SIMILAR EFFECTS OF VARIOUS LOW-MOLECULAR-WEIGHT ENZYME INHIBITORS ON ENZYME NETWORKS IN DYSTROPHIC MICE

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We compared the therapeutic effects of various low-molecular-weight enzyme inhibitors on dystrophic mice. Leupeptin, bestatin, forphenicinol and forphenicine significantly affected the enzymatic activities in the dystrophic muscles. The pattern of enzymatic changes in the muscles of forelimb and hindlimb caused by these inhibitors were similar in spite of the variety of their inhibitory spectra in vitro. However, comparing the pattern of enzymatic changes in spleen, forphenicinol differed from the other inhibitors tested. This may be related to the peculiar effects of this inhibitor on immunologically responsive cells.

Keywords — dystrophy; leupeptin; bestatin; forphenicinol; forphenicine

INTRODUCTION

Our serial studies suggested the possibility that not only the abnormalities in endopeptidases but also the abnormalities in exopeptidases might play some roles in pathogenesis of muscular dystrophy.1-3 This promoted the trial of the inhibitors leupeptin and bestatin for the treatment of murine muscular dystrophy. It has been shown that these inhibitors suppress the occurrence of dystrophy.4,5 Judging from the extensive spectrum of the enzymatic changes induced by the low-molecular-weight enzyme inhibitors in comparison to their in vitro actions, a priori, there seemed to be no need to limit the therapeutic trials to the inhibitors of endopeptidases or exopeptidases. The present study indicates that forphenicine, an inhibitor of alkaline phosphatase, and forphenicinol, not an enzyme inhibitor, exert effects similar to those of leupeptin and bestatin.

MATERIALS AND METHODS

Animals — C57BL/6J dy/dy male dystrophic mice (3 week-old) were obtained from the Central Institute for Experimental Animals, Kanagawa, Japan. Mice were killed by cervical dislocation 3 h after the final injection, and organ homogenates were prepared in phosphate buffered saline (PBS) using a tissue homogenizer, Ultraturrax, at the maximum speed for 1 min. The homogenate was centrifuged (3,000 × g, 20 min) and the supernatant fluid was withdrawn for the measurement of enzymatic activities.

Inhibitors — The inhibitors used in this study were leupeptin, bestatin, forphenicinol and forphenicine (Fig. 1). Leupeptin is an inhibitor of trypsin, plasmin, papain and cathepsin B.6 Bestatin is an inhibitor of aminopeptidase B (AP-B), Leu-AP and triaminopeptidase.7 Forphenicinol was prepared by reduction of the formyl group of forphenicine which is an inhibitor of alkaline phosphatase of chicken intestine.8 These inhibitors were dissolved in 0.2 ml of PBS, and were given daily intraperitoneally for 8 d in the following doses: leupeptin 500 µg, bestatin 200 µg, forphenicinol 500 µg and forphenicine 500 µg. The control animals were given 0.2 ml PBS daily.

Substrates for Enzyme Assay — The sources of substrates were as follows (see Table I for
**FIG. 1. Structures of Various Inhibitors**

**TABLE I. List of the Enzymes Measured and Their Substrates**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Abbreviation</th>
<th>Substrate</th>
<th>Reference for assay method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartate aminopeptidase (EC 3.4.11.7)</td>
<td>AP-A</td>
<td>Glu·NA</td>
<td>1, 9</td>
</tr>
<tr>
<td>Arginine aminopeptidase (EC 3.4.11.6)</td>
<td>AP-B</td>
<td>Arg·NA</td>
<td>1, 7</td>
</tr>
<tr>
<td>Proline aminopeptidase</td>
<td>Pro-AP</td>
<td>Pro·NA</td>
<td>1</td>
</tr>
<tr>
<td>Leucine aminopeptidase (EC 3.4.11.1)</td>
<td>Leu-AP</td>
<td>Leu·NA</td>
<td>1, 7</td>
</tr>
<tr>
<td>Formylmethionine aminopeptidase</td>
<td>fMet-AP</td>
<td>fMet·NA</td>
<td>1, 10</td>
</tr>
<tr>
<td>Phenylalanine aminopeptidase</td>
<td>Phe-AP</td>
<td>Phe·NA</td>
<td>1</td>
</tr>
<tr>
<td>Tripeptide aminopeptidase (EC 3.4.11.4)</td>
<td>Gly-Pro-Leu-AP</td>
<td>Gly-Pro-Leu·NA</td>
<td>1, 11</td>
</tr>
<tr>
<td>Serine proteinase (EC 3.4.21)</td>
<td>Trypsin-like</td>
<td>BAEE</td>
<td>1, 6</td>
</tr>
<tr>
<td>Serine proteinase (EC 3.4.21)</td>
<td>Chy-try-like</td>
<td>ATEE</td>
<td>1, 6</td>
</tr>
<tr>
<td>Serine proteinase (EC 3.4.21)</td>
<td>Elastase-like</td>
<td>Ac (Ala)_3·ME</td>
<td>1</td>
</tr>
<tr>
<td>Carboxyl proteinase (EC 3.4.23.5)</td>
<td>Cathepsin D</td>
<td>Hemoglobin</td>
<td>1, 12</td>
</tr>
<tr>
<td>α-D-Glucosidase (EC 3.2.1.20)</td>
<td>Glucosidase</td>
<td>NP-Glc</td>
<td>1, 13</td>
</tr>
<tr>
<td>α-D-Mannosidase (EC 3.2.1.24)</td>
<td>Mannosidase</td>
<td>NP-Man</td>
<td>1, 13</td>
</tr>
<tr>
<td>N-Acetyl-β-D-glicosaminidase (EC 3.2.1.30)</td>
<td>GlcNAc-ase</td>
<td>NP-GlcNAc</td>
<td>1, 13</td>
</tr>
<tr>
<td>Creatine kinase (EC 2.7.3.2)</td>
<td>CK</td>
<td>1-14C-creatine</td>
<td>14</td>
</tr>
<tr>
<td>Alkaline phosphatase (EC 3.1.3.1)</td>
<td>Phosphatase</td>
<td>NPP</td>
<td>1, 15</td>
</tr>
<tr>
<td>Arylesterase (EC 3.1.1.2)</td>
<td>Esterase</td>
<td>NPA</td>
<td>1, 15</td>
</tr>
</tbody>
</table>
Effect of Enzyme Inhibitor in Dystrophy

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abbreviations): Glu · NA, Arg · NA, Pro · NA and Met · NA from Mann Research, New York, U.S.A.; Leu · NA, Phe · NA and Ac(Ala)₃ · ME from Bachem Fienchemikalien AG, Budendorf, Switzerland; Gly-Pro-Leu · NA from Peptide Institute, Osaka, Japan; BAEE from Sigma Chemical Co., Saint Louis, Missouri, U.S.A.; ATEE and NPA from Tokyo Kasei Kogyo Co., Tokyo, Japan; NP-Glc, NP-Man, and hemoglobin from Calbiochem-Behring Co., San Diego, California U.S.A.; NP-GlcNAc and NPP from BDH Chemicals Ltd., Poole, England; 1-[¹⁴C] creatine from Daiichi Chemical Co., Tokyo, Japan. fMet · NA was synthesized in our laboratory.

Buffers — The buffers used were as follows: 0.02 M phosphate-buffered saline (PBS, pH 7.2) for all enzyme systems; 0.2 M sodium acetate buffer (pH 3.2) for cathepsin D; 0.5 M sodium glycine buffer (pH 9.0) for creatine kinase.

Determination of Enzymatic Activities — The supernatant fluids of the homogenates were dispensed into test tubes (1.5 × 10 cm) with PBS containing the respective substrates. The assays were carried out at pH 7.2 except for cathepsin D and creatine kinase, which were assayed at pH 3.2 and 9.0, respectively. The test tubes were incubated for 1 h at 37°C. All enzyme assays were in triplicate and their errors were within 10% of the average values. The values presented are the averages of triplicate assays. The references for the assay methods and the substrates used and abbreviations are listed in Table I. For most assays the units of the enzyme activities are expressed as nmol of reaction products generated in 1 min of incubation per mg protein (nmol/ min/mg protein). The absorbance values actually measured were translated into those units on standard curves which had been prepared in advance.

For the assays of aminopeptidases, the reaction mixture was prepared by mixing 0.25 ml 2 mM β-naphthylamide derivatives, 0.65 ml PBS, and 0.1 ml diluted homogenates in a series of test tubes. After the reaction, the mixture was processed as described elsewhere¹,²,³,⁹,−¹¹ and the absorbance at 525 nm of the final mixture was determined. For serine proteinase assays, 0.1 ml 75 mM ester of peptide or N-acylamino acid was used as substrate. After the reaction, the mixture was processed as described elsewhere¹,⁶ and the absorbance at 525 nm was determined. For cathepsin D assay, 1.0 ml 0.5% hemoglobin solution (pH 3.2) was used as the substrate. Two milliliters of 1.7 M perchloric acid was added after the reaction, and the mixture was kept 1 h at room temperature. The supernatant fluid was withdrawn by centrifugation (2 000 × g, 5 min) and the absorbance at 280 nm was determined.¹,² For glycocidase assays, 0.1 ml 25 mM p-nitrophenol derivative was used as the substrate. After the reaction, the mixture was processed as described elsewhere¹,³ and the absorbance at 400 nm was determined. For creatine kinase assay, 0.1 ml 1 mM creatine containing 1-[¹⁴C]- creatine (50 000 dpm) was used as the substrate. After the reaction, the radioactivity of the product in the unabsorbed fraction of Dowex 50 (H⁺) was determined.¹⁰ For the assays of phosphatase and esterase, 0.1 ml 15 mM p-nitrophenol derivatives were used as the substrate. After the reaction, the mixture was processed as described elsewhere¹,¹⁳ and the absorbance at 400 nm of the final mixture was determined.

Protein Determination — The method of Lowry et al.¹⁶ was used, with bovine serum albumin as the standard.

Statistical Method — The observed values of enzyme activities in the muscles of the forelimbs and hindlimbs, and spleen administrated the various inhibitors were treated by Student's t-test and Spearmann’s rank correlation.¹⁷

RESULTS

Table II shows the effects of the four inhibitors on the hydrolytic enzymes in forelimb muscle of dystrophic mice. Leupeptin significantly suppressed the activities of AP-A, Leu-AP, phosphatase and esterase. On the contrary, the activities of fMet-AP, Chy-try-like and cathepsin D were increased. Bestatin suppressed the activities of AP-A, Leu-AP, Phe-AP,
trypsin-like, glucosidase, creatine kinase, phosphatase and esterase. Only the activity of fMet-AP was significantly increased. Forphenicolin and forphenicin also suppressed many hydrolytic enzymes including AP-A, Leu-AP, trypsin-like, glucosidase, GlcNAc-ase, phosphatase and esterase. Forphenicin also suppressed the activity of Phe-AP, Chy-try-like and cathepsin D. The activities of fMet-AP and Chy-try-like were elevated by the effect of forphenicin.

Table III shows the effects of the inhibitors on enzymes in hindlimb muscle. The enzymatic changes which occurred were much different from the ones in forelimb muscle. The activity of Leu-AP was increased by the effects of any of the inhibitors tested. The activity of fMet-AP was increased in most cases. The activity of trypsin-like was elevated by the effect of leupeptin but was suppressed by that of forphenicin. The activities of cathepsin D and GlcNAc-ase were elevated by leupeptin. The activities of AP-B, Phe-AP, trypsin-like, Chy-try-like, creatine kinase and esterase were suppressed by the effect of forphenicin.

Table IV lists the enzymatic changes in spleen. Leupeptin significantly suppressed trypsin-like but elevated Pro-AP, Phe-AP, Gly-Pro-Leu-AP, cathepsin D and phosphatase. Bestatin suppressed trypsin-like and Chy-try-like but elevated AP-B, Pro-AP, Leu-AP, Phe-AP and Gly-Pro-Leu-AP. The effects of forphenicin were similar to those of bestatin in the fact that it suppressed the activities of trypsin-like, Chy-try-like and mannosidase but elevated Pro-AP, Leu-AP, Phe-AP, Gly-Pro-Leu-AP, glucosidase, phosphatase and esterase. Forphenicin suppressed AP-B and glucosidase but elevated many others including Phe-AP, Gly-Pro-Leu-AP, mannosidase, phosphatase and esterase.

### Table II. Changes in Enzymatic Activity of Forelimb Muscle of Dystrophic Mice Induced by Low Molecular Weight Inhibitors

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>None (N=10)</th>
<th>Leupeptin</th>
<th>Bestatin</th>
<th>Forphenicolin</th>
<th>Forphenicin</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP-A</td>
<td>1.63 ± 0.22</td>
<td>0.95 ± 0.60&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.26 ± 0.22&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.28 ± 0.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.24 ± 0.31&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>AP-B</td>
<td>10.45 ± 1.53</td>
<td>8.39 ± 2.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.22 ± 1.14</td>
<td>9.08 ± 1.42</td>
<td>10.04 ± 1.36</td>
</tr>
<tr>
<td>Pro-AP</td>
<td>2.65 ± 1.25</td>
<td>2.42 ± 0.49</td>
<td>1.45 ± 0.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.54 ± 0.85</td>
<td>3.67 ± 1.18</td>
</tr>
<tr>
<td>Leu-AP</td>
<td>8.96 ± 0.93</td>
<td>5.68 ± 3.45&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.67 ± 0.74&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.58 ± 0.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.65 ± 1.40&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>fMet-AP</td>
<td>4.03 ± 0.36</td>
<td>6.34 ± 1.41&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5.17 ± 0.67&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.60 ± 0.07&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.42 ± 0.80</td>
</tr>
<tr>
<td>Phe-AP</td>
<td>14.23 ± 1.31</td>
<td>10.87 ± 3.32&lt;sup&gt;g&lt;/sup&gt;</td>
<td>10.93 ± 2.31&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13.59 ± 0.58</td>
<td>10.54 ± 2.25&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gly-Pro-Leu-AP</td>
<td>1.16 ± 0.18</td>
<td>0.94 ± 0.48</td>
<td>0.97 ± 0.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.03 ± 0.15</td>
<td>1.19 ± 0.26</td>
</tr>
<tr>
<td>Trypsin-like</td>
<td>72.79 ± 8.72</td>
<td>102.07 ± 47.95&lt;sup&gt;a&lt;/sup&gt;</td>
<td>54.42 ± 21.40&lt;sup&gt;b&lt;/sup&gt;</td>
<td>55.11 ± 13.43&lt;sup&gt;c&lt;/sup&gt;</td>
<td>43.09 ± 16.95&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chy-try-like</td>
<td>74.98 ± 14.62</td>
<td>134.26 ± 25.32&lt;sup&gt;d&lt;/sup&gt;</td>
<td>63.33 ± 23.28</td>
<td>90.06 ± 5.96&lt;sup&gt;b&lt;/sup&gt;</td>
<td>48.01 ± 19.05&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Elastase</td>
<td>49.00 ± 7.51</td>
<td>48.74 ± 18.50</td>
<td>45.76 ± 18.82</td>
<td>54.11 ± 7.22</td>
<td>45.36 ± 15.29</td>
</tr>
<tr>
<td>Cathepsin D</td>
<td>0.0096 ± 0.0007</td>
<td>0.008 ± 0.0009&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.007 ± 0.0009</td>
<td>0.0066 ± 0.0010</td>
<td>0.0048 ± 0.0017&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glucosidase</td>
<td>0.71 ± 0.24</td>
<td>0.74 ± 0.16</td>
<td>0.40 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.43 ± 0.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.34 ± 0.07&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mannosidase</td>
<td>0.17 ± 0.15</td>
<td>0.31 ± 0.28</td>
<td>0.14 ± 0.03</td>
<td>0.19 ± 0.05</td>
<td>0.14 ± 0.001</td>
</tr>
<tr>
<td>GlcNAc-ase</td>
<td>1.75 ± 0.55</td>
<td>2.22 ± 1.03</td>
<td>1.22 ± 0.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.13 ± 0.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.61 ± 0.25&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Creatine kinase</td>
<td>22.51 ± 4.76</td>
<td>19.75 ± 10.43</td>
<td>16.05 ± 2.74&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.27 ± 3.48</td>
<td>18.01 ± 2.85&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Phosphatase</td>
<td>4.34 ± 0.92</td>
<td>1.67 ± 1.23&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.46 ± 0.20&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.42 ± 0.19&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.40 ± 0.29&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Esterase</td>
<td>131.56 ± 45.58</td>
<td>67.62 ± 16.80&lt;sup&gt;b&lt;/sup&gt;</td>
<td>62.57 ± 2.85&lt;sup&gt;c&lt;/sup&gt;</td>
<td>57.31 ± 4.81&lt;sup&gt;c&lt;/sup&gt;</td>
<td>43.77 ± 12.97&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> p < 0.1, <sup>b</sup> p < 0.05, <sup>c</sup> p < 0.01, <sup>d</sup> p < 0.001.
Table V compares the patterns of enzymatic changes caused by each of the agents tested in the present study. It is clearly seen that the effects of these agents resembled each other in the muscles of forelimb and hindlimb. However, forphenicinol differed from the others in its affect on the enzyme networks in spleen.

**DISCUSSION**

The activities of many hydrolytic enzymes are known to be increased in the muscular tissues of dystrophic mice and chickens.\(^1\,^2\) Thus it was suggested that low-molecular-weight enzyme inhibitors discovered in our institute would be useful in the treatment of this disease.\(^3\) However, because of the extensive spectrum of the enzymatic changes induced by the inhibitors in vivo, the selection of the most appropriate inhibitors is not simple.

Previously, we demonstrated that various inhibitors have such extensive physiological and pharmacological actions in vivo as would not be expected from their actions in vitro.\(^14\) Also, we recently found that bestatin, one of the enzyme inhibitors, induces unique oscillative fluctuations of various enzymatic activities in spleen.\(^18\) Such a phenomenon cannot be explained without introducing the concept of homeostasis in the body. Living organisms have a complicated network of metabolism, the framework of which consists of numerous chains of enzymatic reactions. If one enzyme is inhibited by a specific inhibitor, such an action would initiate a chain of events which affects the whole network. In fact, the low-molecular-weight enzyme inhibitors exert various physiological effects simply not explainable by their direct inhibitory actions.\(^14\,\,19\) These findings prompted us to apply the various inhibitors for modulation of the abnormalities of immunological networks in autoim-

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**TABLE III. Changes in Enzymatic Activity of Hindlimb Muscle of Dystrophic Mice Induced by Low Molecular Weight Inhibitors**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Specific activity ± SD</th>
<th>N=5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>None (N=10)</td>
<td></td>
</tr>
<tr>
<td>AP-A</td>
<td>1.60 ± 0.23</td>
<td></td>
</tr>
<tr>
<td>AP-B</td>
<td>10.07 ± 1.35</td>
<td></td>
</tr>
<tr>
<td>Pro-AP</td>
<td>3.63 ± 0.78</td>
<td></td>
</tr>
<tr>
<td>Leu-AP</td>
<td>4.56 ± 0.37</td>
<td></td>
</tr>
<tr>
<td>fMet-A</td>
<td>3.60 ± 0.31</td>
<td></td>
</tr>
<tr>
<td>Phe-AP</td>
<td>13.41 ± 2.12</td>
<td></td>
</tr>
<tr>
<td>Gly-Pro-Leu-AP</td>
<td>1.28 ± 0.17</td>
<td></td>
</tr>
<tr>
<td>Trypsin-like</td>
<td>36.40 ±10.94</td>
<td></td>
</tr>
<tr>
<td>Chy-try-like</td>
<td>40.17 ± 8.53</td>
<td></td>
</tr>
<tr>
<td>Elastase-like</td>
<td>29.68 ± 4.94</td>
<td></td>
</tr>
<tr>
<td>Carboxypeptidase</td>
<td>0.0042 ± 0.0005</td>
<td></td>
</tr>
<tr>
<td>Glucosidase</td>
<td>0.49 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>Mannosidase</td>
<td>0.21 ± 0.05</td>
<td></td>
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<tr>
<td>GkNACase</td>
<td>0.86 ± 0.15</td>
<td></td>
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<tr>
<td>Creatine kinase</td>
<td>35.68 ± 4.56</td>
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</tr>
<tr>
<td>Phosphatase</td>
<td>1.90 ± 0.79</td>
<td></td>
</tr>
<tr>
<td>Esterase</td>
<td>46.42 ± 7.50</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Leupeptin</th>
<th>Bestatin</th>
<th>Forphenicinol</th>
<th>Forphenicinole</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP-A</td>
<td>1.44 ± 0.22</td>
<td>1.54 ± 0.09</td>
<td>1.28 ± 0.38(^a)</td>
<td>1.36 ± 0.48</td>
</tr>
<tr>
<td>AP-B</td>
<td>9.60 ± 1.55</td>
<td>10.78 ± 0.56</td>
<td>9.76 ± 0.66</td>
<td>8.35 ± 1.06(^b)</td>
</tr>
<tr>
<td>Pro-AP</td>
<td>3.14 ± 0.84</td>
<td>3.48 ± 0.22</td>
<td>4.22 ± 0.56</td>
<td>3.99 ± 0.44</td>
</tr>
<tr>
<td>Leu-AP</td>
<td>6.46 ± 0.20(^a)</td>
<td>7.12 ± 0.37(^a)</td>
<td>6.15 ± 1.49(^c)</td>
<td>5.93 ± 1.44(^b)</td>
</tr>
<tr>
<td>fMet-A</td>
<td>4.87 ± 0.88(^a)</td>
<td>4.70 ± 0.27(^a)</td>
<td>5.24 ± 1.40(^a)</td>
<td>3.82 ± 0.83</td>
</tr>
<tr>
<td>Phe-AP</td>
<td>11.36 ± 0.78(^a)</td>
<td>12.11 ± 0.70</td>
<td>11.86 ± 1.24</td>
<td>9.38 ± 2.69(^c)</td>
</tr>
<tr>
<td>Gly-Pro-Leu-AP</td>
<td>1.38 ± 0.11</td>
<td>1.32 ± 0.09</td>
<td>1.15 ± 0.15</td>
<td>1.08 ± 0.25(^a)</td>
</tr>
<tr>
<td>Trypsin-like</td>
<td>52.35 ± 8.58(^b)</td>
<td>38.05 ± 5.01</td>
<td>33.81 ± 12.51</td>
<td>22.51 ± 4.33(^b)</td>
</tr>
<tr>
<td>Chy-try-like</td>
<td>50.92 ± 14.82(^a)</td>
<td>40.90 ± 6.09</td>
<td>51.95 ± 12.75(^a)</td>
<td>25.21 ± 4.03(^c)</td>
</tr>
<tr>
<td>Elastase-like</td>
<td>36.06 ± 14.86</td>
<td>34.20 ± 3.64(^a)</td>
<td>31.55 ± 5.29</td>
<td>26.00 ± 6.00</td>
</tr>
<tr>
<td>Carboxypeptidase</td>
<td>0.0056 ± 0.0005(^d)</td>
<td>0.0047 ± 0.0006</td>
<td>0.0043 ± 0.0009</td>
<td>0.0034 ± 0.0011(^a)</td>
</tr>
<tr>
<td>Glucosidase</td>
<td>0.52 ± 0.09</td>
<td>0.52 ± 0.03</td>
<td>0.45 ± 0.09</td>
<td>0.37 ± 0.15(^a)</td>
</tr>
<tr>
<td>Mannosidase</td>
<td>0.21 ± 0.02</td>
<td>0.23 ± 0.02</td>
<td>0.18 ± 0.03</td>
<td>0.19 ± 0.04</td>
</tr>
<tr>
<td>GkNACase</td>
<td>1.20 ± 0.19(^a)</td>
<td>0.98 ± 0.19</td>
<td>0.96 ± 0.09</td>
<td>0.71 ± 0.26</td>
</tr>
<tr>
<td>Creatine kinase</td>
<td>34.83 ± 1.78</td>
<td>35.33 ± 10.41</td>
<td>25.71 ± 2.43(^a)</td>
<td>25.71 ± 2.43(^a)</td>
</tr>
<tr>
<td>Phosphatase</td>
<td>1.43 ± 0.50</td>
<td>1.71 ± 0.26</td>
<td>1.44 ± 0.63</td>
<td>2.71 ± 0.51(^a)</td>
</tr>
<tr>
<td>Esterase</td>
<td>49.95 ± 5.95</td>
<td>49.11 ± 6.36</td>
<td>50.71 ± 9.04</td>
<td>34.65 ± 9.20(^b)</td>
</tr>
</tbody>
</table>

\(^a\) p < 0.1, \(^b\) p < 0.05, \(^c\) p < 0.01, \(^d\) p < 0.001.
mune diseases. We found that several inhibitors effectively suppressed the development of experimental allergic encephalomyelitis in guinea pigs. Also, therapeutic trials are now being done on lupus nephritis in New Zealand black and white mouse.

The present results demonstrated the similarity of various inhibitors on enzyme networks in dystrophic mice. Especially, it is noteworthy that forphenincolin presented a peculiarity in its effects on the enzyme networks in spleen. This may be related to its unique immunological actions and may warrant its potential usefulness for the therapy of some intractable diseases.

Acknowledgement This work was partly supported by a grant-in-aid for New Drug Development Research from the Ministry of Health and Welfare, Japan.

### TABLE IV. Changes in Enzymatic Activity of Spleen of Dystrophic Mice Induced by Low Molecular Weight Inhibitors

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Specific activity ± SD</th>
<th>N = 5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>None (N = 10)</td>
<td>Leupeptin</td>
</tr>
<tr>
<td>AP-A</td>
<td>0.52 ± 0.18</td>
<td>0.43 ± 0.05</td>
</tr>
<tr>
<td>AP-B</td>
<td>16.74 ± 1.15</td>
<td>15.78 ± 2.60</td>
</tr>
<tr>
<td>Pro-AP</td>
<td>1.47 ± 0.04</td>
<td>1.60 ± 0.15(^b)</td>
</tr>
<tr>
<td>Leu-AP</td>
<td>4.38 ± 0.98</td>
<td>5.11 ± 1.48</td>
</tr>
<tr>
<td>fMet-AP</td>
<td>11.94 ± 2.13</td>
<td>12.96 ± 2.97</td>
</tr>
<tr>
<td>Phe-AP</td>
<td>4.29 ± 0.12</td>
<td>5.73 ± 1.10(^d)</td>
</tr>
<tr>
<td>Gly-Pro-Leu-AP</td>
<td>0.55 ± 0.01</td>
<td>1.03 ± 0.35(^d)</td>
</tr>
<tr>
<td>Trypsin-like</td>
<td>231.33 ± 52.17</td>
<td>160.73 ± 73.59(^b)</td>
</tr>
<tr>
<td>Chy-try-like</td>
<td>351.13 ± 113.35</td>
<td>299.83 ± 119.69</td>
</tr>
<tr>
<td>Elastase-like</td>
<td>147.43 ± 97.42</td>
<td>126.57 ± 55.77</td>
</tr>
<tr>
<td>Carthepsin D</td>
<td>0.0303 ± 0.0129</td>
<td>0.0384 ± 0.0134(^b)</td>
</tr>
<tr>
<td>Glucosidase</td>
<td>1.05 ± 0.32</td>
<td>0.76 ± 0.34</td>
</tr>
<tr>
<td>Mannosidase</td>
<td>0.74 ± 0.30</td>
<td>0.49 ± 0.34</td>
</tr>
<tr>
<td>GlcNAc-ase</td>
<td>9.11 ± 9.81</td>
<td>3.43 ± 1.16</td>
</tr>
<tr>
<td>Phosphatase</td>
<td>1.94 ± 0.32</td>
<td>2.43 ± 0.48(^b)</td>
</tr>
<tr>
<td>Esterase</td>
<td>15.27 ± 1.10</td>
<td>16.32 ± 1.27(^a)</td>
</tr>
</tbody>
</table>

\(^a\) p < 0.1, \(^b\) p < 0.05, \(^c\) p < 0.01, \(^d\) p < 0.001.

### TABLE V. Correlations among Enzymatic Changes Induced by Low Molecular Weight Inhibitors

<table>
<thead>
<tr>
<th>Organs</th>
<th>Leupeptin</th>
<th>Bestatin</th>
<th>Forphenicol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forelimb</td>
<td>0.69(^a)</td>
<td>0.94(^b)</td>
<td>0.92(^b)</td>
</tr>
<tr>
<td>Hindlimb</td>
<td>0.75(^b)</td>
<td>0.84(^b)</td>
<td>0.86(^b)</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.92(^b)</td>
<td>0.89(^b)</td>
<td>0.08</td>
</tr>
</tbody>
</table>

\(^a\) p < 0.05, \(^b\) p < 0.01.
REFERENCES


