INFLUENCE OF CARBIDOPA, AN AROMATIC AMINO ACID DECARBOXYLASE INHIBITOR, ON THE DEVELOPMENT OF AUTOIMMUNE HEMOLYTIC ANEMIA IN NZB MICE

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Abstract—Causal relationships of carbidopa and its related drugs on the development of spontaneous autoimmune hemolytic anemia (AIHA) in NZB mice were studied, and the following results were obtained: 1) Long term treatment with carbidopa (3 mg/kg/day) and levodopa (30 mg/kg/day) neither accelerated nor suppressed the development of spontaneous AIHA in NZB mice. 2) In mice treated with carbidopa/levodopa (3/30 mg/kg/day), microhematocrit levels were lower than those in the control mice on and after 20 weeks of age and showed a significant decrease at 66 weeks of age (P<0.05). The average anti-RBC antibody titers reached the maximum level 8 weeks earlier than the control group. 3) Microhematocrit levels in the α-methyldopa (60 mg/kg/day)-treated group were higher than those in the control group, and at 66 weeks of age, they were decreased below that in the control group. The elevation of anti-RBC antibody titers was slower than that in the control group. As the reason for the weak effectiveness of α-methyldopa on the incidence of AIHA, it might be considered that the dosage employed was not sufficiently high enough and/or it may be due to the species difference between man and animals. Further studies are necessary in order to draw a conclusion on the AIHA-inducing ability of carbidopa.

Materials and Methods

1. Animals
Forty mice (20 females and 20 males) of
the NZB/BINJ strain were obtained from the Jackson Laboratory (U.S.A.). Inbreeding and raising of NZB mice were performed at a temperature of 23±2°C and a humidity of 55±10%. Weanling NZB mice were separated at 3 weeks of age from their parents and fed a solid diet for breeding (CMF, Oriental Yeast Co.) throughout their lives. Water was given by an automatic water-supply system ad libitum. Male mice at 6 weeks of age which belonged to the 2nd and 3rd generation were divided into 5 groups, consisting of 15–18 mice in each group. The groups were comparable with respect to litter, body weights and generation. Animals in each group were also housed individually and distinguished from each other by the animal number marked on each animal cage.

2. Drugs and dosages

Carbidopa [S(-)-2-hydrazino-3,4-dihydroxy-α-methylhydrocinnamic acid monohydrate, Merck & Co.], levodopa (Sankyo) and α-methyldopa (Merck & Co.) were used. Test drugs were suspended in a 0.5% solution of methylcellulose just before use. The dose levels selected for carbidopa, levodopa, and α-methyldopa corresponded to the maximum doses for man: carbidopa, 3 mg/kg/day; levodopa, 30 mg/kg/day; carbidopa/levodopa, 3/30 mg/kg/day; and α-methyldopa, 60 mg/kg/day. Mice were treated orally by gavage with drug suspensions in a volume of 10 ml/kg once a day on a schedule of 6 consecutive daily doses per week. The treatment was initiated at 6 weeks of age and continued up to 66 weeks of age, and body weights of each animal were measured prior to drug administration. Control mice were treated orally with 0.5% methylcellulose solution as above.

3. Evaluation methods

The effects of test drugs on the development of AI HA in NZB mice were evaluated by the following 4 parameters: 1) the direct Coombs' test which detects autoantibody against red blood cells (RBCs) and its titer, 2) microhematocrit levels as an index of hemolysis, 3) urinary protein concentrations as an index of the presence of glomerular nephritis which is associated with the spontaneous occurrence of AIHA, and 4) mortality rate due to hemolytic anemia.

The measurements were initiated at 8 weeks of age, that is, at 2 weeks after the commencement of drug administration and continued afterwards at serial intervals of approximately 4 weeks.

1) Microhematocrit levels and direct Coombs' test: Two hundred to 400 μl of blood was obtained by orbital sinus puncture using heparinized capillary tubes for microhematocrit determination (Terumo Co.) and then centrifuged at 11,000 rpm for 5 min. Percent packed RBC volumes were measured by a standard microhematocrit method. The RBCs from the treated and control mice were used for agglutination with antiserum to mouse immunoglobulins. The RBCs were washed three times with 0.138M phosphate buffered saline (PBS, pH 7.4) and resuspended in PBS to make a 2% suspension. Rabbit anti-mouse immunoglobulin G sera (Miles Laboratories, Inc., U.S.A.) were inactivated at 56°C for 30 min. A direct Coombs' test was performed using microtiter plates with 25 μl of 2% RBCs suspension and 25 μl of a serial two-fold dilution of the antiserum. After being mixed on a plate mixer, the plates were left undisturbed at 37°C overnight. Reading of hemagglutination was performed stereoscopically. The reciprocal of the highest antiserum dilution giving an unequivocally positive reaction was recorded as the anti-RBC antibody titer.

2) Urinary protein concentrations: Urinary protein was assayed using Hema-Combistix II (reagent strips integrated with tetrabromo-phenol-blue, pH 3.0, Miles-Sankyo Co.). The urinary protein concentrations manifested by the changes of color was graded as follows:
- = none, + = trace, + = 30 mg/dl, ++ = 100 mg/dl and +++ = 300 mg/dl.

3) Mortality rate: Autopsies of the dead animals were performed for a histopathologic examination to investigate the possible cause of their deaths.

4. Statistical analysis

Differences in anti-RBC antibody titers, microhematocrit levels and body weights between each group were compared every 4 weeks using a one-way analysis of variance and multiple comparisons based on the application of Bonferroni’s inequality. Since anti-RBC antibody titers \( (2^2, 2^3, 2^4, \ldots) \) did not show a normal distribution in the present analysis, their exponents \( (2, 3, 4, \ldots) \) were substituted for the titers. Probability values of less than 0.05 were considered significant.

Results

1. Influence on body weights and mortality rate: The change of body weights is shown in Table 1. The mean body weights of mice in each group were 27.0–27.5 g at the beginning of the treatment, i.e., at 6 weeks of age. No significant difference in body weights between the control group and each treated group was observed up to 66 weeks of age. At 64 and 66 weeks of age, however, body weights in the carbidopa-treated and carbidopa/levodopa-treated groups were significantly heavier in comparison with the control group (\( P<0.05 \)). During the experimental period, 4 to 6 mice died in each group, and the mortality rate in the treated groups was about the same as that in the control group. The findings obtained in histopathological examinations of the dead animals in each group were non-specific for the drug-treatment and corresponded to those which are often observed in non-treated aging NZB mice. The possible causes of their deaths judged from histopathological examinations are shown in Table 2. Except for animal No. 10–29–80–6, which died from asphyxia when being bled, and 12–15–80–3 in the levodopa-treated group, 12–15–80–15 and 9–22–80–6 in the carbidopa/levodopa-treated group and 12–15–80–5 in the α-methyldopa-treated group, which were not examined because of postmortem autolysis, the possible causes of their deaths were judged to be pyelonephritis, urethremphraxis, tubular nephrosis, necrotic sytisitis, hyperplastic nodele of liver, reticulum cell sarcoma of liver, peritonitis, catarrhal pneumonia and/ or hemolytic anemia. Therefore, the dosages of test drugs used in this experiment seem to have had no apparent toxic effects on the mice. The findings, including extramedullary hematopoiesis in the liver and spleen, hemosiderin deposition in Kupffer’s cell, hematopoiesis in bone marrow and pyelonephritis, which suggest the development of AIHA, were obtained in these histopathological examinations on and after 44 weeks of age.

2. Influence on microhematocrit levels (Table 3): Significant reductions in microhematocrits were observed with increasing age in all groups. The mean microhematocrit levels in the control group were 55.2±0.61% at 8 weeks of age, 44.2±1.22% at 44 weeks of age and 40.3±1.36% at 66 weeks of age. The mean microhematocrit levels in both carbidopa-treated and levodopa-treated groups were 54.3±0.75% and 54.4±0.61% at 8 weeks of age, respectively. Thereafter, progressive decreases of microhematocrits in both treated groups were similar to those in the control group.

In the carbidopa/levodopa-treated group, the mean microhematocrit level was 56.6±0.43%, a little higher than that in the control group, at 8 weeks of age. On and after 20 weeks of age, however, the levels were slightly but consistently lower than those in the control group, and at 66 weeks of age, it showed a significant decrease (\( P<0.05 \)). The mean microhematocrit level (55.3±
Table 1. Body weight changes in male NZB mice treated chronically with test drugs

| Drug          | Daily dose (mg/kg, p.o.) | No. of animals | Mean body weight ± S.E. (g) at age: |
|---------------|--------------------------|----------------|------------------------------------|
|               |                          |                | 6 8 12 16 20 24 28 32 36 40 44 48 52 56 60 64 66 Weeks | |
| 0.5% MC       | 10 (ml/kg)               | 18             |                                      |
|               |                          |                | 27.3 ± 0.59 31.1 ± 0.64 35.5 ± 0.68 38.3 ± 0.79 40.5 ± 1.04 42.2 ± 1.26 43.2 ± 1.47 45.3 ± 1.59 46.8 ± 1.71 45.7 ± 1.85 45.8 ± 2.06 46.0 ± 2.19 44.3 ± 2.56 43.3 ± 1.90 41.0 ± 1.65  |
| Carbidopa    | 3                        | 16             |                                      |
|               |                          |                | 27.3 ± 0.04 21.1 ± 0.60 35.7 ± 0.01 39.1 ± 0.77 40.0 ± 0.79 42.7 ± 0.88 44.0 ± 1.09 45.3 ± 1.18 46.6 ± 1.22 46.6 ± 1.38 46.6 ± 1.55 46.7 ± 1.92 47.4 ± 1.81 46.7 ± 1.80 46.3 ± 1.73 46.0 ± 1.97 45.0 ± 1.81 |
| Levodopa     | 20                       | 15             |                                      |
|               |                          |                | 27.3 ± 0.43 31.0 ± 0.39 36.8 ± 0.52 38.8 ± 0.61 41.0 ± 0.84 42.1 ± 0.68 43.8 ± 0.83 45.1 ± 0.73 46.4 ± 0.85 46.5 ± 1.05 46.6 ± 1.16 46.6 ± 1.25 46.2 ± 1.42 45.6 ± 1.71 45.1 ± 1.42 44.4 ± 1.41 |
| Carbidopa/Lev | 3/20                    | 17             |                                      |
| Levodopa     |                          |                | 27.5 ± 0.23 31.4 ± 0.51 35.6 ± 0.34 38.8 ± 0.48 41.1 ± 0.38 42.7 ± 0.07 44.7 ± 0.80 44.8 ± 0.76 46.6 ± 0.84 46.5 ± 0.99 46.6 ± 1.04 46.5 ± 1.10 47.9 ± 1.11 47.3 ± 1.04 46.8 ± 1.07 45.6 ± 1.12 |
| e-Methyllevo  | 60                       | 17             |                                      |
|               |                          |                | 27.0 ± 0.04 31.1 ± 0.45 35.1 ± 0.51 38.5 ± 0.65 40.4 ± 0.71 41.8 ± 0.65 43.2 ± 0.91 43.7 ± 0.96 44.9 ± 1.01 44.6 ± 1.16 44.9 ± 1.13 44.8 ± 1.15 44.6 ± 1.11 44.4 ± 1.54 41.7 ± 1.13 41.3 ± 1.08 |

Figure in parenthesis indicates the number of dead animals. Animal No. 1) 12-15-60-11, 2) 9-10-60-11, 3) 10-10-60-10, 4) 10-22-60-1 and 10-22-80-8, 5) 12-15-80-10, 6) 12-15-80-11, 7) 11-7-60-2 and 9-10-80-10, 8) 10-60-60-9, 9) 10-22-80-6, 10) 10-22-80-3, 11) 12-15-80-3, 12) 12-15-80-9, 13) 9-22-60-3, 14) 10-10-60-5, 15) 12-15-80-16, 16) 9-22-80-6 and 12-15-80-13, 17) 12-15-60-7, 10-22-80-2 and 12-15-80-5, and 18) 10-22-80-1. 1) This animal died from asphyxia when being bled by orbital sinus puncture. *: Significant difference from the 0.5% MC group (P<0.05).
### Table 2. Possible cause of death judged from results of histopathologic examination

| Drug      | Daily dose (mg/kg, p.o.) | Animal No. | Possible cause of death |
|-----------|--------------------------|------------|-------------------------|
| 0.5% MC   | 10 (ml/kg)               | 12-15-80-11 9-10-80-11 10-22-80-01 10-10-80-10 10-22-80-08 | Pyelonephritis Pyelonephritis Pyelonephritis Unknown |
|           |                          | 12-15-80-10 12-15-80-02 10-10-80-01 11-07-80-02 9-10-80-10 9-10-80-02 | Pyelonephritis Pyelonephritis Neutrophilic abscess of liver and/or tubular nephrosis |
| Carbidopa | 3                        | 10-29-80-06 10-22-80-06 12-15-80-03 10-29-80-03 10-22-80-09 9-22-80-03 | Asphyxia Urethrephraxis and/or peritonitis<sup>a</sup> N.E. Reticulum cell sarcoma of liver and/or tubular nephrosis Hyperplastic nodule Pyelonephritis and/or purulent cystitis |
| Levodopa  | 30                       | 10-10-80-05 12-15-80-15 9-22-80-06 12-15-80-13 | Pyelonephritis N.E. N.E. Catarrhal pneumonia |
| Carbidopa/Levodopa | 3/30                 | 12-15-80-07 10-22-80-02 12-15-80-05 10-29-80-01 | Pyelonephritis Hyperplastic nodule of liver N.E. Necrotic cystitis |

<sup>a</sup> Since peritonitis was not found in the histopathologic examination because of postmortem autolysis, this diagnosis was made from macroscopic findings. N.E.: Not examined because of postmortem autolysis.
### Table 3. Effects of test drugs on microhematocrit levels in NZB mice

| Drug         | Daily dose (mg/kg, p.o.) | No. of animals | Mean microhematocrit ± S.E. (%) at age: |
|--------------|--------------------------|----------------|----------------------------------------|
|              |                          |                | 8 | 12 | 16 | 20 | 24 | 28 | 32 | 36 | 40 | 44 | 66 Weeks |
| 0.5% MC      | 10 (ml/kg)               | 18             | 55.2 | 52.1 | 50.4 | 50.6 | 49.4 | 48.3 | 47.0 | 46.6 | 45.6 | 44.2 | 40.3 |
|              |                          |                | ±0.61 | ±0.42 | ±0.50 | ±0.44 | ±0.51 | ±0.42 | ±0.70 | ±0.49 | ±0.81 | ±1.22 | ±1.36 |
| Carbidopa    | 3                        | 16             | 54.3 | 52.7 | 50.5 | 49.6 | 49.0 | 47.8 | 48.0 | 46.7 | 44.8 | 42.9 | 38.4 |
|              |                          |                | ±0.75 | ±0.40 | ±0.53 | ±0.50 | ±0.42 | ±0.53 | ±0.56 | ±0.56 | ±1.01 | ±1.09 | ±1.56 |
| Levodopa     | 30                       | 15             | 54.4 | 50.7 | 51.6 | 49.4 | 49.5 | 48.7 | 47.3 | 45.3 | 45.4 | 44.0 | 37.7 |
|              |                          |                | ±0.61 | ±0.47 | ±0.46 | ±0.51 | ±0.48 | ±0.73 | ±0.40 | ±0.83 | ±0.71 | ±1.12 | ±0.77 |
| Carbidopa/Lv | 3/30                     | 17             | 56.6 | 51.9 | 50.9 | 49.1 | 49.1 | 47.9 | 46.8 | 45.6 | 44.1 | 43.3 | 35.7* |
|              |                          |                | ±0.43 | ±0.28 | ±0.47 | ±0.39 | ±0.57 | ±0.47 | ±0.65 | ±1.04 | ±1.09 | ±0.90 | ±1.37 |
| α-Methyladopa| 60                       | 17             | 55.3 | 52.8 | 52.0 | 51.2 | 50.7 | 49.0 | 48.6 | 47.9 | 47.2 | 44.7 | 38.2 |
|              |                          |                | ±0.40 | ±0.50 | ±0.44 | ±0.51 | ±0.44 | ±0.47 | ±0.50 | ±0.45 | ±0.63 | ±1.19 | ±1.52 |

*: Significant difference from the 0.5% MC group (P < 0.05)

### Table 4. Development of positive RBC antiglobulin reactions in NZB mice treated with test drugs

| Drug            | Daily dose (mg/kg, p.o.) | No. of animals | Percent of mice with positive* direct antiglobulin reactions at age: |
|-----------------|--------------------------|----------------|----------------------------------------------------------|
|                 |                          |                | 16 | 20 | 24 | 28 | 32 | 36 | 40 | 44 | 66 Weeks |
| 0.5% MC         | 10 (ml/kg)               | 18             | 16.7 | 38.9 | 52.9 | 52.4 | 100 | 100 | 100 | 100 | 100 |
| Carbidopa       | 3                        | 16             | 18.8 | 31.3 | 62.5 | 87.5 | 100 | 100 | 100 | 100 | 100 |
| Levodopa        | 30                       | 15             | 13.3 | 46.7 | 66.7 | 71.4 | 100 | 92.9 | 100 | 100 | 100 |
| Carbidopa/Lv    | 3/30                     | 17             | 23.5 | 29.4 | 64.7 | 70.6 | 93.8 | 100 | 100 | 100 | 100 |
| α-Methyladopa   | 60                       | 17             | 17.6 | 47.1 | 64.7 | 70.6 | 94.1 | 94.1 | 100 | 100 | 100 |

*: Serum with an anti-RBC antibody titer of 2^2 or more was regarded as positive.
0.40%) in the α-methyldopa treated group at 8 weeks of age was almost the same level as the control group. Thereafter, the levels were consistently but not significantly higher than those in the control group, and at 44 weeks of age, the levels were about the same as those in the control group. At 66 weeks of age, the levels were lower than those in the control group, but the decrease was not significant.

3. Influence on the direct Coombs' test (Figs. 1–5): The onset of Coombs' positive reactions was similar in all groups with 13.3–23.5% of the animals at 16 weeks of age (Table 4). Thereafter, the rate of appearance of Coombs' positive reactions in each group increased with advancing age. In the control, carbidopa-treated and levodopa-treated groups, positive reactions were observed in all animals at 32 weeks of age. Both carbidopa/levodopa-treated and α-methyldopa-treated groups showed Coombs' positive reactions in all animals at 36 and 40 weeks of age, respectively, later than in the control group.

The titers of anti-RBC antibody which induced Coombs' positive reactions showed
a marked increase on and after 24 weeks of age in all groups. The average anti-RBC antibody titers in the control, carbidopa-treated and levodopa-treated groups reached the maximum level at 40, 44 and 40 weeks of age, respectively. In the carbidopa/levodopa-treated group, the maximum titer was observed at 32 weeks of age, 8 weeks earlier than the control group, contrary to the delayed appearance of Coombs’ positive reactions in some animals in this group. In the α-methyldopa-treated group, the elevation of the antibody titers and the rate of appearance of the Coombs’ positive reaction was slower than the control group, and the average reached the maximum level at 44 weeks of age.

For the anti-RBC antibody titers, there was no significant difference between groups, with the exception of a significant difference (P<0.05) between the α-methyldopa-treated group and the carbidopa/levodopa-treated group at 36 weeks of age.

4. Influence on urinary protein concentrations (Table 5): In the control group, animals whose urinary protein concentrations were continuously 100 mg/dl and more appeared on and after 44 weeks of age. In the carbidopa-treated, levodopa-treated and α-methyldopa-treated groups, animals which had 100 mg/dl and more of urinary protein concentrations appeared on and after 52, 44 and 52 weeks of age, respectively; but in the carbidopa/levodopa-treated group, the animals appeared on and after 40 weeks of age, 4 weeks earlier than the control group. Furthermore, there was a marked elevation of urinary protein concentrations in the combination-treated group in comparison with those in the other groups. It is considered, therefore, that the formation of glomerular nephritis associated with the spontaneous occurrence of AIHA was enhanced to a certain extent in the carbidopa/levodopa-treated animals.

Discussion

A positive direct Coombs’ test and rare
| Drug       | Daily dose (mg/kg, p.o.) | No. of animals | Urinary protein levels* | Percent of animals in each urinary protein level at age: |
|------------|--------------------------|----------------|-------------------------|--------------------------------------------------------|
|            |                          |                | 0 | 12 | 16 | 20 | 24 | 28 | 32 | 36 | 40 | 44 | 48 | 52 | 56 | 60 | 64 | 68 |
|            |                          |                | - |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 0.5% MC    | 10 (mg/kg)               | 18             | - | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
|            |                          |                | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
|            |                          |                | ++| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
|            |                          |                | +++| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Carbidopa  | 3                        | 16             | - | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
|            |                          |                | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
|            |                          |                | ++| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Levodopa   | 50                       | 15             | - | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
|            |                          |                | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
|            |                          |                | ++| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
|            |                          |                | +++| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Carbidopa/ | 2                        | 17             | - | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Levodopa   | 30                       |                | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
|            |                          |                | ++| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
|            |                          |                | +++| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0-MethylDop| 60                       | 17             | - | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
|            |                          |                | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
|            |                          |                | ++| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
|            |                          |                | +++| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* Urinary protein levels: - = none, ± = trace, + = 30 mg/dl, ++ = 100 mg/dl, and +++ = ≥300 mg/dl.
AIHA are known complications of α-methyldopa treatment, particularly when it is given at higher dosage levels. The mechanism of this effect is not known (13, 14). The fact that drug-associated AIHA has not been reproduced unequivocally in animal experiments also seems to be one of the reasons why the mechanism is still unknown. Peck et al. in Merck Sharp & Dohme Research Laboratories performed chronic toxicity studies with 20 and 1,000 mg/kg/day of α-methyldopa in rats, dogs and monkeys in order to investigate the relationship of hemolytic anemia and positive Coombs' test with the administration of α-methyldopa (unpublished data). However, they found neither hemolytic anemia nor positive Coombs' tests in any of the test animals. Consequently, it was considered to be difficult to investigate drug-associated AIHA using normal animals. In the present study, therefore, the AIHA inducing ability of carbidopa was investigated by testing its influence on the development of spontaneous AIHA in NZB mice.

The results of the present studies indicate that increases in anti-RBC antibody titers and reductions in microhematocrit levels in both carbidopa-treated and levodopa-treated groups were not significantly different from those in the control group. In the carbidopa/levodopa-treated group, microhematocrit levels were lower than those in the control group and after 20 weeks of age and showed a significant decrease at 66 weeks of age (P<0.05). Also, the elevation of anti-RBC antibody titers in the combination group was earlier than that in the control group, and the titer reached the maximum levels at 32 weeks of age. Furthermore, in the combination group, the marked elevation of urinary protein levels in comparison with that in the other groups suggests the formation of glomerular nephritis associated with the spontaneous occurrence of AIHA. It is possible that these findings obtained in the combination group occurred by chance. However, when the fact that levodopa chemotherapy induces AIHA in a few patients (15–17) is taken into account, it is possible that these changes are due to levodopa treatment.

In the α-methyldopa-treated group, microhematocrits showed higher levels than those in the control group; and at 66 weeks of age, the levels decreased below that in the control group. In addition, the increase of anti-RBC antibody titers in the treated group was slower than that in the control group.

Jarvinen et al. (18) also investigated the influence of long term administration of α-methyldopa in NZB mice. They treated NZB mice either orally or by subcutaneous injection with α-methyldopa hydrochloride. Mice treated orally received a constant dose of 0.4 mg/ml of drinking water for the life of the animal, and animals treated by injection received one daily dose of 5 mg subcutaneously for a period of 6.7 months. Their data showed that there was no significant differences in microhematocrit levels and the number of mice with positive direct antiglobulin reactions between treated and control groups. They concluded that age and a genetic predisposition to autoimmune disorders are not sufficient to induce α-methyldopa-associated AIHA in mice even with long term high dose drug treatment. In their studies, the incidence of direct anti-globulin reactions was measured, but the movement of anti-RBC antibody titers was not; furthermore, prolongation of the survival time was found in the subcutaneously treated NZB mice in comparison with the control mice. Consequently, when the influences of α-methyldopa on NZB mice are considered on the basis of our data as well as theirs, it is possible that α-methyldopa acts to suppress a genetic predisposition to antibody production. Nevertheless, these results are contrary to the effects of α-methyldopa that
have been shown in man and the conclusion that \(\alpha\)-methyldopa inhibits suppressor T-lymphocyte function by causing a persistent increase in lymphocyte cyclic-AMP content (19). Therefore, the phenomena observed in NZB mice treated with \(\alpha\)-methyldopa merit further investigation.

Thus, although the AIHA-inducing ability of carbidopa, levodopa and \(\alpha\)-methyldopa was investigated by testing their effects on the spontaneous AIHA in NZB mice, none of the drugs exhibited remarkable drug-associated AIHA. It might be suggested that the dosages of these drugs employed were not sufficiently high enough and/or the weak effect may be caused by the species difference between man and animals. Therefore, further studies are necessary to draw a conclusion on the AIHA-inducing ability of carbidopa.

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