Biochemical Pharmacology, Vol. 26, pp. 71-76. Pergamon Press, 1977. Printed in Great Britain.

PRELIMINARY COMMUNICATIONS

TOXICITIES OF ADENOSINE AND 2'-DEOXYADENOSINE IN L CELLS TREATED WITH INHIBITORS OF ADENOSINE DEAMINASE*

Lillie Lapi† and Seymour S. Cohen‡

Department of Microbiology,

University of Colorado School of Medicine,

Denver, Colo. 80262, U.S.A.

(Received 20 September 1976; accepted 26 October 1976)

Effective inhibitors of mammalian adenosine deaminase first became available within the past 3 years. The first of these, synthesized by Schaeffer and Schwender (1), was erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA). This is a reversible inhibitor with K_i of about 10^{-8} M (1). This compound was shown in this laboratory (2) to protect 9-β-D-arabinofuranosyl adenine (ara-A) from very extensive deamination by mouse fibroblasts (L cells) and thereby increases the lethality of ara-A at least 20-fold. The combination was effective in mice bearing Ehrlich ascites tumor also, markedly increasing the survival of the mice as compared to the effects of ara-A alone (3). It was demonstrated that 10^{-5} to 10^{-7} M EHNA potentiated the toxicity of 10^{-4} M 3-deoxyadenosine (cordycepin) as well (3); in the presence of EHNA, this nucleoside became extremely lethal and completely inhibited the synthesis of both RNA and DNA in growing L cells.

Another even more recently recognized inhibitor of the deaminase is 2'-deoxycoformycin (DCF), which is essentially irreversible in its inactivation of the mammalian enzyme, the K_i being less than 10^{-10} M (4). Not only has DCF been observed to potentiate the antitumor and antiviral effects of ara-A, as summarized in Ref. 5, but at 0.5 mg/kg, i.e. 2×10^{-6} M, it also significantly increased the anticellular toxicity of cordycepin, as well as the antitumor activity of the latter in mice bearing P_{388} ascites leukemia (6). A comparison of the structures of EHNA and DCF is presented in Fig. 1.

*This work was supported by Grant 1-R01-11636 from the National Institute of Allergy and Infectious Diseases.

†Miss L. Lapi, Commander, U.S. Naval Reserve Nursing Corps, died on July 16, 1976.

‡The new address of this author is Department of Pharmacological Sciences, School of Basic Health Sciences, State University of New York, Stony Brook, Long Island, N.Y.

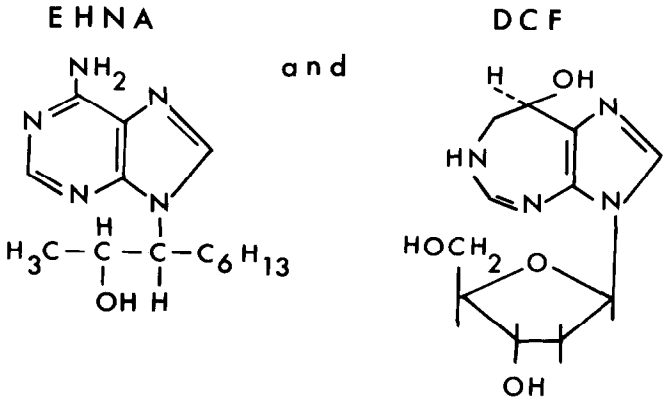

Fig. 1. Structures of the inhibitors of adenosine deaminase, erythro-9-(2-hydroxy-3-nonyl)-adenine (EHNA) and 2'-deoxycoformycin (DCF).

We have concluded, therefore, that the deamination of analogues of adenine nucleosides is a major reason for the limited activity in vivo of many of these substances given alone (2) and suppose that with the use of these deaminase inhibitors it may be possible to obtain insights concerning the true toxic potential of compounds such as ara-A and cordycepin in the chemotherapy of viruses and tumors. At this point, however, we should ask several questions concerning the use of such inhibitors of the deaminase in conjunction with the presumed chemotherapeutic agent. An obvious question is that of the toxicity of such a combination in man. Will the protection by EHNA or DCF of a toxic nucleoside, such as ara-A and cordycepin, markedly affect the therapeutic index and convert these nucleosides into agents whose lethal and toxic action is heightened far more than their therapeutic action? We have not addressed ourselves to this question but have asked some related questions approachable in tissue culture systems.

We now have an embarrassment of riches, in possessing two potent inhibitors of the adenosine deaminase. In addition to the apparent irreversibility of the action of DCF on the enzyme, we have wondered if its deoxynucleoside structure also confers a toxicity to this inhibitor as a result of possible incorporation into cellular nucleic acid. In studies on the growth of L cells in liquid suspension culture (2) in the presence of either inhibitor, it was shown that such cells will grow and multiply as well in the presence of 10^{-5} M EHNA or DCF as in the absence of these substances. In addition to following cell number and cell size with measurements in a Coulter counter (7), the ability of individual cells to form colonies (2) was determined. No significant change was detected in number, size or in the efficiency of plating of the cells; the latter

value remained between 60 and 70 per cent of cell number in cultures, which continued to increase exponentially from 5 x 10^4 viable cells/ml to 2 x 10^5 viable cells/ml in a 31-hr interval after the addition of the deaminase inhibitors.

In a 25-hr interval, the same cells continued to enlarge in the presence of 10^{-4} M ara-A, although this substance had killed 40-50 per cent of the cells. However, in the presence of 10^{-4} M ara-A plus 10^{-5} M EHNA or DCF, the cells were killed at a much greater rate; in 25 hr only 1 per cent of the originally viable cells were able to produce colonies, although cell number and the distribution of sizes of the cells were essentially the same in cultures containing ara-A with or without deaminase inhibitor. In this experiment, therefore, it appeared that, although both deaminase inhibitors were equally effective at 10^{-5} M in exacerbating ara-A toxicity for L cells, neither appeared toxic alone on a short-term or long-term basis for the growth and multiplication of these cells.

It was demonstrated also that under the conditions of these experiments in which the deaminase inhibitors were added simultaneously with the ara-A, EHNA or DCF similarly protected exogenous radioactive ara-A from deamination. These metabolic experiments were carried out as described earlier (2,8). In the normal culture (10^5 cells/ml), 30 per cent of the ara-A disappeared from the medium in a 6-hr interval, with the concurrent appearance of 25 per cent of the radioactivity as ara-Hx. Less than a fourth of this deaminase was due to serum deaminase in the presence of EHNA or DCF; only 10-14 per cent of the ara-A left the medium with the accumulation of 4-7 per cent of the radioactivity as ara-Hx.

Although the K_i of ara-ATP in inhibition of mammalian DNA polymerase is about 10^{-6} M (9), ara-A is barely toxic to L cells at 10^{-4} M and unable to sustain lethality after 30-hr, when all the ara-A in the medium has disappeared. These cells are rich in an active adenosine deaminase (2), and it is thought likely that the normal role of the enzyme may be the inactivation of the "normal" nucleosides, 2'-deoxyadenosine and adenosine. After conversion to the triphosphate, the former is a powerful inhibitor of ribonucleotide reductase and mimics the toxicity of hydroxyurea in this respect. Adenosine is toxic to several tissue culture lines in the absence of exogenous uridine (10) and is also inhibitory as such to cAMP phosphodiesterase, to platelet aggregation and causes vasodilatation of the mammalian heart. These toxic effects are markedly decreased by deamination. In some as yet obscure way, genetically determined immunoincompetence has been found to correlate with the absence of adenosine deaminase. It appeared desirable, therefore, to explore the effects of EHNA and DCF on the toxicities of these "normal" metabolites.

As shown in Fig. 2, the "normal" nucleosides at 10^{-4} M are not inhibitory to the growth

of deaminase-rich L cells. However, these substances are toxic in the presence of 10^{-5} M inhibitor of the deaminase. In the presence of EHNA, both adenosine and deoxyadenosine are found to be inhibitory but do not kill. In the presence of DCF, 2'-deoxyadenosine is quite lethal, whereas adenosine is cytostatic.

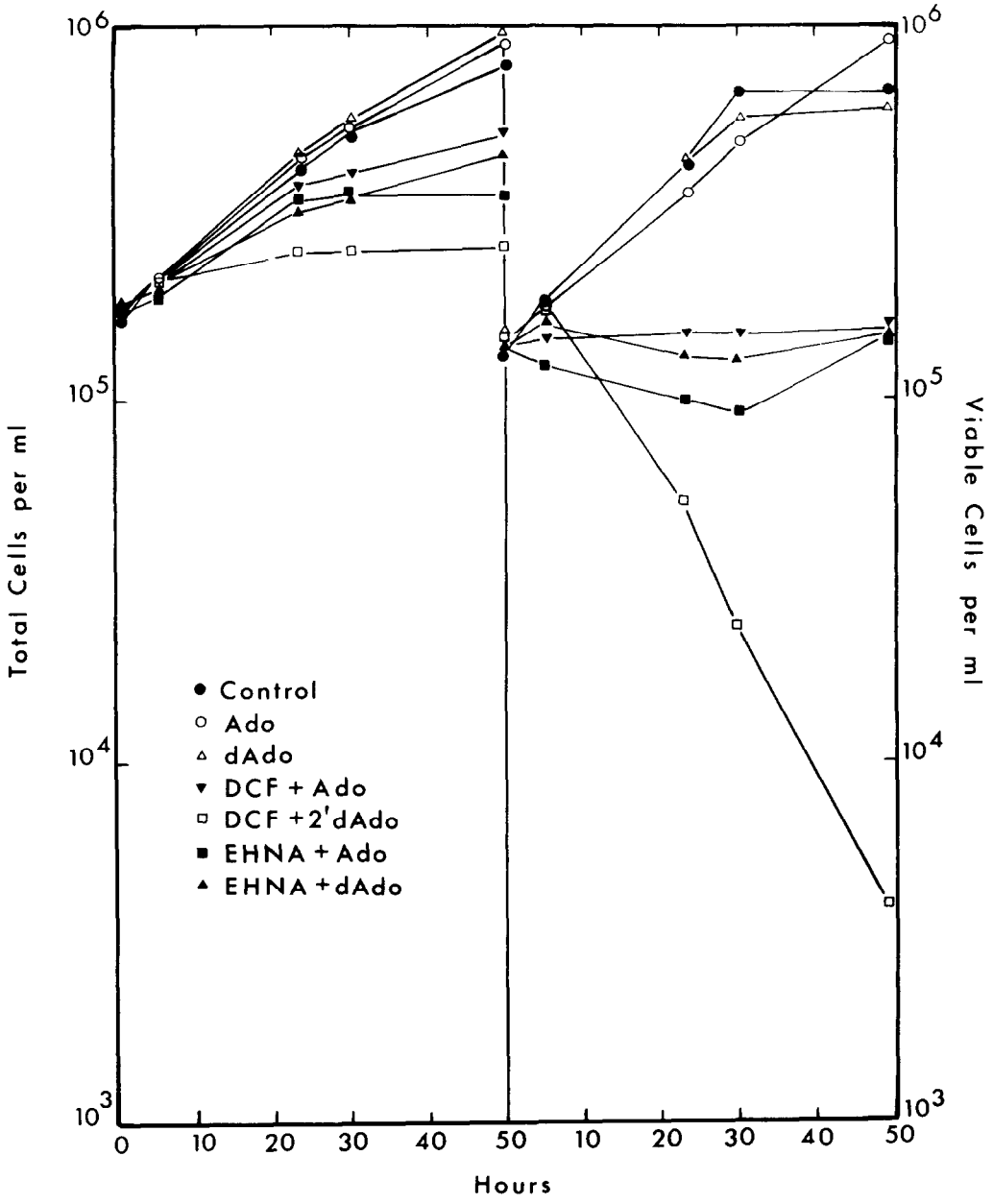

Fig. 2. Growth and viability of mouse fibroblasts exposed to adenosine and 2'-deoxyadenosine in the presence or absence of inhibitors of adenosine deaminase. Adenosine (Ado) and 2'-deoxyadenosine (dAdo) were initially present at 10^{-4} M. The inhibitors were present at 10^{-5} M.

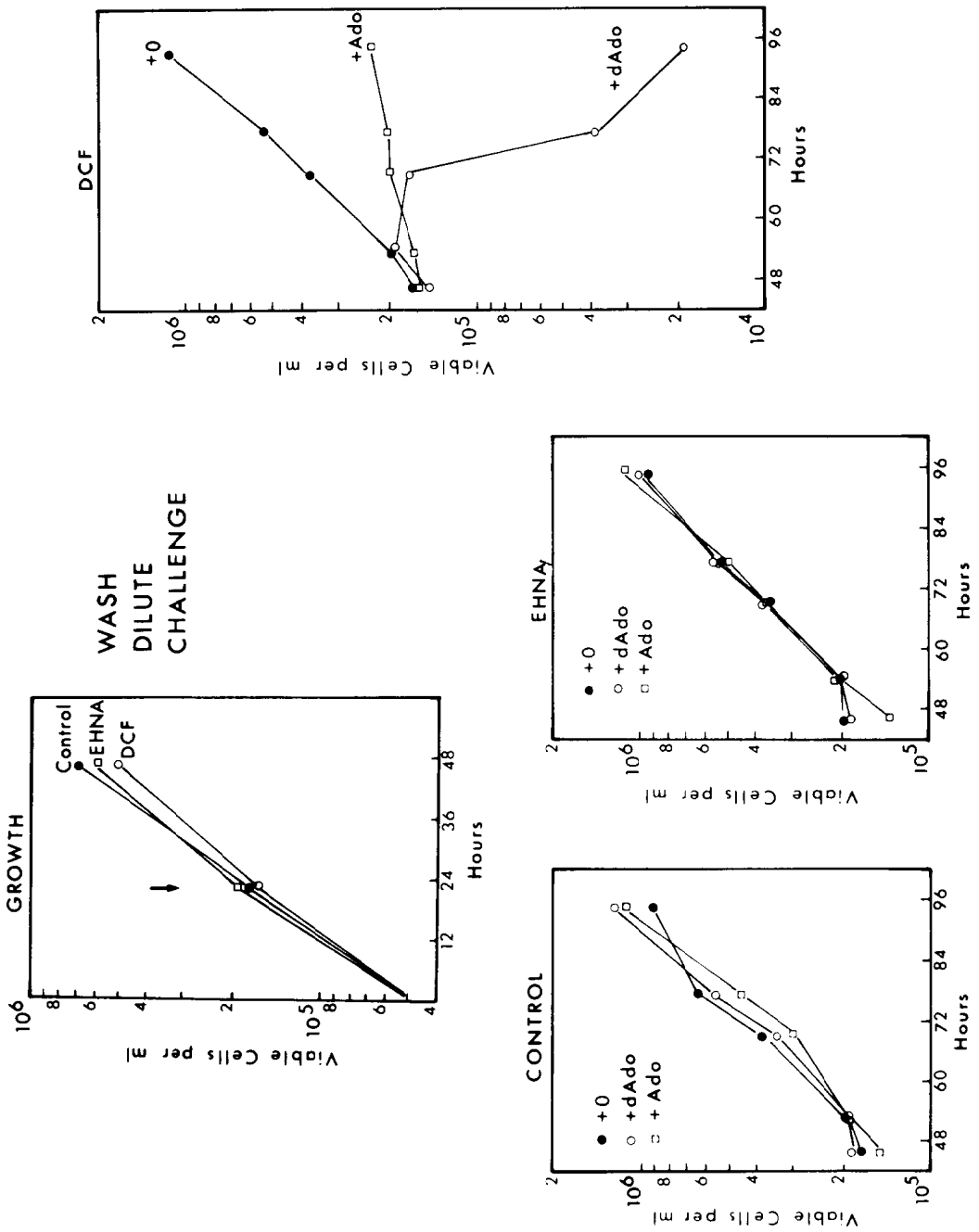

Fig. 3. Growth and viability of L cells in the presence of inhibitors of adenosine deaminase (EHNA and DCF) and subsequent challenge of the washed cells by nucleosides (Ado and dAdo). The inhibitors were present initially at 10^{-5} M. Adenosine (Ado) and 2'-deoxyadenosine (dAdo) were present initially at 10^{-4} M.

We then asked if this more pronounced effect of DCF was potentially hazardous, in the sense that treated cells might not easily regain the enzymatic activity which is protective against this type of challenge by "normal" nucleosides. Cells were exposed to EHNA or DCF for 24-hr and were washed to reduce the external concentration of inhibitor greater than 1000-fold. These cells, as well as a control culture, were then exposed to 10^{-4} M nucleoside. In Fig. 3, it can be seen that cells exposed to EHNA could, after washing, then grow normally in the presence of adenosine or deoxyadenosine. On the other hand, cells exposed to DCF and washed were nevertheless killed in the presence of 2'-deoxyadenosine and were markedly inhibited by adenosine. It can be concluded then that DCF is not easily eliminated from the cellular system and may present a hazard with respect to a possible later challenge by these "normal" toxic nucleosides.

In making a decision, then, as to which deaminase inhibitor should be explored in increasing the therapeutic efficacy of compounds such as ara-A, it should be asked if hazards such as these, including that of immunoincompetence, may not be introduced in the test animal by DCF or EHNA.

REFERENCES

1. H.J. Schaeffer and D.F. Schwender, J. Med. Chem. 17, 16 (1974).
2. W. Plunkett and S.S. Cohen, Cancer Res. 35, 1547 (1975).
3. S.S. Cohen and W. Plunkett, Ann. N.Y. Acad. Sci. 255, 269 (1975).
4. S. Cha, R.P. Agarwal and R. E. Parks, Jr., Biochem. Pharmac. 24, 2187 (1975).
5. S.S. Cohen, Med. Biol., in press.
6. D.G. Johns and R.H. Adamson, Biochem. Pharmac. 25, 1441 (1976).
7. A. Doering, J. Keller and S.S. Cohen, Cancer Res. 26, 2444 (1966).
8. W. Plunkett and S.S. Cohen, Cancer Res. 35, 415 (1975).
9. J. Furth and S.S. Cohen, Cancer Res. 28, 2061 (1968).
10. H. Green and T.S. Chan, Science, N.Y. 182, 836 (1973).