PROCAINAMIDE AUGMENTS VACCINATION EFFECT

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ABSTRACT

Procainamide (PA), an anti-arrhythmic agent, was recently found to be a specific inhibitor of suppressor T cells. The effects of PA and X-ray irradiation were studied on both plaque forming cells (PFC) and antibody titer using sheep red blood cells and vaccinia virus as antigens. A single injection of PA (50 μg) augmented both PFC and antibody titer; the increase was more than twice that seen after X-ray irradiation. A single injection of higher doses and repeated PA injections did not further augment antibody production. These data indicate that PA could be introduced as the sensitizing step in some experimental antibody-dependent anticancer regimens.

X-ray irradiation and administration of cyclophosphamide are often used to enhance the immune response in mice by inhibiting suppressor T cell activity (3, 4, 12, 13, 15, 18, 22). The potential lethality of this approach restricts its use to animal experimental systems. Procainamide (PA) hydrochloride, used in humans for a long time as an anti-arrhythmic agent, was recently found to be a specific inhibitor of suppressor T cells (11). This study compares the administration of PA with conventional X-ray irradiation therapy upon antibody production in mice.

MATERIALS AND METHODS

Mice

Two hundred five-week-old male C3H/He mice (Charles River, Japan) were acclimatized for two weeks and then used in the experiment. Groups of 5 mice from each major group were sacrificed on days 2, 5, 10 and 20. Body weight, spleen weight and spleen cell number were measured; antibodies in sera and spleen cells were characterized.

Vaccinia Virus (VV)

Vaccinia virus (Ikeda strain) was grown in chorioallantoic membranes (CAM) of chick embryos. Infected CAM was homogenized in Eagle’s minimum essential medium (1 sheet of CAM/ml) and centrifuged at 1,600 g at 4°C for 15 min. The supernatant was stocked as the virus suspension at -80°C. The infectious titer of the stocked virus suspension was usually 1×10⁹ plaque forming units (PFU)/ml. At the time of the injection it was adjusted to 1×10⁶ PFU/0.2 ml with Hanks’ balanced salt solution (HBSS).

Procainamide (PA)

Procainamide hydrochloride (Amisalin Injection: Daiichi Seiyaku, Tokyo) was diluted to 50, 100, 250 and 500 μg/0.2 ml in phosphate-buffered saline without Ca²⁺ and Mg²⁺ (PBS(−)).

Immunization of Sheep Red Blood Cells (SRBC) and VV

On day 0, mice were immunized with SRBC and VV; 1×10⁶ PFU of VV intraperitoneally (I.P.) and 0.2 ml of 20% (v/v) SRBC subcutaneously (S.C.) in the axillary region.
Procainamide Administration

The 200 mice were divided into four major groups: two groups received PA, one X-ray irradiation and one vehicle only. Each of the two PA groups comprised four subgroups. Each of the eight PA subgroups and the other two groups contained 20 mice.

The first group received a single PA injection (PA-SI); PA (50, 100, 250 and 500 μg/mouse) was administered I.P. two days before immunization. The second group received multiple PA injections (PA-MI); PA (50, 100, 250 and 500 μg/mouse) was administered I.P. two days before immunization and at six subsequent times over three weeks. The third group (X-ray) received no injections but the mouse’s whole body was irradiated with 150 rad two days before immunization. The fourth group (CTL) was injected once with vehicle only 2 days before immunization.

Hemolytic Plaque Forming Cell (PFC) Assay for SRBC

The method of Jerne et al. was used (8). Spleen cells obtained from mice were adjusted to $2 \times 10^8$/ml in HBSS. Agarose (0.5%; 0.4 ml), 20 μl of 20% SRBC suspension, and 100 μl of spleen cell suspension were mixed, plated on a glass slide and incubated at 37°C for 60 min. After incubation, guinea-pig complement (1:30) (Biken, Osaka, Japan) was added and the glass slide incubated for an additional 60 min. Plaques were counted and both PFC/10^6 spleen cells and PFC/spleen were calculated with subtracting PFC of non-treatment (non-immunization) group from PFC of each experimental group.

Anti-SRBC Antibody Titer

Anti-SRBC antibody titer of mice sera stocked at $-60°C$ was measured by the hemagglutination method (14). Glutaraldehyde-treated SRBC suspension (25 μl) adjusted to $2 \times 10^8$ cells/ml and 25 μl of diluted serum were mixed, incubated at 37°C for 60 min and hemagglutination assessed.

Anti-VV Antibody Titer

Fluorescent antibody technique was used (5, 21). Vero cells (monkey kidney cells) grown fully on a cover slip were infected with VV at multiplicities of infection of 0.01, incubated at 37°C for 20 h, fixed with acetone at $-20°C$ and covered with a serum sample. After incubation at 37°C for 30 min, the cover slip was washed thoroughly in PBS(−) and fluorescein isothiocyanate-conjugated goat anti-mouse IgG (Cappel Laboratories, Cochranville, PA, U.S.A.) was added. After incubation at 4°C for 30 min, it was washed again and examined with a fluorescent microscope for the titration.

Statistical Analysis

Data were expressed as mean ± SE and analyzed by Welch’s t-test.

RESULTS

Effect of PA and X-ray Irradiation on Spleen

There was no significant difference in body weight between all groups throughout the study. A similar pattern was recorded for spleen weights and spleen cell numbers although some differences were noted on day 5. Spleen weight in the X-ray-irradiated group was significantly decreased (52% of CTL) as was the spleen cell number (47% of CTL) (Table 1). Spleen weights and spleen cell numbers were similar in all PA groups and CTL. On days 2, 10 and 20, no significant differences in spleen weight and spleen cell number were measured between all groups.

Effect of PA on PFC

The serial change of mean PFC/spleen of PA-SI/50, PA-MI/50 and CTL is depicted in Fig. 1. PFC/spleen began to appear in all groups on day 2, increased markedly on day 5 and then decreased rapidly on days 10 and 20. On day 5, when PFC/spleen was maximal in each group, the enhancing effect of PA on PFC was most marked; mean PFC/spleen of PA-SI/50 and PA-MI/50 were 2.3 and 2.1 times higher than CTL. On days 2, 10 and 20, no significant difference in PFC/spleen was observed in these three groups.

On day 5, mean PFC/spleen of PA-SI/50 and PA-MI/50 were higher than CTL, whereas PA-SI/500 and PA-MI/500 were lower (Table 1).

Effect of PA on Antibody Titer

Anti-VV antibody titer Fig. 2 shows the serial change of mean anti-VV antibody titers of PA-SI/50, PA-MI/50 and CTL. On day 2, no antibody was detected in any group; mean anti-VV antibody titers of CTL increased over days 5 and 10 and reached a plateau by day 20. A marked increase in mean antibody titers for PA-SI/50 was seen on each day, especially on day 20. PA-
MI/50 had a similar increase on day 20, but this only doubled CTL.

Anti-VV antibody titers of all groups were compared using the sera from day 20 (Table 2). Mean antibody titers of PA-SI/50, 100 and 500 were significantly increased. None of the PA-MI groups showed a significant increase.

Anti-SRBC antibody titer Mean antibody titer of PA-SI/50 was the only one higher than CTL; PA-MI/100 and 500 had significant decreases (Table 2).

Comparison between X-ray Irradiation and PA Injection
The administration of PA (50 μg/mouse) had a significant effect on the augmentation of PFC, anti-VV antibody and anti-SRBC antibody (Tables 1 and 2); X-ray irradiation had an effect only on the augmentation of anti-VV antibody.

DISCUSSION
To enhance vaccination efficacy is clinically useful for two reasons: first, for the prophylactic vaccination to microorganisms which are difficult to raise a high antibody titer (2, 19) and, second, for specific anticancer immunotherapy against some malignant tumors which have been experimentally demonstrated in mice (3, 4, 7, 16, 18, 22). The first step to accomplish this objective was the examination of the effect of PA on the antibody-dependent system. The experimental system was fundamentally the same as the sensitizing step of the specific anticancer immunoprevention with vaccinia virus (4, 18, 22) which is effective partially through the antibody-dependent system.

We observed only the effect of PA on direct PFC which is IgM-PFC (6, 8). The serial change of PFC of CTL was a normal pattern for IgM-PFC. PA has the same inactivating effect on suppressor T cells in both IgM-PFC and IgG-PFC (11). Therefore, we studied only anti-SRBC IgM-PFC; PA-SI/50 had a significant effect on anti-SRBC PFC. Three of the PA single injection groups, especially PA-SI/50, showed a significant increase in anti-VV antibody titer, however, augmentation of anti-SRBC antibody titers was significantly demonstrated only in PA-SI/50.
Table 1  *Spleen Weight, Spleen Cell Number and PFC Number on Day 5*

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Spleen weight (g)</th>
<th>Spleen cell number (×10⁶)</th>
<th>PFC per 10⁸ spleen cells</th>
<th>PFC per 10⁹ spleen (×10⁴)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Mean  SE</td>
<td>Mean SE</td>
<td>Mean SE</td>
<td>Mean SE</td>
</tr>
<tr>
<td>I</td>
<td>PA (µg) 1 injection</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.25 ±0.018</td>
<td>2.1 ±0.16</td>
<td>735*±107</td>
<td>16.0*±2.8</td>
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<td></td>
<td>100</td>
<td>0.24 ±0.026</td>
<td>1.8 ±0.13</td>
<td>622 ±305</td>
<td>10.0 ±4.5</td>
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<td></td>
<td>250</td>
<td>0.22 ±0.016</td>
<td>1.3 ±0.37</td>
<td>684 ±186</td>
<td>7.7 ±1.8</td>
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<tr>
<td></td>
<td>500</td>
<td>0.17 ±0.008</td>
<td>1.1 ±0.14</td>
<td>36*±11</td>
<td>0.6*±0.1</td>
</tr>
<tr>
<td>II</td>
<td>PA (µg) 7 injections</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.19 ±0.003</td>
<td>1.2 ±0.10</td>
<td>1,140 ±330</td>
<td>14.5 ±4.4</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.24 ±0.020</td>
<td>1.5 ±0.14</td>
<td>482 ±107</td>
<td>7.4 ±1.6</td>
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<tr>
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<td>250</td>
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<td>375 ±113</td>
<td>4.0 ±1.4</td>
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<tr>
<td></td>
<td>500</td>
<td>0.22 ±0.018</td>
<td>1.4 ±0.12</td>
<td>91**±32</td>
<td>1.5**±0.4</td>
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<tr>
<td>III</td>
<td>X-ray irradiation</td>
<td>0.13*±0.005</td>
<td>0.8**±0.12</td>
<td>682 ±201</td>
<td>4.3 ±1.2</td>
</tr>
<tr>
<td>IV</td>
<td>Control</td>
<td>0.25 ±0.008</td>
<td>1.7 ±0.32</td>
<td>353 ±93</td>
<td>7.1 ±2.0</td>
</tr>
</tbody>
</table>

*P<0.01, **P<0.05

Table 2  *Anti-VV and Anti-SRBC Antibody Titer on Day 20*

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Anti-VV antibody titer Mean SE</th>
<th>Anti-SRBC antibody titer Mean SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>PA (µg) 1 injection</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>×2,560**±713</td>
<td>×1,280*±143</td>
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<tr>
<td></td>
<td>100</td>
<td>×1,280**±512</td>
<td>×160 ±25</td>
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<td></td>
<td>250</td>
<td>×640 ±207</td>
<td>×160 ±50</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>×1,280**±429</td>
<td>×40 ±29</td>
</tr>
<tr>
<td>II</td>
<td>PA (µg) 7 injections</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>×640 ±187</td>
<td>×80 ±22</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>×320 ±96</td>
<td>×40**±10</td>
</tr>
<tr>
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<td>250</td>
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</tr>
<tr>
<td></td>
<td>500</td>
<td>×160**±24</td>
<td>×20**±2</td>
</tr>
<tr>
<td>III</td>
<td>X-ray irradiation</td>
<td>×1,280 ±384</td>
<td>×80 ±27</td>
</tr>
<tr>
<td>IV</td>
<td>Control</td>
<td>×320 ±90</td>
<td>×320 ±118</td>
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</table>

*P<0.01, **P<0.05

single administration of 50 µg of PA had a stronger effect on the augmentation of PFC, anti-VV antibody titer and anti-SRBC antibody titer than X-ray irradiation, without influencing body weight, spleen weight and spleen cell number. These data indicate that a single PA injection at a proper dosage is able to inactivate suppressor T cells without any negative biological effects.

As PA is a reversible inhibitor of suppressor T cells in vitro (11), we speculated that continual administration during the immunization of an antigen would be necessary to inactivate suppressor T cells. Only one injection at the lowest PA dosage, PA-SI/50, produced the best result; higher dosages as well as multiple injections were less effective. In particular, the administration of 500 µg of PA, PA-SI/500 and PA-MI/500, showed a significant decrease in PFC and antibody titers. It was found that an administration of the optimal dosage of PA could inactivate suppressor T cells effectively in vivo. Non-specific inactivation of all immune cells occurs at higher PA dosages in vitro (Ochi et al., unpublished data), and accumulation of PA in tissues as seen in patients with drug-induced lupus (9).

Although PA has an adverse side effect of a drug-induced lupus (1, 10, 17, 20), it has been used for a long time to treat many patients for heart disease, especially arrhythmia. Thus, this method of enhancing the effects of vaccines by PA administration is feasible in the clinical situation. Its use could strengthen the effects of vac-
cines and perhaps make specific anticancer immunotherapy developed in experimental systems available for clinical trials.

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REFERENCES


