The Effects of a Series of 9-Alkylpurines on the Growth of Sensitive and 6-MP-resistant H.Ep. #2 Cells*

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SUMMARY

A systematic study of the activity of 6-mercaptopurine, hypoxanthine, adenine, and 6-chloropurine substituted at the 9-position with normal alkyl and cycloalkyl groups indicates that the butyl, pentyl, cyclopentyl, hexyl, cyclohexyl, heptyl, and octyl derivatives of these purines exerted an inhibitory effect on a line of H.Ep. #2 sensitive to MP and on two sublines resistant to MP. Additionally, the data indicate that generally the MP-1 line was inhibited by the 9-alkylpurine derivatives to a greater degree than was the MP-2 line. Although both of the resistant lines were more sensitive to the short-chain normal alkyl derivatives and all the cycloalkyl derivatives than was the parent line, there was little difference in the sensitivity of all three of the cell lines to the longer-chain normal alkyl derivatives. Of the compounds tested, the most effective were the cyclohexyl derivatives, which were consistently more inhibitory against all three of the cell lines. Except for the long-chain normal alkyl purines, the derivatives of MP were more inhibitory than the corresponding alkyl derivatives of hypoxanthine, which, in turn, were more inhibitory than the corresponding derivatives of adenine and chloropurine.

From a cell line of human epidermal carcinoma (H.Ep. #2) have been isolated two sublines highly resistant to 6-mercaptopurine (MP) (6). These two sublines, differing in morphology and rate of growth, have furnished additional biological systems for studying metabolic differences between parent and resistant cells. In the course of studying the comparative effects of various compounds on the growth of these cell lines, it was observed that a number of 9-substituted purines were inhibitory to all three lines and that some of the compounds exerted a selective inhibition on the MP-resistant lines. The effect these compounds exerted on the parent and resistant cells constitutes the subject of the present paper. Similarly, LePage and Jones (7) have found that 9-alkylated purinethiols were inhibitory to a thioguanine-resistant cell line.

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MATERIALS AND METHODS

The parent cell culture used throughout the course of these experiments was the H.Ep. #2 line of human epidermoid carcinoma (14) from which were isolated two mutant sublines: H.Ep. #2/MP-1 and H.Ep. #2/MP-2. The morphological and growth characteristics of these resistant cells have been described previously (6). The genetic stability of the resistant lines was demonstrated in cloning experiments with cells from cultures of MP-resistant lines that had been grown for 150 generations (over 9 months) in the absence of MP. When these cells were cultured in the presence of MP they cloned with a plating efficiency as high (90–95 per cent) as that of cells grown from the outset in the presence of MP.

All H.Ep. #2 stock cultures, sensitive and resistant, were propagated in SRI-14 complete growth medium (6), with the exception that 160 μg/ml MP was routinely added to the growth medium of the resistant cells. All stock cell cultures, sensitive to or resistant to MP, were grown as confluent monolayers in 32-oz. prescription bottles and as fluid-suspension cultures in 1500-ml. flat-bottomed boiling flasks. Serial transplantation and

propagation of the cells were accomplished as previously reported (5).

Experimental runs were designed so that each compound was tested under conditions as nearly identical as possible on the MP-sensitive and MP-resistant cells. Approximately 2×10^7 cells were inoculated into 100 ml. of the complete growth medium to which the candidate inhibitor had been added to yield a concentration of 10 μg/ml. The medium and the cells were contained in a 500-ml. flat-bottomed boiling flask, mounted on a New Brunswick gyrotory shaker, and maintained as fluid-suspension cultures for the duration of the experiment. The total cell count of each flask was made with a hemacytometer at the beginning of the experiment and after each 24-hour interval of growth until the experiment was terminated, which was usually 48–72 hours following inoculation. In each experiment the controls consisted of sensitive and MP-resistant cultures each grown in the absence and the presence of MP. References to the synthesis of the compounds used in this study are included in Table 1.

RESULTS

In Table 1 are tabulated the effects which a number of derivatives of MP and closely related compounds exerted on the H.Ep. #2 parent line and the MP-resistant sublines. All results are those obtained after a growth period of 48 hours, and, in most instances, the value given is the mean value for a number of experiments. The R value in which these results are reported was obtained from the formula

$$\frac{\text{Cell count after 48 hours}}{\text{Cell count at 0 hour}} = R.$$

Therefore, the R value 1.00 is an indication of stasis; a number larger than 1.00 indicates an increase in the number of viable cells, whereas a fractional number indicates a decrease in cell population. The data for the growth controls in normal medium show the relative rate of growth of the three cell lines. The data also indicate the inhibitory effect of MP on the parent line and the absence of any inhibition on the two resistant sublines.

From the data may be determined the effect of placing various substituent groups in the 9-position of the MP molecule. The ethyl and propyl derivatives had little effect upon the parent line and the MP-2 subline, but the ethyl derivative inhibited the MP-1 subline to a small degree. The butyl compound, however, was more active and inhibited all three lines, with the greatest inhibition being exerted upon the two resistant cultures. Results similar to that found with the 9-butyl MP were obtained with the pentyl and the cyclopentyl derivatives of MP. However, when a hydroxyl group was placed in the 2′ position of the cyclopentyl compound, whether *cis* or *trans* to the purine moiety, the inhibitory activity was destroyed. Although the hexyl derivative of MP was a potent inhibitor of all three lines, it was not as inhibitory as the cyclohexyl derivative which had, after 48 hours' contact with the cultures, killed all the MP-1 cells, virtually all the MP-2 cells, and 50 per cent of the cells of the parent line. Cyclohexenyl 6-MP was also an active inhibitor but not as active as the cyclohexyl derivative. As in the case of the cyclopentyl MP, when a hydroxyl group was placed in the 2′ position of the cyclohexyl compound, whether *cis* or *trans*, the inhibitory activity was lost. The heptyl and octyl derivatives of MP exerted an inhibitory activity comparable to the inhibition caused by the hexyl MP.

The 9-β-D-ribofuranosyl derivative of MP inhibited the growth of the sensitive line and inhibited the growth of the MP-1 subline, although to a much lesser degree. However, the inhibition of the resistant cells was transitory, and at the end of the 72-hour growth period the extents of growth of the MP-1 cells in the presence of the 9-β-D-ribofuranosyl MP or in the absence of this compound were similar. The effects of the 5′ and the 2′(3′)-ribonucleotides of MP on the three cell lines resembled the effects exerted by MP riboside. Since it was possible that the presence of the terminal phosphate groups might interfere with the entrance of the ribonucleotides into the cells, the terminal phosphate groups were masked by esterification with another ribonucleoside moiety. In one compound the phosphate of MP ribonucleotide was esterified with MP ribonucleoside and in the other with thymidine. It was hoped that these compounds would enter the cell intact and be cleaved by intracellular enzymes to MP ribonucleotide, which would inhibit the growth of the resistant cells. However, the data which were obtained indicated that these compounds did not have the desired inhibitory effect upon the resistant lines, although they inhibited the sensitive line in an amount comparable to that exerted by the MP riboside and ribotides.

In an effort to determine the specificity of the 9-position, 7-butyl MP was synthesized, and, although it proved to be inhibitory, it was not as effective an inhibitor as was the 9-butyl MP. More compounds of this type are being synthesized with larger substituent groups in the 7-position.

Although hypoxanthine (Hx) did not inhibit the

TABLE 1

CYTOTOXICITY OF 9-ALKYLPURINES AGAINST H.EP. #2 CELLS AND SUBLINES RESISTANT TO 6-MERCAPTOPURINE*

Purine	NSC no.	9-Substituent	H.Ep. #2	H.Ep. #2/MP-1	H.Ep. #2/MP-2	Ref. to cmpd. synthesis
None		None	3.84††‡	2.43#	2.89#	
6-MP	755	None	0.72	2.48	2.80	
	14575	Ethyl	3.15	1.70	3.50	10
	61965	Propyl	3.43	2.35	2.46‖	
	19488	Butyl	2.10	0.30	0.96	11
	407749	7-Butyl§	1.85	1.26	1.55	8
	60638	Pentyl	0.29	0.31	1.57‖	
	19487	Cyclopentyl	2.05	0.15	0.50	11
	33194	*cis*-2-Hydroxycyclopentyl	3.20	2.00	2.90	15
	30607	*trans*-2-hydroxycyclopentyl	3.50	1.70	2.75	15
	407423	Hexyl	0.76	0.35	0.51‖	
	19202	Cyclohexyl	0.50	0.00	0.10	11
	25746	*cis*-2-Hydroxycyclohexyl	4.06	2.47	3.00	16
	23081	*trans*-2-Hydroxycyclohexyl	3.82	1.37	2.32	16
	22239	2′-cyclohexenyl	1.66	0.61	0.72	16
	407420	Heptyl	0.71	0.40	0.70‖	
	60632	Octyl	0.48	0.58	0.47‖	
	4911	β-D-Ribofuranosyl	0.85	1.35	2.90	4, 2
	46024	β-D-Ribofuranosyl-5′-PO_4	1.15	1.70	3.10	12, 3
	405278	β-D-Ribofuranosyl-2′(3′)PO_4	0.85	1.85	3.10	9
	407427	Bis (thioinosine)5′-5‴-phosphate#	1.00	1.87	2.76	13
	407338	Thymidylyl (3′→5′)thioinosine#	1.00	1.64	2.65	13
Hypoxanthine		None	4.00	2.50	2.96	
	61978	Propyl	4.20	2.43	2.08‖	
	19207	Butyl	2.15	0.50	1.50	11
	60633	Pentyl	0.31	0.30	1.00‖	
	19205	Cyclopentyl	2.00	0.80	1.40	11
	34856	*cis*-2-Hydroxycyclopentyl	4.01	1.68	2.48	15
	35002	*trans*-2-Hydroxycyclopentyl	4.12	2.61	2.13	15
	407342	Hexyl	0.79	0.46	0.54‖	
	18925	Cyclohexyl	0.90	0.05	0.25	11
	25745	*cis*-2-Hydroxycyclohexyl	4.07	2.43	3.50	16
	22244	*trans*-2-Hydroxycyclohexyl	4.25	1.73	3.00	16
	22240	2′-cyclohexenyl	4.06	1.94	2.57	16
	407401	Heptyl	0.27	0.16	0.35‖	
	407421	Octyl	0.29	0.46	0.86‖	
	Adenine	None	2.92	2.31	2.82	
	61961	Propyl	4.34	2.36	2.88‖	
	19094	Butyl	2.85	1.10	2.25	11
	60637	Pentyl	1.57	1.21	1.74‖	
	19486	Cyclopentyl	2.25	1.40	2.15	11
	34037	*cis*-2-Hydroxycyclopentyl	4.32	1.77	3.88	15
	31140	*trans*-2-Hydroxycyclopentyl	3.87	2.45	3.29	15
	407402	Hexyl	1.01	0.48	0.58‖	
	19201	Cyclohexyl	1.65	0.20	0.55	11
	29406	*cis*-2-Hydroxycyclohexyl	3.34	1.73	1.92	16
	22241	2′-Cyclohexenyl	2.80	2.32	3.55	16
	407422	Heptyl	0.53	0.30	0.52‖	
	60634	Octyl	0.54	0.99	0.64‖	
Chloro	7354	None	3.43	2.71	2.65	
	16129	Ethyl	3.51	1.36	3.00	10
	61964	Propyl	4.14	2.32	2.24‖	
	19204	Cyclopentyl	2.50	0.61	2.15	11
	34455	*cis*-2-Hydroxycyclopentyl	3.87	1.76	4.04	15

* All compounds were tested at 10 μg/ml.

† $\frac{\text{Cell count after 48 hrs}}{\text{Cell count at 0 hr}} = R$. Therefore, 1.00 would indicate stasis. A number larger than 1.00 would indicate an increase in the number of viable cells, whereas a fractional number would indicate a decrease in cell population.

‡ This is a mean value of more than 90 experiments having a range of 3.32–4.18 for H.Ep. #2; 2.02–2.68 for H.Ep. #2/MP-1; 2.53–3.10 for H.Ep. #2/MP-2.

§ For comparison purposes the butyl group was substituted at the 7-position instead of on the 9-carbon of the purine.

The full name rather than the 9-substituent is given.

‖ C. Temple, Jr., C. L. Kussner, and J. A. Montgomery, unpublished data.

TABLE 1—*Continued*

Purine	NSC no.	9-Substituent	H.Ep. #2	H.Ep. #2/MP-1	H.Ep. #2/MP-2	Ref. to cmpd. synthesis
Chloro—*Continued*	31136	*trans*-2-Hydroxycyclopentyl	3.17	1.97	2.94	15
	407425	Hexyl	1.35	1.02	0.64‖	
	18924	Cyclohexyl	2.85	0.83	1.47	11
	25712	*cis*-2-Hydroxycyclohexyl	4.50	1.84	2.77	16
	21309	*trans*-2-Hydroxycyclohexyl	3.68	2.77	3.14	16
	22237	2′-Cyclohexenyl	3.55	1.21	2.50	16
	407424	Heptyl	2.59	3.33	1.59‖	
	60639	Octyl	1.91	1.09	1.38‖	
8-Azaguanine	749	None	0.50	1.90	1.60	
	23905	Ethyl	3.61	2.03	2.83	17
	31737	Butyl	2.88	1.34	2.11	17
Thioguanine	752	None	0.50	1.27	0.40	
	39336	Butyl	4.36	1.45	3.73	1

growth of any of the cell lines used during these experiments and, in fact, caused slight stimulation, the 9-alkyl Hx compounds exerted an inhibitory effect resembling that of the 9-alkyl MP derivatives. The growth of the resistant lines was generally inhibited to a greater degree than were the cells of the sensitive line, and to about the same extent as they were inhibited by the 9-alkyl MP compounds. However, the cyclohexenyl derivative exhibited no inhibitory activity, and once again the 2-hydroxycyclo alkyl compounds were devoid of activity.

In spite of the fact that adenine was somewhat toxic to the cells of all three lines, the 9-alkyl derivatives of adenine yielded results similar to those found for the 9-alkyl hypoxanthine compounds; however, the extent of inhibition was less. Again the resistant lines were apparently more sensitive to inhibition by the 9-alkyl adenines than was the parent line, and, here also, the addition of a hydroxyl in the 2′ position of the 9-cycloalkyl adenine derivatives destroyed the inhibitory effect.

Another purine antagonist, 6-chloropurine, did not inhibit the parent line or either of the two resistant sublines; however, a few of the 9-alkyl-6-chloropurine derivatives did cause some inhibition. The ethyl derivative inhibited the MP-1 line to a limited extent, and the cyclopentyl compound caused some death of the cells of all three lines, with MP-1 most severely affected. The best overall inhibition of all three lines was caused by the hexyl, cyclohexyl, and the octyl derivatives of 6-chloropurine. However, the inhibition was not as profound as with the 9-alkyl derivatives of the other purines tested.

From these data there would appear to be an indication that the degree of inhibition was influenced both by the substituent at the 6-position and the substituent at the 9-position, but at present there is no evidence that all the compounds function by the same mechanism. It is highly unlikely that dealkylation of these derivatives occurs, since the resultant purines have been shown to be noninhibitory to the resistant cells. Several types of experiments are now being performed in an effort to gain more indication of the metabolic sites involved and the mode of action of these compounds.

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