test chemical from the surface.

Table 2 Test chemicals

No.	Name	C/NC	Comments
1	Potassium hydroxide(10%aq)	C	Positive control
2	Sulfuric acid(10% wt)	C	
3	Octanoic (Caprylic) acid	C	
4	Sodium hydroxide(4.88%)	C	
5	Phenol	C	
6	Chromium trioxide	C	
7	Phosphoric acid	C	
8	Sodium perborate	NC	
9	Tetrachloroethylene	NC	
10	Potassium hydroxide(5% aq)	NC	
11	4-Amino-1,2,4-triazole	NC	
12	L-Lactic acid	NC	
13	Isopropanol (2-propanol)	NC	

Calculation of cell viability

The effects of the test chemicals on cell viability were determined using an MTT reduction assay. After blotting, the models were incubated in 0.3 mL (EpiDerm™) or 1 mL (Vitrolife-Skin™) of each DMEM-based assay medium containing 0.5 mg of MTT for an additional three hours at 37°C and 5% CO_2. Living cells were dyed dark-violet by the MTT reagents. After the models were washed with PBS, biopsies of Vitrolife-Skin™ models were taken using a biopsy punch (6 mm diameter), although this operation is not used in EpiDerm™ models. The biopsies were separated from the models using forceps, and placed into acidified isopropanol (2.0mL: EpiDerm™,1.0 mL: Vitrolife-Skin™), after removing excess water by placing the samples on absorbent paper. Precipitated formazan was extracted overnight at room temperature with protection from light. The absorbance of the extracts was measured at 570 nm using a UV-VIS spectrophotometer. Adequate absorbance of spectrophotometers was checked using 0.1mg/mL solution of MTT formazan prior to the validation study. Cell viability of EpiDerm™ models determined by the MTT reduction assay method was expressed as follows:

$$\text{Cell viability} = \frac{A_t}{A_c} \times 100\,(\%), \qquad (1)$$

where At and Ac are the absorbancies of the extracts when test chemicals and a negative control, respectively, are applied to the cultured skin model.

In case of Vitrolife-Skin™, additional tests using collagen sponges without cells were performed, with the potential to interfere with the MTT assay, and thus cell viability was expressed as follows:

$$\text{Cell viability} = \left(\frac{A_t - A_{bt}}{A_c - A_{bc}} \right) \times 100\,(\%), \qquad (2)$$

where At and Ac are absorbancies of the extracts

when test chemicals and a negative control, respectively, are applied to the viable Vitrolife-Skin™ model, and A_{bt} and A_{bc} are the values obtained for a blank test using a test chemical and the negative control, respectively, with a collagen sponge without cells.

Prediction models

Predictions of *in vitro* corrosiveness/non-corrosiveness were made according to the refined final prediction model (PM2) used in phase III of the EpiDerm™ skin corrosivity test (Liebsch *et al.*, 2000). Hence, chemicals that reduced cell viability to less than 50% upon exposure to the Vitrolife-Skin™ model for three min. were predicted to be 'corrosive' *in vivo*. If 3 min. exposure produced cell viability of ≥ 50%, the chemical was classified as 'non-corrosive' after a 3 min. exposure, but the same chemical was still be classified as 'corrosive' if viability after a 60 min. exposure was below 15%. The results obtained using the EpiDerm™ and Vitrolife-Skin™ models in this study were compared with the results of ECVAM validation studies using EpiDerm™ (Liebsch *et al.*, 2000) and EPISKIN ™ (Fentem *et al.*, 1998) for skin corrosivity testing.

This test was repeated twice. If different results from the two tests were obtained, a third test was performed at each laboratory and used for final judgment.

Table 3 Data from each laboratory

Chemical	Potassium hydroxide (10%), Corrosive						Sulfuric acid (10%), Corrosive					Tetrachloroethylene, Non-Corro				
Lab.	NIHS	NN	NS	NM	SC	IET	NIHS	NN	NS	NM	SC	NIHS	NN	NS	NM	IET
Blind No.	1	2	3	4	5	6	13	14	15	16	17	18	19	20	21	22
EpiDerm -test 1-	C	C	C	C	C	C	NC	NC	NC	C	C	NC	NC	NC	NC	NC
EpiDerm -test 2-	C	C	C	C	C	C	NC	C	C	C	C	NC	NC	NC	NC	NC
EpiDerm re-trial	C	C	C	C	C			C	C		C					
Judge	C	C	C	C	C	C	NC	C	C	C	C	NC	NC	NC	NC	NC
Vitrolife-Skin -	C	C	C	C	C	C	C	C	C	C	C	NC	NC	NC	NC	NC
Vitrolife-Skin -	C	C	C	C	C	C	C	C	NC	C	C	NC	NC	NC	NC	NC
Vitrolife-Skin re-	C	C	C						C							
Judge	C	C	C	C	C	C	C	C	C	C	C	NC	NC	NC	NC	NC

Chemical	Octanoic acid, Corrosive					Potassium hydroxide(5%), Non-					Sodium hydroxide(4.88%) Corrosive				
Lab.	NIHS	NN	NS	NM	IET	NIHS	NN	NS	SC	IET	NIHS	NN	NS	SC	IET
Blind No.	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37
EpiDerm -test 1-	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
EpiDerm -test 2-	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
EpiDerm re-trial		C	C				C	C							
Judge	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
Vitrolife-Skin -	C	C	NC	C	C	C	C	C	C	C	C	NC	C	C	C
Vitrolife-Skin -	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
Vitrolife-Skin re-			C				C	C				C			
Judge	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C

Chemical	4-Amino-1,2,4-triazole, Non-Corro					Phosphoric acid, Corrosive					L-Lactic acid, Non-Corro.				
Lab.	NIHS	NN	NM	SC	IET	NN	NS	NM	SC	IET	NIHS	NS	NM	SC	IET
Blind No.	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52
EpiDerm -test 1-	NC	NC	NC	NC	NC	C	C	C	C	C	NC	C	C	NC	C
EpiDerm -test 2-	NC	NC	NC	NC	NC	C	C	C	C	C	C	C	C	C	C
EpiDerm re-trial											C	C	C	C	
Judge	NC	NC	NC	NC	NC	C	C	C	C	C	C	C	C	C	C
Vitrolife-Skin -	NC	NC	NC	NC	NC	C	C	C	C	C	C	C	C	C	C
Vitrolife-Skin -	NC	NC	NC	NC	NC	C	C	C	C	C	C	C	C	C	C
Vitrolife-Skin re-											C	C	C		
Judge	NC	NC	NC	NC	NC	C	C	C	C	C	C	C	C	C	C

Chemical	Isopropanol, Non-Corro					Phenol, Corrosive					Sodium perborate, Non-Corro.					Chromium trioxide, Corrosive				
Lab.	NN	NS	NM	SC	IET	NIHS	NN	NM	SC	IET	NIHS	NN	NS	NM	SC	NIHS	NS	NM	SC	IET
Blind No.	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72
EpiDerm -test 1-	NC	NC	NC	NC	NC	C	C	C	C	C	NC	NC	NC	NC	NC	C	C	C	C	C
EpiDerm -test 2-	NC	NC	NC	NC	NC	C	C	C	C	C	NC	NC	NC	NC	NC	C	C	C	C	C
EpiDerm re-trial							C													
Judge	NC	NC	NC	NC	NC	C	C	C	C	C	NC	NC	NC	NC	NC	C	C	C	C	C
Vitrolife-Skin -	NC	NC	NC	NC	NC	C	C	C	C	C	NC	NC	NC	NC	NC	C	C	C	C	C
Vitrolife-Skin -	NC	NC	NC	NC	NC	C	C	C	C	C	NC	NC	NC	NC	NC	C	C	C	C	C
Vitrolife-Skin re-	NC											NC								
Judge	NC	NC	NC	NC	NC	C	C	C	C	C	NC	NC	NC	NC	NC	C	C	C	C	C

Laboratory:
NIHS: Div. Toxicol., NIHS
NS: ODAWARA Res. Center, Nippon Soda Co., Ltd.
SC: Environmental Health Science Lab., Sumitomo Chemical Co., Ltd.
NN: Res. Div., Nihon Nohyaku Co., Ltd.
NM: Res. Lab., Nippon Menard Cosmetic Co., Ltd.
IET: Toxicol. Div., The Inst.Environmental Toxicol.

C: Corrosive, NC: Non-Corrosive

Results

This validation study was not performed under GLP. However, all data obtained in each laboratory followed GLP compliance and spirit. Their records (data and detailed documents) could be checked after the assays, and raw data was sent to Tokyo Univ. of Science for analysis by biostatisticians. All documents were checked by the chairperson, biostatisticians and chemical distributors, and are stored in the NIHS.

Predictivity

Using cell viability after exposure to test chemicals for three or 60 min., the chemical classifications according to the EpiDerm™ prediction model are shown in Table 3. Data for positive controls in the two models were evaluated correctly at all laboratories. The EpiDerm™ data summarized in Table 4 excluded the positive control data. Of 30 classifications of six chemicals in the corrosive class, 29 classifications of EpiDerm™ were correctly predicted to be corrosive, and sensitivity was 96.7%. All six chemicals in the corrosive class were correctly predicted excluding one laboratory. Lab.1 gave a negative classification of sulfuric acid from two data sets, but this chemical is corrosive. Cell viability values after expose to sulfuric acid for 60 min. were 18.54% and 38.80%, and these values

Table 4 Contigency table for EpiDerm™ predictions

Vitro / *Vivo*	Corrosive	Non-Corrosive
Corrosive	29	1
Non-Corrosive	10 (5% KOH, Lactic Acid)	20

Table 5 Contingency table for Vitrolife-Skin™ predictions

Vitro / *Vivo*	Corrosive	Non-Corrosive
Corrosive	30	0
Non-Corrosive	10 (5% KOH, Lactic Acid)	20

Table 6 Key statistical parameters for the four tests

	EpiDerm™	Vitrolife-Skin™	EpiDerm™ (ECVAM)	EPISKIN™ (ECVAM)
No. of Chemicals	12	12	24	60
Sensitivity	100% (12/12)	100% (12/12)	92%	82%
Specificity	66.7% (4/6)	66.7% (4/6)	83%	84%
Accuracy	83.3% (10/12)	83.3% (10/12)	92%	83%
False positive rate	16.7% (2/12)	16.7% (2/12)	17%	19%
False negative rate	0% (0/12)	0% (0/12)	8%	14%

were slightly high compared to 15%, which is the border line. On the other hand, of 30 classification of six chemicals in the non-corrosive class, 20 classifications of EpiDerm™ were correctly predicted to be non-corrosive, and specificity was 66.7%, but two were false positives. There were 5% potassium hydroxide and lactic acid. All the laboratories gave them positive classifications from two data sets, which is a non-corrosive chemical. Positive predictivity was 74.4% (29 true corrosive classifications / 39 corrosive classifications in this assay). Negative predictivity was 95.2% (20 true non-corrosive classifications / 21 non-corrosive classifications in this assay). The total consistency rate was 81.7% (49 true classifications / 60 classifications in this assay).

The Vitrolife-Skin™ data are summarized in Table 5, excluding the positive control data. Of 30 classifications of six chemicals in the corrosive class, 30 of Vitrolife-Skin™ were correctly predicted to be corrosive, and sensitivity was 100%. All six chemicals in the corrosive class were correctly predicted.

On the other hand, of 30 classification of six chemicals in the non-corrosive class, 20 of Vitrolife-Skin™ were correctly predicted to be non-corrosive, and specificity was 66.7%, but two were false positives. They were 5% potassium hydroxide and lactic acid, which all laboratories gave a positive classification from two data sets. This chemical is non-corrosive. Positive predictivity was 75% (30 true corrosive classifications / 40 corrosive classifications in this assay). Negative predictivity was 100% (20 true non-corrosive classifications / 20 non-corrosive classifications in this assay). The total consistency rate was 83.8% (50 true classifications / 60 classifications in this assay).

Predictability of these two models was similar to the results obtained by the ECVAM validation study.

Intralaboratory variation

Most chemicals did not show any great differences in scores on tests repeated at each laboratory. Different classifications of EpiDerm™ accounted for 6.66% (4/60). These data are not shown in the Tables. Cell viabilities of sulfuric acid after exposure for 60 min. in Lab. 2 were 17.26%, 9.46% and 12.02%, and those in Lab.3 were 15.72%, 10.58% and 9.01%, respectively. On the other hand, cell viabilities of lactic acid after exposure for 60 min. in Lab. 1 were 16.55%, 13.39% and 7.19%, while those in Lab.5 were 15.85%, 12.01% and 15.89%, respectively. These cell viabilities were around 15% after exposure for 60 min. (the success criteria).

Different classifications of Vitrolife-Skin™ accounted for 5.0% (3/60). Cell viabilities of sulfuric acid after exposure for 60 min. in Lab. 3 were 5.90%, 16.09% and 6.34%, while after exposure to octanoic acid for 60 min in Lab.3 were 21.37%, 11.77% and 10.71%. These cell viabilities were around 15% after exposure for 60 min (the success criteria). Meanwhile, cell viabilities of sodium hydroxide (4.88%) after exposure for 3 min. in Lab. 2 were also 55.12%, 15.41% and 17.51%. These cell viabilities were around 50% after exposure for 3 min. (the success criteria).

These cell viabilities were in an extremely narrow range despite the different classifications. Therefore, intralaboratory variation between the two models is presumed to be small.

Interlaboratory variation

In EpiDerm™, inter-laboratory variation was significant for only sulfuric acid. The classification of sulfuric acid in Lab. 1 was different from the data in the other four laboratories. In the data of Lab.1, not shown in the Tables, cell viabilities after exposure for 60 min. were 18.54% and 38.80%, and these values were almost the same as the positive classification. For Vitrolife-Skin™, inter- laboratory variation was not significant. From these results, the feasibility of using EpiDerm™ and Vitrolife-Skin™ was suggested by the experiment.

Discussion

From the obtained data, we confirmed the potential of using EpiDerm™ and Vitrolife-Skin™ as methods to evaluate the corrosivity of a chemical. We consider the data form these models has high predictivity, and low intra- and inter-laboratory variation.

With Vitrolife-Skin™, however, it is necessary to use limited blank data using collagen sponges without cells.

Modified points of Vitrolife-Skin™ from the ECVAM skin corrosivity validation study

Application volume

Although the surface of the Vitrolife-skin™ model (0.5 cm^2) is similar to that of EpiDerm™ (0.63cm^2), 50 μL of Liquid chemical was often insufficient for the surface. In this study, therefore,

the application volume of liquids was increased from 50 μL, the volume used in the phase III protocol in the EpiDerm™ skin corrosivity test, to 100 μL. For the same reason, 50 mg of solid chemical was applied and 50 μL of water was added to ensure good contact with the surface (in contrast to the Phase III protocol, in which 25mg of solid and an additional 25 μL of water were applied. Additional tests using collagen sponges without cells, the Vitrolife-Skin™ model uses a collagen sponge without cells to construct the dermal layer, and this allows test chemicals to be easily absorbed and bound, compared with epidermal models consisting of only an epidermal layer and supporting material. In a previous study, tests using collagen sponges without cells, instead of non-viable Vitrolife-Skin™ models, were performed for several test chemicals with the potential to interfere with the MTT assay (Mirokawa, 2006). For 3- methoxypropylamine and n-heptylamine, these experiments suggested about 50-60% and 80% "viability", respectively, due to a chemical reaction with the MTT medium. Hence, the 70-80% viability obtained for 3-methoxypropylamine with the Vitrolife-Skin™ model should be corrected to about 20%. In the same way, the 120% viability obtained for n-heptylamine should be decreased to about 40%. Therefore, these two chemicals, which were incorrectly classified as negatives by testing without using blank collagen sponges, should correctly be classified as corrosive by adding blank collagen sponges, in agreement with the results from the EpiDerm™ model. The additional test for the other six chemicals gave results of around 15% "viability", such that the Vitrolife-Skin™ *in vitro* prediction of corrosivity was not changed.

Therefore, we obtained blank data using collagen sponges without cells in the validation of Vitrolife-Skin™. In this validation study, we detected solibilization, swelling and color change after exposure to chemicals, and the need to use blank collagen sponges without cells.

Comparison of skin models

As shown in Table 6, there was no difference in sensitivity, specificity, accuracy, false positive rate or false negative rate between EpiDerm™ and Vitrolife-Skin™ in this validation study. The result in this validation study may be due to no difference in structure between a two-layer skin model consisting of a dermis and epidermis (Vitrolife-Skin™) and epidermal models (EpiDerm™). The barrier function of cornified layers of the cultured epidermal and skin model is less effective compared with human skin tissue (Kojima *et al.*, 2000). In addition, as chemical exposure times become longer, stronger cytotoxicity occurs due to the accumulation of chemicals which permeate the cornified layer of the skin model. However, it is considered the barrier function of these model is similar.

The sensitivity was 92% in phase III of the EpiDerm™ study and 82% in EPISKIN™ study, and the present values (100%) were higher than data of these previous validation assays. The specificity, however, was 83% in phase III of the EpiDerm™ study and 84% in the EPISKIN™ study, and the present ones (66.7%) were lower than those. We consider these accuracy and false positive rates to be no different between the present validation and previous validation study. On the other hand, none of the false negative rates in present validation study were lower than data from previous validation studys. This issue must be handled carefully, because this assay is a catch-up validation trial, and the number of chemicals and classes is small.

Though peer review of these models is in progress, the ad hoc. committee of toxicology at MHLW in Japan has approved the utilization of these models to evaluate the corrosivity of a chemical.

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座談会

アレルゴロジーvsトキシコロジー

われわれを取り巻く環境には，免疫系に影響を与える物質が多く存在している。そのなかで，アレルゴロジーに対する取り組みは進展してきたが，トキシコロジーについては十分な取り組みがなされてきたとはいえない。今回は，アレルゴロジーとトキシコロジーの概念および接点を確認しながら，現在に至る経緯と今後の課題について考えてみたい。

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（発言順，敬称略）

撮影／畑 博己

わが国においては，
アレルゴロジーに比べて
トキシコロジーに対する意識が
まだまだ低い状況です

1 言葉の概念と歴史およびトピックス

松永（司会）──「皮膚アレルギーの旅」は2002年創刊以来年4回発行され，今までアレルギーに関するさまざまな情報を提供してきました。本来アレルギーとそうでない反応では何が違うのか，その両者のリスクを評価する方法は現在どこまで進んでいるのか，世界の情勢はどうなのか，そしてわが国が果たすべき役割は何なのか。今回は年初にあたりそのようなことを掘り下げてみたく，免疫毒性の研究を中心にご活躍の相場節也先生ならびに動物実験代替法の研究でご活躍の小島 肇先生を交えて「アレルゴロジーvs トキシコロジー」と題して討議したいと思います。アレルゴロジーとトキシコロジーがversusかandの関係かについてはいろいろな考え方があると思いますが，まず相場先生にアレルゴロジーについての解説

をお願いします。

相場――Allergology（アレルゴロジー）というのは，“-ology”という語尾が付いていることから「アレルギーを研究する学問」であり，具体的にはアレルギーの病態および診断・治療などについて研究するものといえるでしょう。現在アレルギーという言葉は「私は○○に対してアレルギーがある」というような形で一般的用語ともなっていますが，そもそも医学用語としてのアレルギーは，石坂公成先生が抗原に反応して生じるレアギン抗体がIgE抗体であると発見された時点から始まり，CoombsとGellが〔アレルギー＝過敏反応〕としてその発症機構の違いにより4型に類型化したことからアレルギーの概念が広がったように思います。

本来アレルギーとは，“自己”に対する過敏反応である自己免疫疾患とは対照的な概念で，動物性の蛋白質や他人の細胞あるいは化学物質など“非自己”に対して過敏に反応する状態と定義され，それを探求する学問がアレルゴロジーだと考えられます。過敏反応が起こる基礎にはやはり免疫反応が主として存在し，免疫学の進歩に伴ってアレルギーの考え方も変遷してきたわけですが，最近トピックスとして取りあげられているのがregulatory T細胞（制御性T細胞）です。免疫防御システムにおいてT細胞が防御ネットワークを構築することが知られていますが，このT細胞が自己組織を攻撃しないように制御しているのがregulatory T細胞であり，Foxp3とよばれるmaster gene regulatorにより制御されています。このregulatory T細胞が，自己免疫のみならずアレルギーにも深く関与しているのではないかと考えられ，今後の研究に期待が寄せられています。

松永――続いて小島先生に，トキシコロジーの概念や歴史あるいはトピックスについてお話いただきたいと思います。

小島――Toxicologyは毒物学・中毒学などと訳され，私たちの周りにあるさまざまな毒物を対象としてその作用あるいは生物活性などを研究する生化学，薬理学，病理学などを総括した学問です。いわば基礎的医学の上に成り立っている応用的学問で，欧米では広くかつ深く研究されて専門家も多く育っているのですが，わが国においてはトキシコロジーに対する意識がまだまだ低い状況です。活動主体としては，1973年設立の毒性研究会と1976年設立の毒作用研究会とが1981年に合体して日本毒科学会となり，これが1997年に「日本トキシコロジー学会」と改称されて現在に至っています。トキシコロジーに関する学会は，日本免疫毒性学会，日本産業衛生学会，日本毒性病理学会などもあり，多くの関係者が携わっていますが，残念ながら若い研究者が育っていない印象があります。

トキシコロジーは，サリン事件やタミフル®など何か問題が起こらなければ注目されない分野です。しかし，化粧品，医薬品，食品添加物，農薬，金属，化学薬品，環境汚染物質，産業廃棄物，放射性物質，化学兵器など広範な領域を対象としており，極めて

トキシコロジーを啓発する場としては，「日本皮膚アレルギー・接触皮膚炎学会」が相応しいと考えています。

松永佳世子
まつなが・かよこ

Profile

1976年名古屋大学医学部卒業。三菱名古屋病院研修医。1977年同大学皮膚科入局，研修医。1978～1980年名古屋保健衛生大学医学部皮膚科助手。1980～1991年名古屋大学医学部附属病院および分院医員。1991～2000年藤田保健衛生大学医学部皮膚科学講師，2000年～同教授。2007年～日本皮膚アレルギー・接触皮膚炎学会理事長。

専門分野

皮膚アレルギー，接触皮膚炎の原因解明・防御