Activity of Congeners of Hydroxyurea Against Advanced Leukemia L1210. (30209)

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Studies of the relationship between structure and activity of agents used in cancer chemotherapy are of interest in that they may lead to elucidation of the mechanism of action of known agents as well as to the discovery or synthesis of new agents.

Hydroxyurea, a simple analog of urea first synthesized by Dresler and Stein in 1869(1), has been shown to have various degrees of activity against several rodent tumors(2,3,4, 5) and some human tumors(6,7,8). Studies of the activity of various analogs of hydroxyurea against advanced mouse leukemia L1210 were undertaken in hope of determining the active group or groups on the molecule.

Methods and materials. The compounds studied, structures, and sources are listed in Table I. Hydroxyurea and various analogs were tested for activity against advanced leukemia using methods previously reported(9).

Treatment was initiated on day 5-7 following implantation of mouse leukemia L1210. Doses of compounds studied were administered in water or normal saline daily subcutaneously. Ten mice were used for each dose level, and the optimal dose for each compound is reported. Each compound was screened for activity at least 3 different times. The results are expressed as the percentage increase in median survival time of treated over that of untreated tumor-bearing CDBA mice.

Results and discussion. The results presented confirm the previously reported effectiveness of hydroxyurea against advanced leukemia L1210(4). Significant antitumor activity was shown by N-hydroxyurethane. Dihydroxyurea, urethane, and guanidine showed only a slight degree of effectiveness against the tumor. Isohydroxyurea, semicarbazide, urea, thiourea, selenourea, hydroxylamine, and hadacidin were ineffective even when administered in toxic doses (*i.e.*, above optimal doses reported).

In this series of compounds the essential structural requirement for activity seems to be the integrity of the hydroxamic acid group (-CONHOH) since isohydroxyurea, O-carbamoyl hydroxylamine, was devoid of activity. Replacement of the hydroxamino group by an amino group as in urea or by a hydrazino group as in semicarbazide nullified the antitumor activity. The thio and imino derivatives of urea were inactive, as well as the seleno derivative, although selenium compounds have been reported to depress the growth rate in laboratory animals and inhibit growth in microorganisms and tissue culture (10). Hydroxylamine itself was also inactive. It has been postulated that hydroxyurea is hydrolyzed to hydroxylamine and that the antileukemic activity of hydroxyurea is due to the effect hydroxylamine generates upon various areas of cell function(11). The lack of activity of hydroxylamine against advanced leukemia L1210 and in other systems where hydroxyurea is active does not lend support to this hypothesis(12,13). Urethane, in which an ethoxyl radical takes the place of the hydroxamino group in hydroxyurea, structurally related to isohydroxyurea, was ineffective. However, when the residual amino group of urethane is replaced by a hydroxamino group giving rise to N-hydroxyurethane, which contains the hydroxamic acid group, significant antitumor activity is shown. Hadacidin, a derivative of formylhydroxamic acid, was inactive indicating that the hydroxamino moiety is an essential participant in the antitumor activity. Replacement of the residual amino group in hydroxyurea by a hydroxamino group (dihydroxyurea) results in decreased antitumor effectiveness indicating the substituent on the hydroxamic acid group contributes to the activity of the molecule through electronic interaction, effect on the metabolism and lability of the molecule or attachment of the molecule to enzymes.

TABLE I.

Compound	Structure	Source of compound	Range of doses, mg/kg	Optimal daily dose	% increase in median survival time
Hydroxyurea	$NH_2\text{-}C(=O)\text{-}NH\text{-}OH$	CCNSC	100 - 750	250-500	50-65
Dihydroxyurea	$HO\text{-}NH\text{-}C(=O)\text{-}NH\text{-}OH$	Dr. E. Boyland (Ref 14)	25 - 200	50-100	11
Isohydroxyurea	$NH_2\text{-}C(=O)\text{-}ONH_2$	Synthesized (Ref 15)	12.5 - 100	100*	0
Semicarbazide	$NH_2\text{-}C(=O)\text{-}NH\text{-}NH_2$	Fisher Scientific Co.	12.5 - 150	100	0
Urea	$NH_2\text{-}C(=O)\text{-}NH_2$	Merck & Co.	250 -1000	1000	0
Thiourea	$NH_2\text{-}C(=S)\text{-}NH_2$	Eastman Organic Chemicals	125 -1000	125-250	0
Selenourea	$NH_2\text{-}C(=Se)\text{-}NH_2$	Metallomer Laboratories	6.25- 50	25	0
Guanidine	$NH_2\text{-}C(=NH)\text{-}NH_2$	Eastman Organic Chemicals	125 - 375	250	10
Urethane	$NH_2\text{-}C(=O)\text{-}O\text{-}C_2H_5$	Fisher Scientific Co.	50 -1500	750	0-5
Hydroxyurethane	$HO\text{-}NH\text{-}C(=O)\text{-}O\text{-}C_2H_5$	Aldrich Chemical Co.	50 -1000	400-500	30-40
Hydroxylamine	$NH_2\text{-}OH$	Fisher Scientific Co.	15 - 125	62	0
Hadacidin	$H\text{-}C(=O)\text{-}N(OH)\text{-}CH_2\text{-}C(=O)\text{-}OH$	CCNSC	250 -1500	1000	0

* Divided into 2 daily doses of 50 mg/kg because single injections at 100 mg/kg cause 50% lethality.

Studies of other substitutions on the residual amino group of hydroxyurea will further clarify the structure necessary for antileukemic effect in this series.

Summary. Various analogs of hydroxyurea have been screened for antitumor activity against advanced mouse leukemia L1210. The results indicate that in this series of compounds the essential structural requirement is the hydroxamic acid group.

1. Dresler, W. F. C., Stein, R., Ann., 1869, v150, 242.

2. Goldin, A., Report to Cancer Chemotherapy National Service Center (CCNSC), 1960.

3. Sloan-Kettering Inst., Report to CCNSC, 1960.

4. Stearns, B., Losee, K. A., Bernstein, J., J. Med. Chem., 1963, v6, 201.

5. Adamson, R. H., Yancey, S. T., Ben, M., Loo, T. L., Rall, D. P., Arch. Int. Pharmacodyn., 1965, v153, 87.

6. Thurman, W. G., editor, Symposium on Hydroxyurea, Cancer Chemotherapy Rep., 1964, v40.

7. Fishbein, W. N., Carbone, P. P., Freireich, E. J., Misra, D., Clin. Pharmacol. and Therap., 1964, v5, 574.

8. Origenes, M. L., Jr., Beatty, E. C., Jr., Brubaker, C., Hammond, D., Hartmann, J. R., Shore, N., Williams, K. O., Cancer Chemotherapy Rep., 1964, v37, 41.

9. Goldin, A., Venditti, J. M., Kline, I., Mantel, N., Cancer Chemotherapy Screening Data IV, Cancer Res., 1959, v19, 429.

10. Rosenfeld, I., Beath, O., Selenium, Academic Press, New York, 1964.
11. Fishbein, W. N., Carbone, P. P., Science, 1963, v142, 1069.
12. Gale, G. R., Biochem. Pharm., 1964, v13, 1377.
13. Gale, G. R., Kendall, S. M., McLain, H. H., DuBois, S., Cancer Res., 1964, v24, 1012.
14. Boyland, E., Nery, R., Nature, 1964, v203, 1379.
15. Kofod, H., Huang, T-Y., Acta Chem. Scand., 1954, v8, 485.

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Explanted Nasal Mucosa: Growth Patterns of Cystic Fibrosis and Control.* (30210)

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While studying nasal mucosa from patients with cystic fibrosis of the pancreas (CF), we observed an organoid appearance in the primary and reaggregated tissue cultures. This report summarizes our observations.

Methods. We obtained nasal polyps(1) directly at elective surgery. The resected polyps were skinned by forcing the mucosa flat against a pair of microdissecting scissors and then snipping the translucent mucosal swatch. Those swatches were cut into 1 mm squares, washed 5 times with Hanks' solution plus antibiotics, and divided into groups of 3-4 squares. The antibiotics included polymyxin, neomycin, streptomycin, and penicillin and mycostatin. Each group of squares was briefly dried in a dish of a 2-piece Carrel flask (Bellco #15-031) and then gently squashed with a coverslip. The coverslip remained in the flask thereafter. The dish was flooded with Parker's CMRL-1066 medium (Microbiological Associates) supplemented with 10% calf serum (Colorado Serum Co.). The lid was sealed with a sterile Dow Silicone High Vacuum Grease and the sidearm was capped with a rubber serum stopper(2). All culture procedures were subsequently carried out in the closed containers, by means of disposable plastic syringes. We maintained an approximate pH 6.9 with CO_2.

Results. Explants were taken from 11 patients. Explants from 4 patients yielded good growth in multiple flasks. Explants from 5 patients yielded unsatisfactory growth due to infection with Candida, Staphylococcus or Pseudomonas, which was harbored in the thick mucus of these chronically infected children. Explants from 2 patients were discarded without further culture when no ciliated epithelium was found on any of the 1 mm squares in the first 3 days. Routine histopathological examination of multiple sections of every polyp disclosed no malignant changes. None of the patients was symptomatic for a viral disease within 2 weeks of surgery. All patients had a positive sweat electrolyte test(3) and complete exocrine pancreatic insufficiency. In addition to these studies, a control polyp from a non-cystic fibrosis patient was cultured.

Dissections carried out by micromanipulation during the first 3 days *in vitro* demonstrated an easily identifiable mucosal layer; this consisted mostly of ciliated cells and interspersed nonsecreting crescentic cells. Pavement-like outgrowths of compact cells occurred in 5-8 days from areas where ciliary activity had spontaneously ceased. We did not follow the growth unless it rose from previously ciliated areas. For a given area, outgrowth did not begin until ciliary activity stopped. In other portions of the same speci-

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