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## Effect of diethylcarbamazine on testing serum samples for retroviral antibodies

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Previous investigations have revealed that *in vivo* use of diethylcarbamazine (*N,N*-diethyl-4-methyl-1-piperazine carboxamide; DEC), the mainstay of prevention and therapy for filariases, results in increased titers of antibody to feline oncornavirus-associated cell membrane antigen (FOCMA) in feline leukemia virus (FeLV)-infected cats. In the present study, the effect of *in vitro* DEC on detection of serum antibodies to retroviruses was investigated. The addition of DEC (5 mg ml<sup>-1</sup>, pH 7.3–7.4) to the serum diluent resulted in detection of antibody to FOCMA by indirect membrane immunofluorescence in five of five FeLV-infected cats, whereas samples from all five cats tested negative without the addition of DEC. *In vitro* DEC also increased the absorbance values generated by enzyme immunoassay human immunodeficiency virus type 1 (HIV-1) antibody testing of samples from (a) persons with AIDS-related disease; (b) persons with helper T-lymphocytes < 500 mm<sup>-3</sup> blood; and (c) persons testing positive for antibody to HIV-1 *env*- but not *gag*-related antigens as determined by radioimmunoprecipitation and sodium dodecyl sulfate polyacrylamide gel electrophoresis (RIP-SDS/PAGE). We conclude that *in vitro* as well as *in vivo* use of DEC may alter results of antibody testing for retroviruses.

*Keywords:* retroviral antibodies, diethylcarbamazine, HIV, FeLV

### Introduction

The beneficial effects of *in vivo* diethylcarbamazine (*N,N*-diethyl-4-methyl-1-piperazine carboxamide; DEC) in treatment and prevention of feline leukemia virus infection of cats (one animal model for AIDS) have been reported elsewhere<sup>1,2</sup>. These benefits include an increased titer of serum antibodies to feline oncornavirus-associated cell membrane antigen (FOCMA). High titers of serum antibody to FOCMA in FeLV cats have been associated with resistance to FeLV-related neoplasias<sup>3,4</sup>. Nine cats that tested negative before treatment tested positive [ $\geq 1:10$  serum dilution, geometric mean titer (GMT) = 278] for antibody to FOCMA after DEC treatment. Among 19 cats initially testing positive for FOCMA antibody, higher titers were noted after treatment in 17 (pretreatment GMT of 19 cats = 264, post-treatment GMT = 6158)<sup>1</sup>. Administration of DEC for 2 weeks after one dose of FeLV vaccine increased the titer and duration of

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antibody to FOCMA compared to untreated, vaccinated controls<sup>2</sup>. The DEC treatment also resulted in increased serum titers of antibody of feline infectious peritonitis (FIP), a coronavirus, in seven DEC-treated cats compared with seven untreated cats. FIP-related disease frequently complicates FeLV infection in cats<sup>5</sup>.

These experiments were prompted by data from two earlier investigations suggesting that DEC has an opsonic effect on microfilariae. First, the *in vivo* use of DEC resulted in a rapid fall of circulating microfilariae in cotton rats experimentally infected with *Litomosoides carinii* due to hepatic sequestration<sup>6</sup>. Second, in *in vitro* studies, DEC has been shown to facilitate microfilarial adherence to peripheral blood cells in the presence of serum containing antibodies to microfilarial sheaths<sup>7</sup>. These actions of DEC may be independent from DEC-induced killing of microfilariae<sup>8</sup>.

The above data raise several important questions of immediate relevance to current AIDS research. First, could DEC be of benefit in preventing/treating retroviral infections, either alone or in combination with antiretroviral drugs such as 3'-Azido-3-deoxythymidine (AZT)? Second, will the widely-practiced use of DEC as an antifilarial agent among some African populations alter the serologic outcomes of Africans enrolled in HIV vaccine trials? Third, will the planned substitution of ivermectin, a recently introduced antifilarial agent, for DEC result in increased HIV-related morbidity and mortality in Africa?

To find out more about the possible benefits of DEC, we investigated the effect of the *in vitro* addition of DEC to the serum diluent of serum samples of cats tested for antibody to FOCMA and of humans tested for antibody to the human immunodeficiency virus type 1 (HIV-1).

## Materials and methods

### *Serum samples*

Samples from 10 cats were analysed for antibody to FOCMA. Five of the cats tested positive for FeLV leukocyte antigens (tests were performed by the Laboratory of Veterinary Oncology, Memorial Sloan-Kettering Cancer Center, New York City, U.S.A., as described previously<sup>9</sup>), and all five had high titers of serum infectious virus by the assay of Fischinger<sup>10</sup>. The remaining five cats were from specific pathogen-free breeding colonies and known to be FeLV-naive; all five tested negative for FeLV leukocyte antigens and serum infectious virus.

Of 140 human serum samples tested for antibody to HIV by enzyme immunoassay (EIA), 75 were from asymptomatic persons testing negative for HIV antibody by RIP-SDS/PAGE<sup>11</sup>, including 20 blood bank donors, 30 dialysis patients, 15 hemophiliacs and 10 wives of seronegative hemophiliacs. The remaining 65 samples tested positive for HIV antibody by RIP-SDS/PAGE, and included samples from 57 hemophiliacs, two wives of seropositive hemophiliacs, and six non-hemophiliac patients. Of these 65, 40 were from asymptomatic persons and 25 from patients with AIDS-related disease. Seven additional serum samples from AIDS patients were tested by western blot for HIV antibody profiles.

### *Antibody assays*

Serum antibody to FOCMA was determined by indirect membrane immunofluorescence (IMI) on FL-74 cells, using 10-fold dilutions ( $1:10^1$  to  $1:10^5$ ) of serum in

phosphate-buffered saline after exhaustive absorption of the sample with freshly isolated normal feline lymphocytes. Antibody titers are reported as the highest serum dilution with which >50% of the observed FL-74 cells fluoresced.<sup>3</sup> Serum samples from 140 persons were tested for antibody to HIV both by RIP-SDS/PAGE using H9/HIV-1 (HTLV-III)-infected cells and by the Abbott antibody to HIV-1 (HTLV-III) enzyme immunoassay (EIA). Absorbance values were determined with a Quantum II Analyzer (Abbott Laboratories, North Chicago, IL, U.S.A.). RIP-SDS/PAGE Analysis was performed at the Harvard School of Public Health as described previously<sup>11</sup>.

All 10 feline samples analysed by IMI, 140 human samples analysed by EIA, and an additional seven human samples tested by Western blot (DuPont Company, Wilmington, DE 19898, U.S.A.) were simultaneously tested with the addition of DEC, 5 mg ml<sup>-1</sup>, to the serum diluent (pH corrected to 7.3-7.4 with 4 N NaOH) during the serum incubation phase of the assay. This solution permitted a high concentration of dissolved drug. Both medicated and unmedicated serum diluent solutions were filtered through a 0.45 µm filter prior to use, in order to remove any debris.

#### *T-Cell subsets*

Analyses of T-Cell subsets were performed on blood lymphocyte samples using monoclonal antibodies to Leu-3 (helper/inducer T cells), Leu-2 (suppressor/cytotoxic T cells), and Leu-4 (total T cells).

### Results

*In vitro* addition of DEC resulted in detection of antibody to FOCMA (1:10<sup>1</sup> dilution) in five of five serum samples from FeLV-infected cats, whereas all five samples tested negative (<1:10 dilution) without DEC. Antibody to FOCMA was not detected in serum samples from FeLV-naive cats, with or without addition of DEC to the serum diluent.

The addition of DEC to the serum diluent did not alter the HIV-1 serologic profiles or background upon testing serum samples of seven AIDS patients by Western blot. However, the gp 160 bands on the Western blot strips yielded by four of the seven samples were slightly more intense with the addition of DEC.

Some of the results regarding the addition of DEC to serum diluent during testing of antibody to HIV by EIA appear in Tables 1, 2, and 3. Samples from 65 persons testing positive for antibody to HIV by RIP-SDS/PAGE all tested positive by EIA with or

**Table 1.** Effect of DEC on testing serum samples for antibody to HIV-1 by EIA: relationship to clinical status

| No. tested/<br>clinical<br>status | No. posi-<br>tive by<br>RIP-SDS/<br>PAGE | No. posi-<br>tive by<br>EIA | No. posi-<br>tive by<br>EIA + DEC | Significant<br>effect of DEC<br>on absorbance<br>values of EIA? |
|-----------------------------------|--|-----------------------------|-----------------------------------|---|
| 75/asymptomatic                   | 0  | 17                          | 15                                | No  |
| 25/asymptomatic                   | 25                                       | 25                          | 25                                | No  |
| 15/asymptomatic                   | 15                                       | 15                          | 15                                | Not analysed  |
| 7/AIDS-related disease            | 7  | 7                           | 7                                 | Not analysed  |
| 18/AIDS-related disease           | 18                                       | 18                          | 18                                | Yes, $P < 0.0007$   |

**Table 2.** Effect of DEC on testing serum samples for antibody to HIV-1 by EIA: relationship to HIV-1 antibody profile

| No. tested/HIV-1 antibody profile  | No. positive by RIP-SDS/PAGE | No. positive by EIA | No. positive by EIA + DEC | Significant effect of DEC on absorbance values of EIA? |
|--|------------------------------|---------------------|---------------------------|--|
| 18/testing positive for antibody to gp120/160 and p24                          | 18                           | 18                  | 18                        | No   |
| 25/testing positive for antibody to gp120/160 but negative for antibody to p24 | 25                           | 25                  | 25                        | Yes,<br>$P < 0.03$                                     |

**Table 3.** Effect of DEC on testing serum samples for antibody to HIV-1 by EIA: relationship to the number of helper T-lymphocytes per mm<sup>3</sup> blood

| No. tested/helper T-lymphocyte status                   | No. positive by EIA | No. positive by EIA + DEC | Significant effect of DEC on absorbance values of EIA? |
|---|---------------------|---------------------------|--|
| 29/helper T-lymphocyte counts > 500 per mm <sup>3</sup> | 29                  | 29                        | No   |
| 14/helper lymphocytes < 500 per mm <sup>3</sup>         | 14                  | 14                        | Yes,<br>$P < 0.03$                                     |

without addition of DEC to serum diluent. Of these 65 samples, 22 (15 from asymptomatic persons and seven from patients with AIDS-related disease) yielded absorbance values > 2 (the upper limit of positivity recorded by the Quantum II Analyzer) with and without addition of DEC to the serum diluent, and were omitted from these statistical analyses.

The remaining 43 samples consisted of 25 samples from asymptomatic persons and 18 samples from patients with AIDS-related diseases. The "DEC difference" for a given sample is the number obtained by subtracting the absorbance value yielded by a sample without DEC from the absorbance value generated by the same sample with DEC. The DEC differences were positive for 12 of the 25 asymptomatic cases, and negative for the remainder. The null hypothesis, that the median of the distribution of the DEC differences = 0, was tested against the alternative hypothesis that the median was > 0 by means of a one-tailed sign test (using the normal approximation with continuity correction and level of significance of 0.05). The null hypothesis was not rejected. The DEC differences were positive for 16 of the 18 AIDS-related patients, and negative for the remainder; the corresponding sign test for this group (using the binomial distribution) rejected the null hypothesis ( $P < 0.0007$ ).

These 43 serum samples included 25 patients who tested positive for antibody to HIV-1 *env* glycoproteins but negative for antibody to *gag* gene-encoded p24 by RIP-SDS/PAGE. The DEC differences were positive for 18 of the 25 patients, and negative for the remainder; the corresponding sign test for this group rejected the null hypothesis ( $P < 0.03$ ). The DEC differences were positive for six of the 18 remaining samples that tested positive for both antibody to HIV-1 *gag* and *env* proteins; the null hypothesis was not rejected.

Also included among these 43 serum samples were 14 samples from patients with  $> 500$  T-helper/inducer lymphocytes per  $\text{mm}^3$  blood. The DEC differences were positive for 11 of the 14 samples; the corresponding sign test for this group using the binomial distribution rejected the null hypothesis ( $P < 0.03$ ). The remaining 29 patients had  $> 500$  T-helper/inducer lymphocytes per  $\text{mm}^3$  blood; the DEC differences were positive for 13 of the 29 samples and negative for the remainder; the null hypothesis was not rejected.

Among 75 persons testing negative for antibody to HIV by RIP-SDS/PAGE, 17 tested positive by EIA without DEC and 15 tested positive with DEC. The DEC differences were positive for 37 of the 75 and negative for the remainder; a one-tailed sign test of the null hypothesis that the median was  $= 0$  did not reject the null hypothesis at the 0.05 level of significance.

### Discussion and conclusions

DEC may more consistently enhance detection of antibody to FOCMA in serum samples from FeLV cats because circulating FOCMA antigens have not been reported in such cats. DEC may less dramatically/consistently improve detection of antibodies to HIV because such patients' serum samples may also contain HIV antigens, and DEC may enhance binding of HIV antibodies with HIV serum antigens, in addition to HIV antigens used in commercial assays. However, DEC *in vitro* increases absorbance values generated by HIV antibody testing of serum samples by EIA from (1) patients with AIDS-related disease, (2) patients who test positive for *env* gene-encoded glycoproteins (gp120 and gp160) but not *gag* gene-encoded proteins (p24 and p55) of HIV-1, and (3) patients with  $< 500$  T-helper lymphocytes per  $\text{mm}^3$  blood. These three groups include significantly immunocompromised HIV-1 infected patients<sup>13</sup>. One possible explanation for the above findings is that the rate of *in vivo* HIV-1 genetic changes—approximately  $10^{-2}$  to  $10^{-3}$  nucleotide substitutions per site per year for *env* and 10-fold fewer for *gag*—may prevent HIV-infected immunocompromised patients from mounting an optimal serologic response in the late stages of HIV infection<sup>13</sup>. Such patients' serum samples could contain antibodies that do not bind circulating variant HIV glycoprotein antigens, but can bind less variant HIV glycoproteins used in commercial kits, and DEC could enhance the latter process. This hypothesis could also explain why the gp160 bands on Western blots using serum samples from four of seven AIDS patients were slightly intensified when DEC was added *in vitro*. In this regard, the use of DEC *in vitro* to optimize detection of antibody to HIV-1 glycoproteins may be important to the successful differential diagnosis of patients with AIDS or AIDS-related diseases, or immunocompromised HIV-1 infected patients, since such patients may have low amounts of antibody to gp120 and gp160 and even lower or undetectable amounts of antibody to p24 or p55.

It is conceivable that the observed increases in FOCMA antibody in FeLV cats after *in vivo* DEC treatment may be augmented by increased antigen-antibody interactions

demonstrable *in vitro* with high concentrations of this drug, but data regarding body fluid concentrations of DEC following administration of this drug to cats are not known to us. Whether DEC increases retroviral antigen-immune host cell interactions resulting in increased humoral responses, as has been shown in connection with DEC treatment of humans with filariasis<sup>14</sup>, requires further study; this effect appears likely since DEC increases FOCMA antibody titers in FeLV-vaccinated cats. The mechanisms by which DEC might enhance antigen-antibody and antigen-immune host cell interactions are unknown. Since *in vivo* DEC treatment alters titers of serum antibody to non-retroviruses such as FIP<sup>5</sup>, our results also may have implications for strategies to improve assays for some antibodies to non-retroviral infectious agents or related antigens.

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