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ANTIMITOTIC EFFECTS OF HYDROXYUREA AND ITS DERIVATIVES: STRUCTURE–ACTIVITY RELATIONSHIPS*

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ABSTRACT

A total of 25 hydroxyurea derivatives have been screened on mouse vaginal mucosa for antimitotic effect. Antimitotic properties are preserved in derivatives where a methyl or ethyl group is substituted for hydrogen at position 1 or a methyl group at position 3. Higher alkyl and carbonyl groups at position 1 or 3 are correlated with loss of antimitotic activity. Loss of antimitotic property is interpretable on the basis of steric and inductive effects. The hydroxyl proton may not be substituted by an alkyl group but can be replaced by labile substituents such as carbamoyl or trimethylsilyl which permit formation of hydroxyurea itself in vivo.

Hydroxyurea has been shown to inhibit a variety of transplantable neoplasms in animals [1]. Preliminary trials in patients with neoplastic diseases indicated that hydroxyurea by oral administration is active against adenocarcinomas, melanomas, sarcomas, and acute and chronic leukemia [2, 3].

Studies on mechanism of action have shown that hydroxyurea interferes with the synthesis of DNA in intact mammals [4, 5] and in mammalian cells in vitro [6, 7]. Structure–activity evaluations indicate that the hydroxyl group is required for inhibitory activity on DNA synthesis of Hela cells [8].

It has been shown previously that hydroxyurea exhibits antimitotic effects on both mouse vaginal and rectal mucosae when administered intravaginally (IVag) and intrarectally respectively [9], and when given intraperitoneally exerts antimitotic effects on epithelial cells of both vagina and rectum [10].

Since hydroxyurea is primarily water-soluble it does not penetrate human skin readily. Our search for more lipid-soluble derivatives of hydroxyurea for possible therapeutic topical use in man has prompted us to study the relationships between structure and antimitotic properties of available derivatives and of several synthesized by us for this study. The present work describes these relationships.

MATERIALS AND METHODS

Hydroxyurea and its derivatives were provided by E. R. Squibb and Sons unless otherwise specified. Virginal female ICR mice, 5–7 weeks old, were used to test for antimitotic activity on vaginal epithelium. Ten mice were used for each test compound. Test compounds for IVag administration were prepared in one of the following vehicles: (a) water, (b) acetone:isopropyl myristate, 1:4, or (c) ethanol:propylene glycol:water 1:1:1. Podophyllin for intraperitoneal (IP) injection was prepared in ethanol:propylene glycol 1:4.

Test compounds were instilled IVag at time zero in a volume of 0.05 ml. This dose was repeated 2 hr later at which time 2 mg of podophyllin were injected IP in a volume of 0.1 ml. All mice were killed by cervical dislocation 6 hr later, i.e., 8 hr after initial instillation of test drug. In longer 24-hr screening tests, the same procedure was used except that one dose was delivered 16 hr earlier. The vagina was removed en bloc and fixed in 10% buffered formalin. Transverse sections were cut at 6 μ and stained with hematoxylin and eosin.

The number of mitotic cells per length of mucosa as measured along the basal layer of the mucosa was determined histologically by means of an ocular micrometer and expressed as mitoses/cm.

RESULTS

All vaginal specimens were classified as being in either the estrogenic or progestational phase of the estrus cycle on the basis of histologic characteristics. Only those data from animals with estrogenic vaginal epithelia were taken for comparison. The results are shown in the Table.

Our previous studies [9,10] using the mouse vaginal system to identify drugs with primary antimitotic properties have indicated that a test drug is substantially antimitotic when the median mitotic count is lower than 100/cm.

Of the three 1-alkylated hydroxyurea derivatives, 1-methyl and 1-ethyl hydroxyurea (I, II) were highly antimitotic. Five derivatives, (IV–VIII), each with a carbonyl group substituent at position 1, were not substantially antimitotic. Four 3-alkylated hydroxyurea derivatives (IX–XII) showed no antimitotic activity except that 3-methyl hydroxyurea (IX) at 10% concentration was weakly active. 3-triethylacetyl (XIII) and 3-(α,α-dimethylvaleroyl) hydroxyurea (XIV), each with a carbonyl group at position 3, were totally inactive.

A single substitution of the hydroxyl proton with a methyl group converted the molecule into inac-

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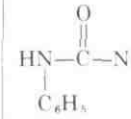
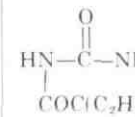
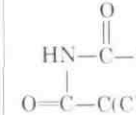
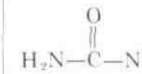
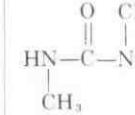
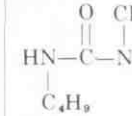
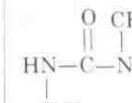
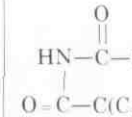
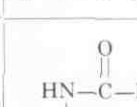
TABLE

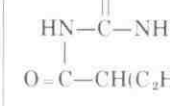
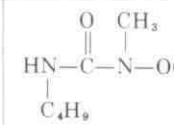
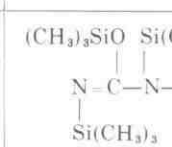
Mitotic counts in estrogenic vaginal epithelium of mice after IVag instillation of test compounds and IP administration of podophyllin

Test compound	Formula	Number of animals	Concentration %	Mitoses/cm	
				Range	Median
Control (podophyllin, IP)[a]		23		67–436	237
Hydroxyurea	$H_2N-C(=O)-NHOH$	7	1	2–126	76
		7	5	6–120	9
		8	10	0–47	9
Hydroxyurea Derivatives: I 1-methyl	$H_2N-C(=O)-N(CH_3)-OH$	6	1	3–69	36
		10	5	2–205	12
II 1-ethyl	$H_2N-C(=O)-N(C_2H_5)-OH$	8	2	14–190	82
		8	5	18–355	45
III 1-n-propyl[b]	$H_2N-C(=O)-N(C_3H_7)-OH$	10	10	16–356	121
IV 1-formyl	$H_2N-C(=O)-N(CHO)-OH$	7	5	34–649	190
		10	2[c]	16–340	147
V 1-acetyl[b]	$H_2N-C(=O)-N(COCH_3)-OH$[d]	9	5	18–287	112
VI 1-carbobenzyloxy[b]	$H_2N-C(=O)-N(COOCH_2C_6H_5)-OH$[d]	10	1	12–303	207
		8	2	18–472	155
		8	5	49–459	152
VII 1-benzoyl[b]	$H_2N-C(=O)-N(COC_6H_5)-OH$[d]	7	2	139–758	352
VIII 1-lauroyl[b]	$H_2N-C(=O)-N(COC_{11}H_{23})-OH$[d]	9	10	14–353	104
		10	5[c]	11–269	146
IX 3-methyl[b]	$HN(CH_3)-C(=O)-NHOH$	10	10	19–245	76
X 3-ethyl[b]	$HN(C_2H_5)-C(=O)-NHOH$	7	10	81–230	172
XI 3-n-butyl	$NH(C_4H_9)-C(=O)-NHOH$	10	1	96–517	160
		10	1[c]	27–333	155
		10	2[c]	42–270	181
		6	5	27–599	213

TABLE—*Continued*

Test compound	Formula	Number of animals	Concentration %	Mitoses/cm	
				Range	Median
XII 3-phenyl[b]		10 10	1 10	36–660 63–254	224 164
XIII 3-triethyl acetyl		8 8	5 2[c]	117–1086 16–267	296 148
XIV 3-(α,α-dimethylvaleroyl)		9	2	28–239	219
XV O-methyl		8 10	2 2[c]	30–530 107–427	165 194
XVI O-carbamoyl		9 10	2 3	13–160 10–152	82 48
XVII 1,3-dimethyl		9 9 10	1 2 2[c]	15–546 29–401 3–187	148 80 61
XVIII 1-methyl-3-n-butyl		5	2[c]	90–290	207
XIX 1-methyl-3-phenyl[b]		8	1	18–486	119
XX 1,0-dimethyl		10	2[c]	64–360	170
XXI 0-methyl-3-diethylcyclohexyl acetyl		5 9	5 1[c]	34–265 60–310	128 143
XXII 0-methyl-3-(α,α-diethyl-β-cyclohexyl propionyl)		9 9	1 1[c]	38–276 67–247	214 189

TABLE—*Continued*

Test compound	Formula	Number of animals	Concentration %	Mitoses/cm	
				Range	Median
XXIII 0-n-butyl-3-(α-ethyl-2-cyclohexyl acetyl)		8	2[c]	69–387	278
XXIV 1,0-dimethyl-3-n-butyl		9	2[c]	36–373	150
XXV tetra (trimethylsilyl)[b]		10	5	23–417	72
		10	5[c]	18–147	41
		8	1[c]	9–51	17

[a] I. P. dose; 2 mg

[b] Synthesized in our Laboratory of Medicinal Chemistry.

[c] Test run for 24 hr.

[d] Preferred chemical structure; another possible structure is 3-substituted.

tive form, e.g., O-methyl hydroxyurea (XV). However, the molecule was antimitotic if the substituent was a carbamoyl group, i.e., in O-carbamoyl hydroxyurea (XVI). Among the 1,3 dialkylated hydroxyurea derivatives (XVII–XIX), 1,3 dimethyl hydroxyurea (XVII) was found to be antimitotically active. All the di- and tri- substituted derivatives (XX–XXIV) with methyl or n-butyl group in the hydroxyl proton were inactive.

Trimethylsilylated derivative of hydroxyurea provides what may be a particular structure–function situation. Whereas hydroxyurea itself is primarily water-soluble, tetra (trimethylsilyl) hydroxyurea (XXV) is highly lipid-soluble. The compound, stable in ether solution that contains a drying agent such as anhydrous sodium sulfate, was antimitotically active possibly because in contact with moisture intravaginally it was slowly hydrolyzed to release hydroxyurea itself.

DISCUSSION

Although it has been shown that hydroxyurea causes a marked decrease in the rate of DNA biosynthesis, the mechanisms involved in the inhibition are unknown. The drug has no effect on incorporation of precursors into RNA or protein [4, 6–8,11–15]. In vitro studies have shown that it has no effect on the activities of DNA polymerase, thymidine kinase, and thymidylate kinase [7]. The reduction of ribonucleotides to their corresponding deoxyribonucleotides, however, is markedly inhibited [11,16–18]. Since a mixture of deoxyribonucleotides does not reverse the blockade of DNA synthesis by hydroxyurea in some systems, an additional unknown site of action other than ribonucleotide reductase has been proposed [14, 19, 20]. Evidence has been provided that hydroxyurea inhibits the formation of histones [5], and the biosynthesis de novo of pyrimidine nucleotides prior to the formation of orotidylic acid [21].

Present results show that certain chemical structures of hydroxyurea derivatives are essential for the molecules to be in active forms as antimitotic agents. All possible structures of hydroxyurea derivatives are from the substitution of protons at positions 1,3 and O (hydroxyl) in the hydroxyurea molecule.

Increased lipophilic properties of hydroxyurea derivatives do not seem to enhance the antimitotic effect of the molecules. For example, 1-ethyl hydroxyurea (II) is more lipid-soluble than 1-methyl hydroxyurea (I) but the latter is a more potent antimitotic agent than the former. Substituents with the same carbon numbers at position 1 of the hydroxyurea molecule do not lead to the same biologic activity. For example, both 1-methyl (I) and 1-ethyl hydroxyurea (II) are antimitotically active but 1-formyl (IV) and 1-acetyl hydroxyurea (V) are inactive forms. Therefore, other factors are involved for the molecule of a hydroxyurea derivative to be in active form.

Both inductive and hindrance properties are introduced when a proton at the position 1 or 3 of hydroxyurea is replaced by an alkyl group. Let us start with the two simplest compounds, hydroxyurea and 1-methyl hydroxyurea (I); the latter is derived when the proton at the position 1 of the former is replaced by a methyl group. Since the methyl group differs from the proton by possessing both an electron-releasing property and a hin-

drance property, the antimitotic effect of l-methyl hydroxyurea (I) depends primarily on the sum of these two factors. The fact that l-methyl hydroxyurea (I) is more antimitotic than hydroxyurea itself indicates that the electron releasing action of the methyl group contributes to an increased antimitotic activity. Although there is a slight increase of hindrance action due to the methyl group, this does not sufficiently suppress the overall antimitotic property of the molecule. However, when a higher alkyl group is substituted for a proton at the position 1 or 3, the hindrance property is more dominant. For example, both the electron releasing action and the hindrance action of the alkyl are in increasing order, i.e., $CH_3 < C_2H_5 < C_3H_7$, and the antimitotic activity is correspondingly diminished, i.e., l-methyl hydroxyurea (I) > l-ethyl hydroxyurea (II) > l-n-propyl hydroxyurea (III). Therefore, antimitotic activity decreases when a bulky group is substituted for the proton at position 1 of hydroxyurea, or when a proton at position 3 of hydroxyurea is replaced by an alkyl group. The latter position seems very sensitive to the hindrance effect; for example, 3-methyl hydroxyurea (IX) is weakly antimitotic but 3-ethyl (X) or 3-n-butyl hydroxyurea (XI) is inactive.

An electron-withdrawing carbonyl group such as a formyl (IV), benzoyl (VII), or lauroyl (VIII) at position 1 or 3 appears to convert the whole molecule into inactive form.

It is generally true that the hydroxyl group of hydroxyurea may not be converted into an ether-type group, which contains a rather stable covalent bond [8], without loss of activity. However, a labile group such as carbamoyl (XVI) or trimethylsilyl group (XXV) can be substituted for the hydroxyl proton of hydroxyurea without loss of activity.

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