[ORIGINAL]

DETECTION OF IgE ANTIBODIES SPECIFIC TO ISONICOTINIC ACID HYDRAZIDE
AND ITS METABOLITE BY ENZYME-LINKED IMMUNOSORBENT ASSAY
AND THE MECHANISM OF SENSITIZATION BY INHALATION
OR INGESTION OF THIS COMPOUND

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A female hospital pharmacist and a nurse developed occupational asthma due to the
inhalation of isonicotinic acid hydrazide (INH) powder. The mechanism of sensitization was
examined in this study. Furthermore, IgE antibodies specific to INH and isonicotinic acid
(INA), the major metabolite of INH, were examined by means of enzyme-linked immuno-
sorbent assay (ELISA).

IgE anti-INH antibody activity was detected by ELISA in sera from 5 out of 8 hospital
workers who inhaled INH powder during the handling of INH, and in sera from 10 out of 142
tuberculosis patients receiving oral INH.

IgE anti-INA antibody activity was detected in sera from 3 of the 8 hospital workers, and
from 7 of the 142 tuberculosis patients. Neither IgE anti-INH nor anti-INA antibody activity
was detected in sera from 45 patients with asthma or from 42 healthy volunteers.

The specificity of the IgE antibodies and the cross-antigenicity between INH and INA were
confirmed by ELISA inhibition studies. Our results indicate that sensitization to INH can
occur not only by inhalation but also by oral administration, and that the asthmatic symptoms
of the patients were caused by an IgE antibody specific to INH as a hapten.

Key words: ELISA — IgE antibody — INH-induced asthma

INTRODUCTION

Isonicotinic acid hydrazide (INH), known also as
isoniazid, has been the most widely used primary
drug for the treatment of tuberculosis. A variety
of adverse reactions to oral INH have been re-
ported, including hepatitis1, fever2,3,4), neurological
disorders5,6,7), renal insufficiency8,9), hematological abnormalities10,11), and skin
rashes12,13). We have encountered two cases of occupa-
tional asthma14,15) caused by the inhalation of
INH powder. The nature and spectrum of INH

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Abbreviations: ELISA enzyme-linked immunosor-
bent assay; INA isonicotinic acid; INH isonicotinic
acid hydrazide; INA-HSA INA-human serum albumin
conjugate; INH-HSA INH-human serum albumin
conjugate; INH-BSA INH-bovine serum albumin con-
jugate; P-K Prausnitz-Küstner

side effects have led to suggestions that they may be
immunologically mediated. We have previously
considered the possibility that INH may act as a
hapten to induce drug-specific antibody responses.
Very few simple tests are available for the detec-
tion of antibodies to drugs or their metabolites in
suspected cases of drug hypersensitivity. In this
paper, we report the use of ELISA for the detec-
tion of anti-drug/metabolite antibody activity, and
for the determination of antigen specificity and
cross-antigenicity by hapten inhibition. In this
study, we evaluated 8 individuals who had inhaled
INH powder and 142 tuberculosis patients receiv-
ing oral INH in order to determine the incidence of
sensitization.

SUBJECTS AND METHODS

Study group

The study group consisted of 8 individuals who
had inhaled INH powder and 142 tuberculosis patients receiving oral INH (Table 1). The 8 subjects in the inhalation group were all hospital workers; two were nurses and six were pharmacists. One pharmacist and one nurse had asthmatic symptoms related to their exposure to INH powder. The average intake of INH among the tuberculosis patients was 0.38 g/day (0.2–0.4 g/day) and the average duration of oral INH therapy was 0.9 years (0.1–5.7 years). As a control group, we investigated 45 patients with asthma and 42 healthy volunteers who had not been treated with INH. Preparation of INH-HSA and INA-HSA conjugates were prepared essentially by the same method as reported by Tse et al. (Fig. 1)\(^{(1)}\).

An amount of 1.0 mol of HSA plus 4 mmol of INH were dissolved in 5.0 ml of distilled water and 125 mg of EDCI was added to the solution. The solution was kept at room temperature for 6 h, dialyzed extensively against distilled water, and then freeze-dried.

INH-BSA was prepared in the same manner.

For the coupling of the isonicotinyl group of INA to the \(\varepsilon\)-amino groups of HSA, the active azide method was used. For this reaction, 1.5 mmol of INH was dissolved in 60 ml of 0.1 N HCl and cooled to 0°C. Then 1.5 mmol of cold sodium nitrite in 6 ml of water was added dropwise with continuous stirring. An amount of 1.5 mmol of cold HSA in 15 ml of 0.2 M borate buffer (pH 9.5) was then added to the acyl azide. The pH of the mixture was maintained at 9.0 by adding 2 N NaOH. After 1 h, the resulting clear solution was dialyzed at 4°C against phosphate-buffered saline (PBS) and then against distilled water before it was freeze-dried.

Spectrophotometry showed a chemical substitution of 10.7 moles of INH per mole of HSA, 8.5 moles of INH per mole of BSA, and 27.1 moles of INA per mole of HSA.

**Skin test**

Skin testing was performed by intracutaneous injection of 0.02 ml of INH-HSA, INA-HSA, and HSA (in concentration of 1 mg/ml).

Weal and flare reactions were read 15 min later. A skin test was regarded as positive when the wheal and flare diameters were greater than 9 by 9 mm and 20 by 20 mm, respectively\(^{(2)}\). The two cases of INH-induced asthma had further skin testing with INH dissolved in saline and INH dissolved in the subject’s serum and incubated at 25°C for 4 hours.

They also underwent prick testing with INH-BSA.
P-K reaction

The P-K reaction test was performed on the 26-year-old female hospital pharmacist (case 1 in Fig. 2).

An amount of 0.1 ml of the subject’s serum was inoculated into the skin of the inner aspect of the arm of the subject’s mother (who had no history of atopy). After 24 hours, 0.02 ml of INH dissolved in saline (10^{-3} gm/ml) and INH dissolved in the subject’s serum and incubated at 25°C for 4 hours (10^{-3} gm/ml) were injected intradermally into the same region of the skin.

Inhalation tests

Inhalation testing was performed in the two cases of INH-induced asthma. The subjects inhaled INH powder, INH dissolved in saline, or INH dissolved in the subject’s serum for 2 minutes using a DeVilbiss 646 nebulizer (DeVilbiss Co., Somerset, Pa.) and air pump (flow rate, 5 L/min).

A reaction was defined as a significant asthmatic response only if it was accompanied by a fall of more than 20% in the FEV_{1.0} compared with the base line value.

Environmental provocation test

One of the two patients with INH-induced asthma was examined to determine the relation-ship between her allergic symptoms and her work during the grinding of INH crystals by machine and the dispensing of the powder with a drug-packaging machine.

<table>
<thead>
<tr>
<th>Skin test</th>
<th>(Case 1)</th>
<th>(Case 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>INH + Saline</td>
<td>+10^{-3}, 45 min</td>
<td>−</td>
</tr>
<tr>
<td>INH + Subject’s serum</td>
<td>+10^{-3}, 15 min</td>
<td>+10^{-3}</td>
</tr>
<tr>
<td>INH - BSA</td>
<td>+prick</td>
<td>−prick</td>
</tr>
<tr>
<td>INH - HSA</td>
<td>+10^{-7}</td>
<td>+10^{-4}</td>
</tr>
<tr>
<td>INH - HSA</td>
<td>+10^{-11}</td>
<td>+10^{-1}</td>
</tr>
<tr>
<td>PK test</td>
<td></td>
<td></td>
</tr>
<tr>
<td>INH + Saline</td>
<td>+, 45 min</td>
<td>N.D.</td>
</tr>
<tr>
<td>INH + Subject’s serum</td>
<td>+, 15 min</td>
<td>N.D.</td>
</tr>
<tr>
<td>Inhalation test</td>
<td></td>
<td></td>
</tr>
<tr>
<td>INH Powder</td>
<td>+</td>
<td>N.D.</td>
</tr>
<tr>
<td>INH + Saline</td>
<td>N.D.</td>
<td>+, 2 hours</td>
</tr>
<tr>
<td>INH + Subject’s serum</td>
<td>+10^{-4}</td>
<td>N.D.</td>
</tr>
<tr>
<td>Environmental provocation test</td>
<td></td>
<td></td>
</tr>
<tr>
<td>INH Powder prescription</td>
<td>+, 21 min</td>
<td>N.D.</td>
</tr>
<tr>
<td>Sensitivity to methacholine (threshold)</td>
<td>750 µg/ml</td>
<td>49 µg/ml</td>
</tr>
</tbody>
</table>

Fig. 2. Allergy tests in two cases of INH-induced asthma.
Case 1 was a female pharmacist and case 2 was a nurse. Both developed occupational asthma due to the inhalation of INH powder. N.D=not done, + = positive, − = negative.
Detection of IgE antibody activity specific to INH-HSA and INA-HSA by ELISA (Fig. 3)

Aliquots of 200 µl of INH-HSA or INA-HSA diluted to a concentration of 50 µg/ml in 0.05 mol/L sodium carbonate buffer (pH 9.6) were placed in each well and incubated overnight at 4°C in micro ELISA plates (Nunc F Immunoplates I, Denmark). Each well was washed three times with PBS (pH 7.2) containing 0.05% Tween-20 (Sigma Chemical Co., St. Louis, Mo.) (PBS-Tween), and a similar wash was repeated between all subsequent steps except after blocking.

Then each well was incubated with 200 µl of 100% FBS at room temperature for 2 hours in order to avoid nonspecific absorption of the serum components of the samples. Then 200 µl samples of serum diluted 1:4 in 100% FBS were added to each well, and incubated at room temperature for 2 hours.

Next, 200 µl/well of alkaline phosphatase-conjugated goat antihuman IgE (Sigma Chemical Co.) diluted to 1:1000 in 50% FBS-PBS-Tween was added and incubation was performed at room temperature for 2 hours.

Finally, 200 µl/well of 1 mg/ml p-nitrophenyl phosphate disodium (Sigma Chemical Co.) substrate solution diluted in 10% diethanolamine buffer solution (pH 9.8) was added and incubated at room temperature for (3 hours for INH-HSA) or (2 hours for INA-HSA). The reaction was stopped with 100 µl/well of 1.5 N NaOH.

Optical density was read on an ELISA analyzer (MR600, Microplate Reader, A Dynatech Product) at 410 nm.

The serum was considered positive for antibody if the O.D. was at least twice that of the O.D. of the negative control.

The control value was the mean of the 42 healthy volunteers who had not taken INH.

ELISA inhibition assays

A fixed dilution of antiserum was incubated at 4°C overnight with a range of concentrations of inhibitor in 100% FBS. Then centrifugation was performed at 3000 ×g for 5 min.

Supernatant fractions were assayed in duplicate for IgE antibody activity by the ELISA described above.

RESULTS

Results of clinical investigations

The two cases of INH-induced asthma were diagnosed on the basis of their case histories and the results of clinical investigations. Allergy test results for these two patients are shown in Figure 2.

Case 1 showed positive reactions for the skin tests, P-K test, inhalation test and environmental provocation test, which were made using several INH preparations. Case 2 showed positive reactions for the skin tests and inhalation test. The P-K test and environmental provocation test were not performed for case 2.

The two patients with INH-induced asthma demonstrated bronchial hypersensitivity to methacholine with threshold values of 750 and 49 µg/ml, respectively. In the skin tests for INH-HSA (1 mg/ml), these two patients showed positive reactions, but the other 6 members of the inhalation group were negative (Fig. 4).

Ten out of 142 tuberculosis patients had a positive skin reaction for INH-HSA (1 mg/ml), but the 45 asthma patients and 42 healthy volunteers were all negative for INH-HSA.

In the skin tests for INA-HSA (1 mg/ml), 3 of the 8 members of the inhalation group were positive, including the two cases of INH-induced asthma. Ten of the 142 tuberculosis patients showed positive reactions for INA-HSA, but the asthma patients and healthy volunteers were all negative for INH-HSA. The 2 patients with INH-induced

<table>
<thead>
<tr>
<th>Inhalation group (n=8)</th>
<th>INH-HSA(1 mg/ml)</th>
<th>INA-HSA(1 mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Tuberculosis (n=142)</td>
<td>*2 cases</td>
<td>6 cases</td>
</tr>
<tr>
<td>Asthma (n=45)</td>
<td>0</td>
<td>45</td>
</tr>
<tr>
<td>Healthy volunteers (n=42)</td>
<td>0</td>
<td>42</td>
</tr>
</tbody>
</table>

Fig. 4. Results of skin testing using INH-HSA and INA-HSA.

* Two cases of INH-induced asthma and 3 cases of tuberculosis had positive skin tests for both INH-HSA and INA-HSA. Seven cases of tuberculosis were only positive for INA-HSA.
asthma and 3 of the tuberculosis patients showed positive skin tests for both INH-HSA and INA-HSA. Therefore, 7 tuberculosis patients had positive skin tests only for INH-HSA and another 7 had positive reactions to INA-HSA.

IgE antibodies specific for INH-HSA and INA-HSA determined by ELISA

The effect of serum dilution on the IgE antibody level determined by ELISA was examined using the serum of case 1 (Fig. 5). The subject had circulating IgE-antibodies against both INA-HSA and INH-HSA. The degree of reaction was assessed and it was determined that INA-HSA reacted more strongly than INH-HSA. This seemed to be in accord with the increase in the amount of hapten drug bound to the HSA molecules.

No reaction occurred in polystyrene tubes coated with the carrier HSA and processed in the same manner.

ELISA inhibition assays and cross-inhibition studies for INH-HSA and INA-HSA

Inhibition experiments were designed to test the apparent anti-hapten specificity using the serum of case 1. Addition of serum following preincubation with several kinds of inhibitors resulted in inhibition of the binding between INH-specific antibodies and the allergen coating during

Fig. 5. Effect of serum dilution on the IgE antibody level in ELISA.

- -O: INA-HSA, serum from case 1.
O-O: INH-HSA, serum from case 1.
X-X: normal serum.

Fig. 6. Inhibition test and cross-inhibition studies for the determination of IgE antibodies to INH-HSA using ELISA.

Four fold-diluted serum from case 1 was incubated overnight at 4°C with a range of concentrations of inhibitors.

After centrifugation, the supernatant fractions were assayed for IgE antibody activity by ELISA. Wells were coated with INH-HSA.

- -O: INH-HSA, O-O: INH,
O-O: INA-HSA, O-O: INA,
X-X: HSA.

Fig. 6. Effect of serum dilution on the IgE antibody level in ELISA.

Fig. 7. Inhibition test and cross-inhibition studies for the determination of IgE antibodies to INA-HSA by ELISA.

Wells were coated with INA-HSA. The inhibition test was performed in the same manner as described in Fig. 6.

O-O: INA-HSA, O-O: INA,
- -O: INH-HSA, O-O: INH,
X-X: HSA.

ELISA (Fig. 6). In this case the wells were coated with INH-HSA. The degree of inhibition was proportional to the amount of allergen added. For the allergens used, the descending order of effec-
tiveness of inhibition of binding was INH-HSA, INH, INA-HSA, and INA. HSA did not produce any inhibition.

The same inhibition assays were also done using wells which were coated with INA-HSA (Fig. 7). The degree of inhibition was also found to be proportional to the amount of allergen added. The descending order of effectiveness of inhibition of binding was INA-HSA, INA, INH-HSA, and

**Fig. 8. IgE antibodies specific for INH-HSA.**

**Fig. 9. IgE antibodies specific for INA-HSA.**

**Table 2 Clinical and immunological data of the 17 cases with positive IgE antibodies specific for INH-HSA and INA-HSA**

<table>
<thead>
<tr>
<th>Case</th>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>Duration of</th>
<th>Daily intake (g/day)</th>
<th>Side effect</th>
<th>Total IgE (10/ml)</th>
<th>Skin test to common allergen</th>
<th>Sensitivity to methacholine</th>
<th>Skin test</th>
<th>O.D. value (specific IgE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>A. I.</td>
<td>♂</td>
<td>27</td>
<td>Inhalation 3Y</td>
<td>/</td>
<td>Asthma</td>
<td>81</td>
<td>(-)</td>
<td>(+)</td>
<td>INH-HSA (10^(-)) 7 x 5</td>
<td>0.321 (+)</td>
</tr>
<tr>
<td>2.</td>
<td>T. N.</td>
<td>♂</td>
<td>54</td>
<td>Inhalation 3Y</td>
<td>/</td>
<td>Asthma</td>
<td>508</td>
<td>(-)</td>
<td>(+)</td>
<td>INH-HSA (10^(-)) 20 x 14</td>
<td>0.225 (+)</td>
</tr>
<tr>
<td>3.</td>
<td>Y. K.</td>
<td>♂</td>
<td>38</td>
<td>Inhalation 3Y</td>
<td>(-)</td>
<td>(-)</td>
<td>11</td>
<td>(-)</td>
<td>(-)</td>
<td>INH-HSA (10^(-)) 3 x 25</td>
<td>0.201 (+)</td>
</tr>
<tr>
<td>4.</td>
<td>K. T.</td>
<td>♂</td>
<td>35</td>
<td>Inhalation 2Y</td>
<td>(-)</td>
<td>(-)</td>
<td>56</td>
<td>(-)</td>
<td>(-)</td>
<td>INH-HSA (10^(-)) 3 x 5</td>
<td>0.185 (+)</td>
</tr>
<tr>
<td>5.</td>
<td>Y. T.</td>
<td>♂</td>
<td>55</td>
<td>Inhalation 5Y</td>
<td>(-)</td>
<td>(-)</td>
<td>75</td>
<td>(-)</td>
<td>(-)</td>
<td>INH-HSA (10^(-)) 20 x 20</td>
<td>0.175 (+)</td>
</tr>
<tr>
<td>6.</td>
<td>A. F.</td>
<td>♀</td>
<td>54</td>
<td>1 Y 2 M</td>
<td>0.4</td>
<td>(-)</td>
<td>150</td>
<td>Candida Aspergilus</td>
<td>N.D.</td>
<td>INH-HSA (10^(-)) 10 x 10</td>
<td>0.267 (+)</td>
</tr>
<tr>
<td>7.</td>
<td>K. N.</td>
<td>♂</td>
<td>62</td>
<td>2 M</td>
<td>0.4</td>
<td>(-)</td>
<td>225</td>
<td>(-)</td>
<td>N.D.</td>
<td>INH-HSA (10^(-)) 10 x 10</td>
<td>0.035 (-)</td>
</tr>
<tr>
<td>8.</td>
<td>N. T.</td>
<td>♂</td>
<td>51</td>
<td>15 Y 7 M</td>
<td>0.3</td>
<td>Skin eruption</td>
<td>65</td>
<td>Candida</td>
<td>N.D.</td>
<td>INH-HSA (10^(-)) 10 x 10</td>
<td>0.299 (+)</td>
</tr>
<tr>
<td>9.</td>
<td>Y. K.</td>
<td>♂</td>
<td>26</td>
<td>8 M</td>
<td>0.4</td>
<td>(-)</td>
<td>115</td>
<td>House dust Candida</td>
<td>N.D.</td>
<td>INH-HSA (10^(-)) 10 x 10</td>
<td>0.029 (-)</td>
</tr>
<tr>
<td>10.</td>
<td>T. I.</td>
<td>♂</td>
<td>77</td>
<td>4 Y</td>
<td>0.4</td>
<td>Liver dysfunction</td>
<td>66</td>
<td>(-)</td>
<td>N.D.</td>
<td>INH-HSA (10^(-)) 10 x 10</td>
<td>0.209 (+)</td>
</tr>
<tr>
<td>11.</td>
<td>Y. S.</td>
<td>♂</td>
<td>30</td>
<td>3 M</td>
<td>0.4</td>
<td>Skin eruption</td>
<td>72</td>
<td>House dust Candida</td>
<td>N.D.</td>
<td>INH-HSA (10^(-)) 10 x 10</td>
<td>0.167 (+)</td>
</tr>
<tr>
<td>12.</td>
<td>N. T.</td>
<td>♂</td>
<td>62</td>
<td>5 M</td>
<td>0.4</td>
<td>(-)</td>
<td>43</td>
<td>House dust Candida</td>
<td>(-)</td>
<td>INH-HSA (10^(-)) 10 x 10</td>
<td>0.255 (+)</td>
</tr>
<tr>
<td>13.</td>
<td>T. N.</td>
<td>♂</td>
<td>68</td>
<td>5 M</td>
<td>0.4</td>
<td>Liver dysfunction</td>
<td>1.161</td>
<td>(-)</td>
<td>(-)</td>
<td>INH-HSA (10^(-)) 10 x 10</td>
<td>0.185 (+)</td>
</tr>
<tr>
<td>14.</td>
<td>K. K.</td>
<td>♂</td>
<td>80</td>
<td>1 Y 7 M</td>
<td>0.3</td>
<td>Liver dysfunction</td>
<td>62</td>
<td>(-)</td>
<td>N.D.</td>
<td>INH-HSA (10^(-)) 10 x 10</td>
<td>0.186 (+)</td>
</tr>
<tr>
<td>15.</td>
<td>S. K.</td>
<td>♂</td>
<td>70</td>
<td>6 M</td>
<td>0.4</td>
<td>Liver dysfunction</td>
<td>45</td>
<td>(-)</td>
<td>N.D.</td>
<td>INH-HSA (10^(-)) 10 x 10</td>
<td>0.154 (+)</td>
</tr>
<tr>
<td>16.</td>
<td>S. N.</td>
<td>♂</td>
<td>64</td>
<td>10 M</td>
<td>0.4</td>
<td>(-)</td>
<td>215</td>
<td>(-)</td>
<td>(-)</td>
<td>INH-HSA (10^(-)) 10 x 10</td>
<td>0.219 (+)</td>
</tr>
<tr>
<td>17.</td>
<td>Y. K.</td>
<td>♂</td>
<td>88</td>
<td>2 M</td>
<td>0.4</td>
<td>Skin eruption</td>
<td>36</td>
<td>(-)</td>
<td>N.D.</td>
<td>INH-HSA (10^(-)) 10 x 10</td>
<td>0.173 (+)</td>
</tr>
</tbody>
</table>

N.D.: not done, (+): positive, (-): negative.
The Japanese Society of Allergology

Terufumi Shimoda

DISCUSSION

Most commonly used drugs have a relatively low molecular weight. In order to become immunogenic, such low molecular weight compounds must bind with circulating carrier proteins. It has been reported that INH is tightly bound to human serum protein\(^\text{14}\), especially to human serum albumin\(^\text{15}\). The immunological responses to any drug may be quite diverse, and the attendant reactions can become very complex\(^\text{16}\). Anaphylactic, urticarial and asthmatic reactions caused by drugs are type I reactions. Some of these reactions may have an immunological mechanism mediated by IgE. The demonstration of drug-specific IgE antibodies is usually accepted as evidence that an individual may be at significant risk for anaphylaxis and asthmatic reactions if the drug is administered. A number of assays have been described for the measurement of serum antibodies including haemaggulination techniques\(^\text{17}\), the red cell-linked antigen-antiblobulin reaction (RCLAAR)\(^\text{18}\), radioimmunoassay (RIA)\(^\text{19}\), radioallergosorbent test (RAST)\(^\text{20}\), and enzyme-linked immunosorbent assay (ELISA)\(^\text{21,22}\). In this paper we described an ELISA for the detection of IgE antibodies directed against INH and INA (the major metabolite of INH). Furthermore, we examined the hapten specificity and the cross-antigenicity between INH and INA, based on hapten inhibition ELISA assays. The following three criteria have been defined as essential for positive immunoochemical identification of specific antibody activity directed against protein-reactive drugs and haptenes\(^\text{23}\): (1) Immunoglobulin in the test sample binds to the hapten-protein conjugate but not to the same protein in its unconjugated form. (2) Binding is
inhibited by hapten protein conjugates incorporating the test hapten but not unrelated hapten. (3) Binding is inhibited by low molecular weight derivatives of the reactive hapten. In addition, inhibitory conjugates and derivatives of the test hapten must be shown to be inactive in unrelated antigen-antibody interactions.

The present study showed that the anti-INH antibody and anti-INA antibody fulfilled the above three criteria (Fig. 5 and Fig. 6).

There was cross-antigenicity between INH and INA in hapten inhibition ELISA assays, but it was stronger for INH and INH-HSA in INH-HSA-coated wells than for INA and INA-HSA in INH-HSA-coated wells.

This finding suggests that the immunodominant region of this hapten drug includes the terminal nitrogen and that INH is a more important immunogen than INA. An immunogenic INH conjugate may be enzymatically produced in vivo by the coupling of INH through its terminal amino group with high molecular weight compounds similar to albumin. In this context, it has been reported that INH acts as an amine substrate in transaminating reactions catalyzed by liver transglutaminase and transglutaminase has also been shown to bring about the covalent incorporation of INH into a variety of protein acceptors\textsuperscript{15}. In our study, it was shown that sensitization to INH could occur not only by inhalation but also by oral administration.

Two cases (cases 1 and 2) out of 5 INH inhalation subjects who had positive IgE antibodies specific to INH-HSA had asthmatic symptoms, and both those cases showed bronchial hypersensitivity to methacholine (Table 2). Twelve tuberculosis patients (cases 6 to 17) who had positive IgE antibodies specific to either INH-HSA or INA-HSA did not have any asthmatic symptoms. The reasons for this difference may be the following. Firstly, the patients may not have had bronchial hypersensitivity to methacholine, although the test was done in only two cases who did not show hypersensitivity. Secondaly, there may be differences between sensitization by inhalation and by oral administration, with sensitization by inhalation being more likely to cause asthmatic symptoms.

Furthermore, although there were 13 tuberculous patients who had asthma of the atopic or infectious type, none of them had IgE antibodies specific to INH-HSA or INA-HSA. There was also no relationship between the total level of IgE antibodies and specific IgE antibodies to INH-HSA or INA-HSA. Therefore, it was suggested that atopy was not the predisposing factor in sensitization to INH.

As shown in Table 2, there was a good correlation between skin tests for INH-HSA or INA-HSA and the O.D. values in cases 1 and 2, who had asthmatic symptoms caused by INH. However, there was not such a good correlation in the 12 tuberculosis patients (cases 6 to 17) who had positive IgE antibodies specific to INH-HSA or INA-HSA. The reason for this discrepancy is unclear, because it is thought in general that skin testing is more sensitive than ELISA. For high molecular weight drugs, positive immediate wheal and flare skin tests are useful to screen for a potential risk of anaphylactic reaction\textsuperscript{6}. But for the more commonly used lower molecular weight drugs, with the exception of penicillin, the significance of positive skin tests is uncertain. Therefore, whether skin testing is positive or negative, the detection of hapten-specific IgE antibodies is more important in such cases. However, it should be kept in mind that there is often little correlation between the presence of drug-specific IgE antibodies and the occurrence of an allergic drug reaction.

Three cases of INH-induced asthma have been reported so far.

Two cases were reported by us\textsuperscript{9,10}, and the other was reported by Wang et al.\textsuperscript{24}

Only our two cases were examined immunologically and IgE antibodies specific to INH-HSA and INA-HSA were detected.

All three patients had occupational asthma due to inhaling INH powder during handling of the drug. As shown in Figure 9, the titers of IgE antibodies specific to both INH-HSA and INA-HSA have decreased with the passage of time since case 1 was removed from contact with INH powder.

The asthmatic symptoms have also become milder.

In order to prevent or avoid a drug-induced occupational allergy, removal from contact is the most important measure. If it is impossible to do
this, one should change the handling of a powder to handling of tablets or capsules.

In conclusion, we report the existence of specific serum IgE antibodies directed against INH-HSA and INA-HSA in patients with suspected INH allergy due to inhalation and in tuberculosis patients who were treated with oral INH.

ELISA is a good method for detection of anti-drug antibodies. In this study, it was not possible to determine the clinical significance of the IgE antibodies to INH in the tuberculosis patients, because of the low incidence of immediate type adverse reactions observed. However, having established a specific and sensitive assay system for antibodies directed against the hapten, it should now be possible to evaluate the role of drug-specific antibodies in patients taking INH. This will be particularly important in the investigation of rare but serious side-effects such as anaphylaxis. Investigations for other classes of antibodies (IgG, IgM, and IgA) specific to INH-HSA and INA-HSA are now in progress.

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ELISA法によるINHとその代謝物に対する特異IgE抗体の検出

吸入及び内服感作のメカニズム

長崎大学医学部第二内科
下田光文

我々はINH（Isoniazid）粉末の吸入による即時型職業性喘息の2例を経験し、その感作のメカニズムについて検討し、INH並びにその代謝物であるINA（Isonicotinic acid）に対する特異IgE抗体の存在をELISA法にて検索した。更に、INH内服中の肺結核患者に対しても同様に検索し、吸入及び内服による感作の程度についても検討した。その結果、INH特異IgE抗体陽性者は、INH吸入群8名中5名、肺結核患者142名中10名に認められた。INA特異IgE抗体陽性者は、吸入群で3名、内服群で7名に認められた。INH内服既発のない気管支喘息患者45名と健康人42名ではINHあるいはINAに対する特異IgE抗体は検出されなかった。また、ELISA inhibition assay法によりINHとINA間に交差抗原性が認められた。以上より、INH誘発喘息の発症機序はINHをハプテンとするI型アレルギーによることが立証され、INHは吸入及び内服のいずれによっても感作物質となり得る可能性が示唆された。