Effects of 2'-Deoxycoformycin Infusion on Mouse Adenosine Deaminase

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ABSTRACT

The effect of 2'-deoxycoformycin (DCF) on activity levels and physicochemical properties of mouse adenosine deaminase (ADase) and purine nucleoside phosphorylase from nine tissues was characterized. This tight-binding inhibitor of ADase was continuously infused into C57BL mice for five days. Various levels of ADase which were tissue dependent remained after this period. Thymus activity was the most severely inhibited; jejunum, ileum, and spleen activities were only marginally depressed; and stomach activity was not inhibited. The presence of a transplantable colon tumor resulted in significantly lower ADase after infusion, especially in jejunum. Tumor ADase was depressed to approximately 15% of the control value. The extent of inhibition was found to be dependent on the mouse strain, i.e., ADase from tissues of CD-1 mice was generally inhibited to a greater extent than was enzyme from the C57BL strain. Levels of purine nucleoside phosphorylase were relatively insensitive to the infusion. With the exception of the thymus, in vitro inhibition of ADase by DCF exceeded that produced in vivo. Residual levels of activity remaining in both cases were generally less sensitive to inhibition by DCF than untreated enzyme. Ackermann-Poetter plots establish the inhibition as stoichiometric both before and after DCF infusion and provide evidence for a significant decrease in affinity after infusion. The data suggest the possibility of the induction of a form of ADase less sensitive to inhibition than is native enzyme. These results may be of eventual usefulness in the design of combined chemotherapeutic regimens involving adenosine analogs and tight-binding inhibitors.

INTRODUCTION

The ubiquitous enzyme ADase (EC 3.5.4.4) is of special interest because it is the principal in vivo system for catalyzing the deamination and consequent inactivation of several potential antitumor and antiviral agents which are adenosine analogs. These compounds include ara-A, 9-β-o-xylofuranosyl-6-aminopurine, 7-aminoo-3-β-o-ribofuranosylpyrazolo (4,3-d)-pyrimidine, 3'-deoxyadenosine, and 2',3'-dideoxyadenosine (6, 13, 23, 30). It has been suggested that the chemotherapeutic efficiency of these agents could therefore be enhanced by blocking ADase activity with specific inhibitors (9, 11, 23, 30). The most potent of these is the tight-binding DCF. This compound has a sufficiently high affinity for the enzyme that its binding is essentially irreversible and stoichiometric (4), and at low concentrations it appears to be relatively specific for ADase, although other sites of action with a lower affinity for DCF have been reported (3, 15). Indeed, the cytotoxicity of ara-A (6, 25) and 3'-deoxyadenosine (20, 24) is notably enhanced by DCF, and this compound also potentiates the ara-A therapy of mice bearing L1210 leukemia (26).

Previous studies in our laboratory (33) indicate that in response to in vivo administration of the tight-binding but reversible inhibitor erythro-9-(2-hydroxy-3-nonyl)adenine, ADase levels are elevated in certain tissues. These data suggest potential mechanisms of enzyme induction or derepression that must be considered in any attempt to use ADase inhibitors in vivo. The present study documents a tissue-dependent effect of continuous infusion of DCF on mouse ADase and purine nucleoside phosphorylase levels and compares these data to the response in vitro.

A significant conclusion of these studies is that inhibition of ADase after infusion of DCF is not complete but that its extent is tissue dependent. Indeed, the enzyme in vivo appears to be much less sensitive to inhibition than in vitro data predict. We have also documented that the presence of a transplantable tumor can have dramatic influences on the response to DCF. These data may be useful in understanding the clinical response to ADase inhibitors, as well as in elucidating mechanisms of enzyme regulation in general.

MATERIALS AND METHODS

Nucleoside phosphorylase (crystalline ammonium sulfate suspension from calf spleen), xanthine oxidase (crystalline ammonium sulfate suspension from buttermilk), inosine, imidazole, bovine serum albumin, 3-(4,5-dimethylthiazolyl)-2,5-diphenyl tetrazolium, and phenazine methosulfate were purchased from Sigma Chemical Co. (St. Louis, Mo.). Ampholines were obtained from LKB Instruments, Inc. (Hicksville, N. Y.). Acrylamide and bisacrylamide were purchased from Bio-Rad Laboratories (Richmond, Calif.); adenosine, agar (special grade), and sucrose (enzyme grade) were purchased from Schwarz/Mann (Orangeburg, N. Y.); and potassium phosphate was purchased from Mallinckrodt Chemical Works (St. Louis, Mo.). DCF was a generous gift from the National Cancer Institute through the National Large Bowel Cancer Project, by USPHS Grants CA-i 4906 from the National Cancer Institute through the National Large Bowel Cancer Project, by USPHS Grants CA-i 8856 and CA-i 8428, and by Grant BC 202B from the American Cancer Society.

Enzyme Assays. ADase activity in normal and infused tissues was determined by a modification (36) of the assay of Hopkinson et al. (17) in which the inosine produced is converted to uric acid in the presence of excess commercial nucleoside phosphorylase (0.1 unit/ml) and xanthine oxidase (0.2 unit/ml). The uric acid was monitored at 293 nm in a continuous recording Acta III spectrophotometer thermostated at 37°C. Appropriate background blanks due to contaminating ADase in the commercial xanthine oxidase and nucleoside phosphorylase preparations were subtracted. Nucleoside phosphorylase assay was performed similarly, except that 0.2 mM inosine replaced 0.2 mM adenosine, and no commercial nucleoside

1 This research was supported by USPHS Grant CA-i 14906 from the National Cancer Institute through the National Large Bowel Cancer Project, by USPHS Grants CA-i 18856 and CA-i 18428, and by Grant BC 202B from the American Cancer Society.
2 To whom requests for reprints should be addressed. Recipient of American Cancer Society Faculty Research Award FRA i 82.
3 The abbreviations used are: ADase, adenosine deaminase; ara-A, 9-β-o-arabinofuranosyl-6-aminopurine; DCF, 2'-deoxycoformycin, (R)-3-(2-deoxy-β-o-erythro-pentofuranosyl)-3,6,7,8-tetrahydroimidazo[4,5-d][1,3]diazepin-8-ol.

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phosphorylase was used. For both assays, a millimolar extinction coefficient of 12.2 (21) was used to convert absorbance to μmol of adenosine. For determination of the inhibition by DCF in vitro, the adenosine to inosine conversion was monitored at 37° by continuous recording at 265 nm. A millimolar extinction coefficient of 8.33 (22) was used to convert absorbance to μmol of adenosine deaminated.

A unit of enzyme activity is defined as the amount of enzyme that catalyzes the conversion of 1 μmol of substrate to product under the specified steady state assay conditions. For specific activity determinations, protein was quantitated by the method of Lowry et al. (27) with bovine serum albumin as standard.

Isoelectric Focusing. Analytical isoelectric focusing was performed in a 5% polyacrylamide flat bed containing 2% ampholines, pH 4 to 6, and 5% sucrose in an LKB Multiphor apparatus, and stained for enzymatic activity, as previously described (33).

DCF Infusion. CD-1 or C57BL mice, with or without s.c. transplanted colon tumors and weighing 23 to 28 g, were maintained on a diet of Purina rat chow and tap water ad libitum. DCF at concentration of 12.5 μg/ml for a 25-g mouse was infused at room temperature in 0.9% NaCl solution at a constant rate of 0.8 ml/day for 5 days. DCF retained 80 to 90% of its inhibitory capacity after standing for this period. The amount of DCF used was varied in proportion to the weight of the mouse. Weight losses of 4 to 6 g were noted after the 5-day period. Infusion was performed by catheterization through the tail into the abdominal cavity, as previously described (33). Animals were killed by cervical fracture, and tissues were excised, cooled immediately on dry ice, and homogenized at 50% (w/v) solutions in 250 mM sucrose:20 mM imidazole-HCl, pH 7.0. Supernatants obtained after centrifugation at 10,000 × g for 30 min were dialyzed exhaustively against 50 mM imidazole-HCl:100 mM sodium chloride, pH 7.0. Supernatants obtained after centrifugation at 100,000 × g for 30 min were dialyzed exhaustively against 50 mM imidazole-HCl:100 mM NaCl, pH 7.0. The dialysis consisted of a diffusate to retentate ratio of 500:1, with changes of buffer over approximately a 3-day period. ADase and nucleoside phosphorylase activities were generally stable during the dialysis, except for decreases of 20 to 30% sometimes noted in tissues of the gastrointestinal tract. We have also determined the effect of the infusion process per se on these activities by infusing 0.9% NaCl solution over a 5-day period. These activity levels were generally found to be comparable to those from control animals without infusion. A notable exception was a 3- to 4-fold stimulation noted in the stomach ADase of C57BL mice.

RESULTS

Enzyme Tissue Distribution. As a necessary prerequisite to a study of the effects of DCF infusion on activity levels, we have compared the tissue distribution of ADase in control mouse strains C57BL and CD-1 (Table 1). C57BL tissues generally displayed specific activities comparable in magnitude to those we have previously observed in the CD-1 strain (33). Thus, tissues of the gastrointestinal tract and lymphoid system (i.e., thymus and spleen) were up to 2 orders of magnitude greater than were the specific activities of kidney, lung, and liver in both strains. The presence of a colon tumor in the C57BL mice did not substantially alter this order of specific activities, but some activity differences were noted. In particular, a 50% decrease in the thymus activity and increases in lung and liver activities were observed, although the latter was on the borderline of statistical significance.

Nucleoside phosphorylase, the enzyme primarily responsible for further metabolizing the product of the deaminase reaction, did not display a large tissue dependence of specific activity (Table 2). Activities for this enzyme were in the range of 0.02 to 0.12 unit/mg protein, including the tumor. The presence of the tumor in the C57BL strain did not appear to alter these values significantly. Interestingly, the specific activity in the jejunum of the CD-1 mice was approximately an order of magnitude lower than that in the C57BL strain. However, some CD-1 animals examined did display a substantially higher activity than the mean, approaching the value for the C57BL mice. The reason for this variation is at present not understood.

Effects of DCF Infusion. We have compared the effects of DCF infusion on ADase levels in tumor-bearing and normal C57BL mice (Chart 1). Each of the tissue homogenates was exhaustively dialyzed at pH 7.0 before assay. One of the most significant results was that no tissue, including the tumor, displayed zero activity after the infusion. Residual levels of activity were observed which were dependent on the tissue. Thus, for example, in the normal animal the activity of the thymus was the most sharply depressed to less than 10% of the control value, whereas jejunum, ileum, and spleen ADase activities were observed, although the latter was on the borderline of statistical significance.

Table 1

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Normal C57BL</th>
<th>Tumor-bearing C57BL</th>
<th>Normal CD-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jejunum</td>
<td>0.344 ± 0.088</td>
<td>0.321 ± 0.045</td>
<td>0.472 ± 0.055</td>
</tr>
<tr>
<td>Thymus</td>
<td>0.234 ± 0.051</td>
<td>0.117 ± 0.013</td>
<td>0.267 ± 0.056</td>
</tr>
<tr>
<td>Ileum</td>
<td>0.069 ± 0.012</td>
<td>0.077 ± 0.007</td>
<td>0.110 ± 0.015</td>
</tr>
<tr>
<td>Colon</td>
<td>0.049 ± 0.008</td>
<td>0.035 ± 0.009</td>
<td>0.060 ± 0.007</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.049 ± 0.006</td>
<td>0.062 ± 0.003</td>
<td>0.067 ± 0.004</td>
</tr>
<tr>
<td>Stomach</td>
<td>0.017 ± 0.003</td>
<td>0.013 ± 0.004</td>
<td>0.026 ± 0.006</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.008 ± 0.001</td>
<td>0.010 ± 0.001</td>
<td>0.012 ± 0.002</td>
</tr>
<tr>
<td>Liver</td>
<td>0.004 ± 0.001</td>
<td>0.008 ± 0.003</td>
<td>0.011 ± 0.002</td>
</tr>
<tr>
<td>Colon tumor</td>
<td>0.028 ± 0.006</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

These values were obtained from Trotta et al. (33).

Mean ± S.E. for 5 to 8 animals.

Table 2

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Normal C57BL</th>
<th>Tumor-bearing C57BL</th>
<th>Normal CD-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jejunum</td>
<td>0.113 ± 0.026</td>
<td>0.102 ± 0.025</td>
<td>0.011 ± 0.006</td>
</tr>
<tr>
<td>Thymus</td>
<td>0.042 ± 0.003</td>
<td>0.041 ± 0.004</td>
<td>0.034 ± 0.018</td>
</tr>
<tr>
<td>Ileum</td>
<td>0.105 ± 0.013</td>
<td>0.120 ± 0.001</td>
<td>0.017 ± 0.010</td>
</tr>
<tr>
<td>Colon</td>
<td>0.066 ± 0.007</td>
<td>0.061 ± 0.007</td>
<td>0.047 ± 0.012</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.045 ± 0.003</td>
<td>0.052 ± 0.003</td>
<td>0.026 ± 0.006</td>
</tr>
<tr>
<td>Stomach</td>
<td>0.024 ± 0.002</td>
<td>0.029 ± 0.000</td>
<td>0.023 ± 0.004</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.039 ± 0.002</td>
<td>0.042 ± 0.009</td>
<td>0.040 ± 0.012</td>
</tr>
<tr>
<td>Lung</td>
<td>0.039 ± 0.002</td>
<td>0.036 ± 0.002</td>
<td>0.024 ± 0.002</td>
</tr>
<tr>
<td>Liver</td>
<td>0.041 ± 0.009</td>
<td>0.031 ± 0.030</td>
<td>0.024 ± 0.010</td>
</tr>
<tr>
<td>Colon tumor</td>
<td>0.040 ± 0.004</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean ± S.E. for 5 to 8 animals.
showed only marginal reductions. A notable result contrary to the established action of DCF as a tight-binding ADase inhibitor was the dramatic stimulation of approximately 4-fold in the stomach of both normal and tumor-bearing animals. Interestingly, this effect may in some way be related to the infusion process itself, since a similar stimulation was also observed in a number of C57BL mice infused with 0.9% NaCl solution alone. Although the mechanism for this increased activity is not as yet understood, it is clear that DCF does not significantly inhibit stomach ADase.

We have also examined the ADase levels in each tissue before dialysis (Chart 2). The same general trends described above were noted, except that residual levels of specific activity were lower in each case. It was particularly notable that some tissues (e.g., spleen and thymus) displayed significant enzyme levels only after dialysis. These data indicated the presence of a population of molecules with a relatively low affinity for the inhibitor. This conclusion was supported by direct inhibition studies, as described below.

The presence of the transplantable colon tumor produced several dramatic changes in the response (Chart 1). With the exception of the thymus and colon, tissues in the tumor-bearing animals were significantly more inhibited by DCF, with the largest difference being observed in the jejunum. The tumor activity itself was extremely sensitive to DCF inhibition with an approximately 85% loss in specific activity. No significant changes in overall protein concentration were apparently produced by DCF infusions.

We have carried out a similar infusion study in mouse strain CD-1 (Table 3). Most of the results were the same as with the C57BL animals, including residual activity in all tissues, the high degree of inhibition in the thymus, and the failure of the stomach activity to be inhibited. However, some significant quantitative differences were noted between the strains. Thus, several of the CD-1 tissues displayed a higher degree of inhibition after DCF infusion. The greatest increases in inhibition were noted in the jejunum, ileum, and spleen.

In vitro experiments indicated a negligible effect of DCF at concentrations up to 1.0 mM on nucleoside phosphorylase. However, it was of interest to examine whether, as a consequence of the DCF effect on ADase, any change in nucleoside phosphorylase activity might be found in vivo. As shown in Table 4, in general, relatively small changes in specific activity were observed in both strains. However, levels of liver nucleoside phosphorylase in normal and tumor-bearing C57BL mice appeared to be stimulated approximately 2-fold, and similar increases were noted for jejunum, ileum, and stomach activities in the CD-1 strain.

We have examined whether any changes in activity levels could be correlated with the electrophoretic variants of either enzyme. ADase from normal C57BL tissue displayed on analytical isoelectric focusing in polyacrylamide gel (as described in "Materials and Methods") 2 main variants similar in isoelectric point to those observed in CD-1 animals (33). The ratio of these variants in both strains was tissue dependent. DCF
Effects of DCF on ADase

Table 4

**Effect of DCF on nucleoside phosphorylase in mouse strains C57BL and CD-1**

Activities were determined by the spectrophotometric assay at 293 nm with inosine as substrate. Data were obtained as described in Table 3.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Normal</th>
<th>Tumor bearing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Specific nucleoside phosphorylase activity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C57BL</td>
<td>CD-1</td>
</tr>
<tr>
<td>Jejunum</td>
<td>0.100 ± 0.033*</td>
<td>0.027 ± 0.011</td>
</tr>
<tr>
<td>Thymus</td>
<td>0.043 ± 0.010</td>
<td>0.017 ± 0.001</td>
</tr>
<tr>
<td>Ileum</td>
<td>0.123 ± 0.029</td>
<td>0.046 ± 0.047</td>
</tr>
<tr>
<td>Colon</td>
<td>0.067 ± 0.013</td>
<td>0.038 ± 0.003</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.060 ± 0.013</td>
<td>0.077 ± 0.004</td>
</tr>
<tr>
<td>Stomach</td>
<td>0.024 ± 0.005</td>
<td>0.044 ± 0.004</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.050 ± 0.012</td>
<td>0.027 ± 0.003</td>
</tr>
<tr>
<td>Lung</td>
<td>0.046 ± 0.007</td>
<td>0.021 ± 0.003</td>
</tr>
<tr>
<td>Liver</td>
<td>0.097 ± 0.022</td>
<td>0.024 ± 0.006</td>
</tr>
<tr>
<td>Colon tumor</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Mean ± S.E. for 5 to 8 animals.

infusion did not appear to produce any consistent change in the relative intensity of these variants in any of the 9 tissues examined (Table 1), nor were any new forms focusing at an unusual isoelectric point observed. Similarly, nucleoside phosphorylase variants on isoelectric focusing were also found to be tissue dependent in normal animals, but no effect on these patterns was observed after the DCF infusions. Thus, we conclude that none of the electrophoretically distinguishable forms of either enzyme is preferentially inhibited or induced in these tissues.

**In vitro Studies on Inhibition of Mouse ADase by DCF.** We have also determined the response of ADase in the various C57BL mouse tissue homogenates to DCF in vitro and have compared these results to the effects of DCF infusion (Chart 3). Tissue homogenates were inhibited with a molar excess of DCF, followed by exhaustive dialysis. Residual enzymatic activity was observed, the level of which was tissue dependent.

However, the degree of inhibition observed in vitro generally was greater than that obtained after DCF infusion. An exception occurred in the thymus, which displayed approximately the same percentage of activity remaining in both cases. Interestingly, the stomach ADase was over 90% inhibited by DCF in vitro, but yet was either unaffected (CD-1 strain) or elevated (C57BL strain) after infusion (Table 3).

The observation that ADase activity remained after DCF treatment both in vivo and in vitro suggested that this residual activity might be less sensitive to inhibition than control activity. We therefore compared the capacity of DCF to inhibit ADase activity obtained from DCF-infused and control tissues (Chart 4). In general, ADase remaining after the infusion was inhibited to a significantly smaller extent by a fixed concentration of DCF than normal enzyme. Stomach again represented a notable exception since its activity was equally inhibited in both cases. We have also noted a similar trend in our in vitro studies, i.e.,...
activity remaining after treatment of ADase with DCF in vitro followed by dialysis (Chart 3) was generally less sensitive to inhibition by DCF than untreated enzyme. A more rigorous analysis of the apparent change in the affinity of DCF for the enzyme was performed by a comparison of Ackermann-Potter plots (Ref. 1; Chart 5). In these experiments, jejunal ADase and inhibitor were preincubated at varying concentrations of each, followed by assay at a fixed adenosine concentration of 100 μM. The marked curvature observed in these plots both before and after DCF infusion is indicative of a tight-binding, stoichiometric type of inhibition in both cases. Similar results have been previously reported for the inhibition of a partially purified preparation of human erythrocyte ADase by DCF (4) and by coformycin (8). Inspection of these plots clearly indicates that higher concentrations of inhibitor are needed to produce a given percentage of inhibition for enzyme obtained after the infusion (Chart 5B) compared to control ADase (Chart 5A), consistent with the data of Chart 4.

The Ackermann-Potter plot may be further analyzed to yield inhibitor concentrations which reduce the initial velocity by 50% (I50 values) as a function of total enzyme concentration. We have estimated inhibition constants (K values) from the y-intercept of a plot of I50 values versus enzyme concentration (7, 8) as approximately 1.4 × 10^{-11} M before infusion versus approximately 5.0 × 10^{-11} M for the residual activity remaining after infusion. Although the trend of these values does support an alteration in binding affinity, their absolute magnitude may be subject to a significant error due to the difficulty in making such extrapolations that intersect the y-axis close to the origin (4).

**DISCUSSION**

An important result reported here is that although DCF is documented as a tight-binding, essentially irreversible inhibitor (4), high residual levels of ADase remain in various tissues after a long-term infusion. Several mechanisms represent reasonable explanations for these data. Thus, in response to a depressed ADase activity, new enzyme protein may be induced which exceeds the intracellular concentration of inhibitor. This explanation would apply particularly to those tissues which display residual ADase levels before dialysis of excess inhibitor (Chart 2). It is also consistent with our previous findings (33) that in response to infusion of the tight-binding but reversible inhibitor 9-erythro(2-hydroxy-3-nonyl)adenine, several mouse tissues display a dose-dependent increase in ADase activity. L1210 cells are also reported to respond with an increase in ADase in response to DCF inhibition (2).

Alternatively, it must be considered that the intracellular concentration of the total enzyme-drug complex has been elevated. This may result, for example, from an interruption in the normal degradation of the enzyme if the DCF-ADase complex were relatively resistant to proteolysis, as compared to the native enzyme. The residual levels of activity noted would therefore be produced from partial breakdown of the complex either during assay or during the exhaustive dialysis procedure. The latter mechanism is substantiated by the increased activity observed after dialysis, although it cannot be excluded that a breakdown of the complex during the assay is operative as well. Increases in ADase specific activity above the control level were generally not observed, with the exception of the stomach, since the binding of DCF is sufficiently tight as to be poorly reversible by dialysis. This result is in distinction to the ready reversibility of 9-erythro(2-hydroxy-3-nonyl)adenine inhibition (33).

It is of interest that significant differences were found in the response of various tissues to DCF infusion. Tissues of the gastrointestinal tract were generally among those displaying the highest levels of residual ADase activity, especially notable before dialysis. These results may reflect in part the presence of rapidly regenerating cell populations, which have been demonstrated to recover more quickly in response to ADase inhibition than those that are slow to divide or are nondividing (2, 37). Alternatively, there may exist a tissue dependence in the penetration of DCF to the intracellular space. It may be relevant that the binding of DCF to the nucleoside transport system has been demonstrated to be rate limiting for the inhibition of human erythrocyte ADase (5). The mechanism of entry of DCF...
into the cells of the various tissues studied here, however, is yet to be elucidated. It should also be noted that the amount of intracellular enzyme itself may also influence the fraction of inhibitor bound if the local concentration of enzyme should approach the Kᵣ value (7).

These studies also document that DCF has a significantly lower affinity for the enzyme obtained after inhibitor infusion, as compared to normal ADase. Although this population of molecules less sensitive to DCF inhibition was also detectable in lysates from normal animals, its proportion of the total activity was low compared to that obtained after infusion. Since no new ADase species was observed on isoelectric focusing after the infusion, we conclude that this form of the enzyme may actually reside in a subpopulation distinguishable in its uncharged residues. The question of whether active protein synthesis is involved in its production may be resolvable with charged residues. The question of whether active protein synthesis inhibitors or specific ADase antisera. These results are in distinction to the finding in our laboratory that the increase in ADase activity in normal intestinal differentiation may be correlated with the appearance of a particular electrophoretic variant (34, 35).

The fact that the thymus ADase activity shows the largest reduction in activity in normal animals in response to DCF is notable. Much evidence supports the hypothesis that adenosine levels and ADase activity have a profound influence on lymphocyte function and differentiation (18, 19, 29). From studies on severe combined immunodeficiency patients, it appears that lymphoid tissues are by far the most sensitive to an absence of ADase, since there is essentially a complete failure of B- and T-cells to develop in the genetically determined absence of ADase (10, 12, 29). The reason for this particular sensitivity is unknown. One speculation based on the present data is that the thymus may lack the mechanisms of enzyme induction or derepression characteristic of other tissues that may be needed to compensate for the inherited defect.

An interesting feature of the data is the increased sensitivity to DCF in several host tissues of animals bearing the transplantable colon tumor. The tumor thus appears to confer on the whole animal the same high sensitivity to DCF that is characteristic of its own enzyme. These data are reminiscent of several studies which indicate that tumor-bearing animals can acquire levels of enzymes characteristic of hepatomas or immature liver (14, 16, 22, 28, 31, 32, 37). However, it is not yet known whether the results reported here are a specific consequence of the tumor, or are relatively nonspecific, resulting, for example, from stress or an alteration in normal metabolic balance. The presence of a humoral factor that might mediate these responses is a potential mechanism that is currently under investigation.

The fact that nucleoside phosphorylase does not change in most tissues in response to DCF infusion is consistent with the lack of inhibition in vitro. The mechanism for the increase in activity noted in certain tissues is not understood, but the data do suggest that nucleoside phosphorylase itself may be inducible. These changes could result from a disturbance in the balance of adenine and guanine nucleotide concentrations produced by DCF. This alteration in nucleotide pools may be quite extensive since DCF can inhibit other enzymes in addition to ADase, as, for example, adenylate deaminase (3) or the enzymes responsible for the inosinate to adenylate conversion (15).

It is, of course, hoped that these results may be of eventual usefulness if DCF or similar tight-binding inhibitors in conjunction with an adenosine analog are used in the treatment of human cancer. Such an approach has already been successfully used in animals with the antimetabolite ara-A (25, 26). However, it may be significant in this regard that quantitative differences in the response to DCF were noted between the mouse strains CD-1 and C-57BL. Thus, the extrapolation of these data to humans may be hazardous, but the results do emphasize the possibility of different tissue responses, as well as the modulation of the overall clinical effects by the presence of a tumor.

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