Toxicology services
- General toxicology:
  - Rodents
  - Non-rodents: dogs, NHPs and minipigs
- Infusion
- Inhalation
- Dermal
- Ocular
- Immunotoxicology
- Reproductive toxicology including minipigs and NHPs
- Carcinogenicity studies also in rasH2 and p53+/− mice
- Genetic toxicology: ICH compliant package
- In vitro toxicology: BCOP, MUSST, DPRA, Photo 3T3, Episkin™
- Agrochemical / Chemical / REACH
- QSAR
- Physical chemistry
- Ecotoxicology: wide range of test species

Safety pharmacology
- CV telemetry / ECG / BP
- Jacketed External telemetry (JET) / ECG / BP
- Respiratory / plethysmography / JET telemetry
- CNS / EEG
- Early safety pharmacology

DMPK and biomarkers
- Radiolabelled DMPK: in all species
- Bioanalysis LC-MS/MS, GC-MS/MS, LC-ICP/MS, ELISA, RIA
- Toxicogenomics, mRNA: Affymetrix™ / Accredited service provider
- Immunology: 10-color flow cytometer, Luminex, Mesoscale

Specialized expertises
- Juvenile studies including minipigs
- Fertility studies in rodents and NHPs
- Radiation safety and efficacy studies
- Tissue Cross Reactivity: human and animal tissue banks
- Gene therapy vector biodistribution via qPCR
- ES cell testing: devTOX™ and cardioTOX™ (with Stemina)
- Lead optimization and predictive toxicology services: LeadScreen™

Global Expertise, Local Response

The guinea pig systemic anaphylaxis model revisited

Jacques Descotes1, Marie-Eve Duclos2, Valérie Haag2 and Roy Forster2

CiToxLAB Group companies

CiToxLAB France
Phone +33 (0)2 32 29 26 26
Email contact.france@citoxlab.com
BP 365, 27005 Evreux cedex - France

CiToxLAB North America
Phone +1 888 353 2240
Email contact.northamerica@citoxlab.com
445, Armand-Frappier Blvd, Laval, Québec H7V 4B5 - Canada

CiToxLAB Hungary
Phone +36 34 456 500
Email contact.hungary@citoxlab.com
Vezprém, Sisabankugyartás, 8200 - Hungary

CiToxLAB Scantox
Phone +45 56 86 15 00
Email contact.scantox@citoxlab.com
Hestehavevej 36A, Ejby 8643, Lille Skensved - Denmark

Atlantbio
Phone +33 (0)2 51 10 01 00
Email atlantbio@atlantbio.com
1 Rue Graham Bell - 21 de Box BP 42309, 44400 Saint Nazaire Cedex - France

Media Services Ltd Japan
Phone +81 3 3666 9915
Email contact@mediaservices-je.com
10F, Building 16, 1-11-2 Nihonbashii Kayabacho, Chuoi-ku, Tokyo 103-0021 - Japan

Croen Research Inc.
Phone +82 31 888 9390
Email customerservice@croen.co.kr
Advanced Institutes of Convergence Technology - B-6th Fl., 864-1, Irip-ri, Yeongtong-gu, Suwon-si - Gyeonggi-do, 443-270, Korea

Stemina
Phone +1 608 204 0104
Email info@stemina.com
Website www.stemina.com
504 South Rosa Road, Suite 150
Madison, Wisconsin 53719

Also represented by

Partner company

Media Services Ltd Japan
Phone +81 3 3666 9915
Email contact@mediaservices-je.com
10F, Building 16, 1-11-2 Nihonbashii Kayabacho, Chuoi-ku, Tokyo 103-0021 - Japan

Croen Research Inc.
Phone +82 31 888 9390
Email customerservice@croen.co.kr
Advanced Institutes of Convergence Technology - B-6th Fl., 864-1, Irip-ri, Yeongtong-gu, Suwon-si - Gyeonggi-do, 443-270, Korea

Stemina
Phone +1 608 204 0104
Email info@stemina.com
Website www.stemina.com
504 South Rosa Road, Suite 150
Madison, Wisconsin 53719
Obtained in animals sensitized with 0.2 μg OVA.

Materials and methods

Animals

A total of 257 male Dunkin-Hartley guinea pigs corresponding to 12 independent studies were selected for retrospective analysis.

Experimental protocols

Groups of 5 animals received 1 or 2 sensitizing injections of 0.2, 2 or 20 μg OVA by the intraperitoneal (0.5 mL), subcutaneous (0.1 mL) or intradermal route (0.1 mL). The interval between each injection was 7 days, except in some animals (2 injections, 14 days apart).

After a rest period of 7 or 14 days, the animals were challenged with 0.2 or 20 μg OVA (0.1 mL), via the penile vein (or the ear vein in a few animals), except in some animals that were challenged with 10 or 93 μg OVA. Controls received injections of saline using the same route, volume and injection schedule and they were challenged with 20 μg OVA (except in one control group that was challenged with 93 μg OVA). In all cases, challenge was by the intravenous route.

Sensitization was evaluated during a 30-minute period from clinical signs observed including respiratory disorders, retching, defecation, urination, restlessness, stridor, weakness, prostration, and death. Sensitization was evaluated during a 30-minute period from clinical signs observed including respiratory disorders, retching, defecation, urination, restlessness, stridor, weakness, prostration, convulsions, lateral recumbency and death.

Results

The retrospective analysis of the results (pooling all routes of administration for sensitization) showed that the optimal sensitizing dose of OVA was 2 μg since inconsistent results were obtained in animals sensitized with 0.2 μg and, importantly, there were no severe clinical signs suggestive of anaphylaxis (Table 1). Sensitization was evaluated during a 30-minute period from clinical signs observed including respiratory disorders, retching, defecation, urination, restlessness, stridor, weakness, prostration, convulsions, lateral recumbency and death.

Discussion

It can be difficult to determine whether clinical reactions are suggestive of an anaphylactic potential of the test article or not. If such reactions are seen in control (naïve) animals, they may reflect systemic effects, e.g. toxic or pseudo-allergic effects of the test article when given by the intravenous route.

As the aim of this model is to predict the potential for a test article to induce anaphylaxis, it is proposed that one severe anaphylactic reaction among a group of animals tested according to the same protocol is sufficient to conclude that a positive response has been obtained. Vice versa, no severe reaction should be seen in non-sensitized animals after the challenge injection, but mild to moderate reactions may be seen.

The conclusion of this retrospective analysis is that an optimal study plan would consist of 2 sensitizing subcutaneous or intradermal injections of 2 μg OVA with a 7 or 14-day interval followed by a challenge intravenous injection of 20 μg OVA after a 7 or 14-day rest period.

Table 1: Classification of clinical reactions observed in animals after two sensitizing injections and one challenge injection with ovalbumin.

<table>
<thead>
<tr>
<th>Sensitizing dose</th>
<th>Challenge dose: 0.2 μg ovalbumin</th>
<th>Challenge dose: 20 μg ovalbumin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitization: all routes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitizing dose</td>
<td>0.2 μg</td>
<td>2 μg</td>
</tr>
<tr>
<td>No reaction</td>
<td>15/15</td>
<td>11/14</td>
</tr>
<tr>
<td>Severe reaction</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Death</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 2: Clinical reactions observed in animals after 2 sensitizing injections (via different routes of administration) and one challenge intravenous injection of ovalbumin.

<table>
<thead>
<tr>
<th>Sensitizing route</th>
<th>Challenge dose: 20 μg ovalbumin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitization: intraperitoneal route</td>
<td></td>
</tr>
<tr>
<td>Sensitizing dose</td>
<td>0.2 μg</td>
</tr>
<tr>
<td>No reaction</td>
<td>6/6</td>
</tr>
<tr>
<td>Minor reaction</td>
<td>-</td>
</tr>
<tr>
<td>Severe reaction</td>
<td>-</td>
</tr>
<tr>
<td>Death</td>
<td>-</td>
</tr>
</tbody>
</table>

| Sensitization: subcutaneous route | | |
| Sensitizing dose | 0.2 μg | 2 μg | 20 μg | 0.2 μg | 2 μg | 20 μg |
| No reaction | 5/5 | 5/5 | 1/5 | 3/5 | 2/39 | - |
| Minor reaction | - | - | 4/5 | - | 7/39 | 1/5 |
| Severe reaction | - | - | - | - | 12/39 | 2/5 |
| Death | - | - | - | - | 4/39 | 1/5 |

| Sensitization: intradermal route | | |
| Sensitizing dose | 0.2 μg | 2 μg | 20 μg | 0.2 μg | 2 μg | 20 μg |
| No reaction | 4/4 | 2/5 | 3/5 | 1/4 | 1/15 | - |
| Minor reaction | - | 2/5 | 3/5 | 2/4 | 1/15 | - |
| Severe reaction | - | - | - | 1/4 | 3/15 | 1/5 |
| Death | - | - | - | - | 1/15 | 1/5 |

Table 3: Classification of clinical reactions observed in animals according to the sensitization schedule and dose.

<table>
<thead>
<tr>
<th>Sensitizing dose</th>
<th>Challenge dose: 20 μg ovalbumin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitization: intraperitoneal route</td>
<td></td>
</tr>
<tr>
<td>Sensitizing dose</td>
<td>0.2 μg</td>
</tr>
<tr>
<td>No reaction</td>
<td>14/15</td>
</tr>
<tr>
<td>Minor reaction</td>
<td>1/15</td>
</tr>
<tr>
<td>Severe reaction</td>
<td>-</td>
</tr>
<tr>
<td>Death</td>
<td>-</td>
</tr>
</tbody>
</table>

| Sensitization: subcutaneous route | | |
| Sensitizing dose | 0.2 μg | 2 μg | 20 μg | 0.2 μg | 2 μg | 20 μg |
| No reaction | 1/14 | 5/17 | 5/20 | 2/33 | 2/15 | 1/10 |
| Minor reaction | - | 1/20 | 4/20 | 3/33 | - | 1/15 |
| Severe reaction | - | - | 1/20 | 4/33 | 1/15 | 3/10 |
| Death | - | - | - | - | 9/15 | 3/10 |

Conclusion

In view of the current renewed interest in the guinea pig systemic anaphylaxis model, we have reviewed data obtained with the positive reference compound ovalbumin (OVA) from a total of 12 studies with varied study designs. Retrospective analysis of the results supports that the optimal sensitizing dose of OVA was 2 μg. Sensitization was evaluated during a 30-minute period from clinical signs observed including respiratory disorders, retching, defecation, urination, restlessness, stridor, weakness, prostration, convulsions, lateral recumbency and death.

The optimal challenge dose of OVA was 20 μg and no overt differences were seen in animals given 10 (intermediate sensitizing dose) or 35 μg (data not shown), when sensitized with 2 μg OVA.

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