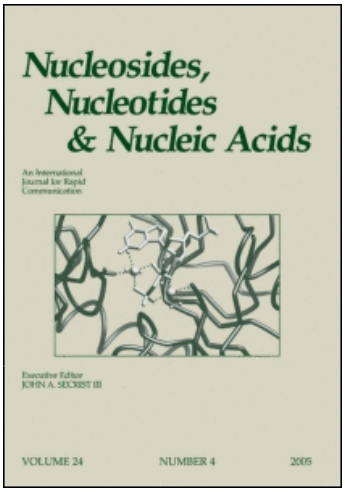

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COMMENTARY

COMMENTARY: METABOLIC IMPEDIMENTS TO THE USE OF NUCLEOTIDE DERIVATIVES TO CIRCUMVENT RESISTANCE TO PURINE AND PYRIMIDINE ANALOGS

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Summary: Many attempts have been made to design derivatives of analog nucleotides that might circumvent resistance due to deficiency of enzymes that convert analogs of purines, pyrimidines and nucleosides to nucleotides. None of these prodrugs that have been evaluated has been active against resistant tumor cells in vivo. The probable reason for this failure is that host cells, but not the resistant cells, have the capacity to form toxic nucleotides from bases or nucleosides resulting from degradation of the prodrugs. These considerations indicate that this strategy for circumvention of resistance will be successful only if the prodrug has some property, other than ability to enter the cell and be converted to toxic nucleotide, that will result in selective toxicity to resistant cells.

Most analogs of purines, pyrimidines, and nucleosides, including clinically useful agents such as 6-mercaptopurine (MP)[+], 5-fluorouracil and arabinosylcytosine, exert their biological activity only after their conversion to nucleotides. The nucleotides are formed by (a) phosphorylation of a nucleoside, catalyzed by a nucleoside kinase, (b) phosphoribosylation of a base, catalyzed by a phosphoribosyltransferase, and (c) a two step conversion of base to

[+]Abbreviations: MP, 6-mercaptopurine; MPR, 9-β-D-ribofuranosyl-6-mercaptopurine; MPRP, 5'-phosphate of 9-β-D-ribofuranosyl-6-mercaptopurine; bis(MPR)P, bis(thioinosine)-5',5'''-phosphate; bis(dibutyryl MPR)P, bis (2',3'-dibutyrylthioinosine)-5',5'''-phosphate; bis(dihexanoyl MPR)P, bis(2',3'-dihexanoylthioinosine)-5',5'''-phosphate; HPRT, hypoxanthine (guanine) phosphoribosyltransferase (EC 2.4.2.8).

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nucleoside to nucleotide catalyzed by the sequential action of a nucleoside phosphorylase and a nucleoside kinase. A frequently observed mechanism of resistance to purine and pyrimidine analogs is a decrease or loss of activities of the enzymes required for their activation[1]. Resistance cannot be overcome by administration of the nucleotide of the analog because nucleotides as such penetrate cell membranes only in small amounts[2]. These facts have led to attempts in several laboratories, including our own, to circumvent this type of resistance by the administration of prodrugs of the analog nucleotides, that is, derivatives of the analog nucleotide that might enter the cell intact, and once within, be converted to the analog nucleotides (Fig. 1). The prodrugs were modified in the phosphate moiety with the expectation that the reduced charge would enable the modified nucleotide to enter the cell. Attempts to develop such prodrugs have been reviewed recently by Tidd[3]. Derivatives that have been studied include amides[4,5], esters[4-16], internal esters (3',5'-cyclic phosphates)[17-19], mixed esters-amides[20-22], and bis esters resulting from the esterification of a nucleotide analog with the corresponding nucleoside analog[23-28]. In cell cultures many of these nucleotide analog derivatives have been active against cells resistant to the base or nucleoside analog[6,12,17,23,26-28] and circumvention of resistance therefore was achieved. However, there is no convincing evidence that any such nucleotide derivative has activity in vivo against a resistant tumor. The purpose of this communication is to point out apparently hitherto unconsidered aspects of this strategy that make it unlikely that such prodrugs will have useful activity against resistant tumor cells in vivo unless they also possess some property, other than capacity to gain cell entry followed by intracellular conversion to nucleotides, that will result in selective toxicity to the resistant tumor cells.

This conclusion is based on the facts that in the tumor-bearing animal (a) some degree of extracellular degradation of prodrug would be expected, and (b) host cells have not become resistant and are not deficient in the enzymes that convert bases and nucleosides to nucleotides. If one assumes, for the sake of argument, that resistant tumor cells and host cells have comparable capacities to take up the prodrug and convert it to the toxic nucleotide, one would still expect that the normal cells, or at least some normal cells, would achieve concentrations of toxic nucleotide higher than those in the resistant cells and that these differences would be determined by the degree of

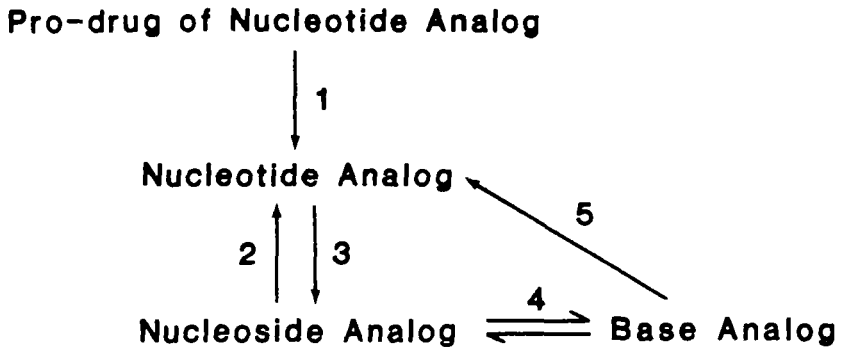

FIG. 1. Pathways for metabolism of a prodrug of a nucleotide analog. Conversions 1-5 are known reactions, and represent the action of the following enzymes: 1, "unmasking" of prodrug by action of phosphodiesterases, esterases, amidases, or other enzymes; 2, nucleoside kinases; 3, phosphatases; 4, nucleoside phosphorylases; 5, phosphoribosyltransferases.

breakdown of the prodrug extracellularly and in the resistant cells. This is because the normal cells would have the capacity to convert base and nucleoside, formed either intracellularly or extracellularly, to toxic nucleotide, whereas resistant cells would not have this capacity. In other words, the intracellular nucleotide analog pool would be higher in the sensitive cells (host cells or parent sensitive tumor cells) than in the resistant tumor cells because sensitive cells can form the nucleotide analog by two routes, unmasking of the prodrug and anabolism of base and nucleoside analogs, whereas resistant cells form analog nucleotide only by unmasking the prodrug (Fig. 1). This metabolic situation can be illustrated by consideration of a specific prodrug, bis(thioinosine)-5',5'''-phosphate (bis(MPR)P) and a specific mechanism of resistance, namely deletion of hypoxanthine (guanine) phosphoribosyltransferase (HPRT). The action of a phosphodiesterase on bis(MPR)P would yield one molecule of 6-MP-ribonucleoside (MPR) and one molecule of 6-MP-ribonucleotide (MPRP) (Fig. 2). MPRP, in addition to exerting its inhibitory action either as such or after further metabolism, can also be dephosphorylated to MPR. MPR, produced both by the action of phosphodiesterase on bis(MPR)P or by dephosphorylation of MPRP, can be converted, via the action of purine nucleoside phosphorylase, to MP. Direct phosphorylation of MPR, indicated by the dotted

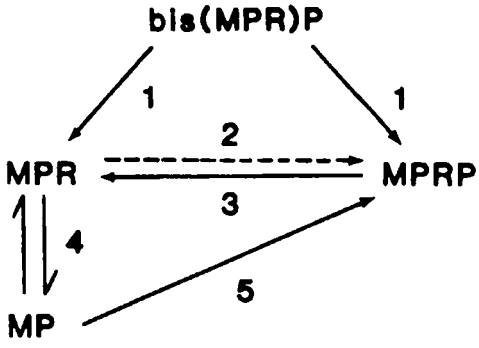

FIG. 2. Pathways of metabolism of bis(MPR)P. The dotted line indicates the absence of significant kinase activity for MPR. Conversions 1, 3 and 4 occur in both host cells and in MP-resistant tumor cells; conversion 5 occurs in host cells only. Reaction 1 is catalyzed by a phosphodiesterase; the identities of the other enzymes are the same as in Fig. 1.

arrow in Fig. 2, does not take place to a significant extent. All of these reactions would take place extracellularly, in host cells, and to some extent in resistant cells. But the capacity to convert MP to MPRP, catalyzed by H(G)PRT, would be present in the host cells, but not in the resistant tumor cells, and hence the prodrug should be more toxic to host cells than to the resistant tumor cells.

The question then arises as to whether the results of published studies in vivo with prodrugs of analog nucleotides can be interpreted in terms of this thesis. Many of the prodrugs show activity in vivo against sensitive tumors[7-11,13,15,20], but surprisingly few have been evaluated in vivo against resistant tumors. The fact that a prodrug is active against sensitive tumor cells yields no information as to the form in which the drug enters cells, and such activity could result entirely from extracellular breakdown to nucleoside or base. The critical test of these prodrugs is evaluation in vivo against drug-resistant tumors, and such results as are available are essentially negative. Thus, two derivatives of 5-fluorodeoxyuridylic acid [5-fluoro-5'-(2-oxo-1,3,2-oxazaphosphorinan-2-yl)-2'-deoxyuridine and 5-fluoro-5'-(2-oxo-1,3,2-dioxaphosphorinan-2-yl)-2'-deoxyuridine] were inactive against a line of P388

mouse leukemia resistant to 5-fluorouracil[20] and the n-butyl esters of araCMP, N^4-palmitoyl-araCMP, and 2,2'-anhydro-3'-O-palmitoyl-araCMP were all inactive against an araC-resistant line of L1210 cells[10]. A positive result was obtained by Turcotte et al.[13], who found that a diacylglycerol ester of araCDP increased the life span of mice bearing araC-resistant L1210 cells by 34-55% (as compared to an increase of 357% produced in sensitive cells); this slight activity was not confirmed in experiments in our laboratory with the same line of araC-resistant L1210 cells[29]. The reasons for the failure of the prodrugs against resistant cells have not been defined. Failure conceivably could be due to (a) unfavorable pharmacokinetics; (b) poor uptake of prodrug; (c) inadequate intracellular activation of the prodrug; (d) extensive extracellular conversion to the nucleotide, nucleoside or base; or (e) selective toxicity to host cells because of the metabolic features discussed above. The information available does not permit exclusion of (a) and (b). However, the inhibition of sensitive cells in vivo indicates that either the prodrug or its breakdown products are available to cells. The fact that resistant cells are inhibited in vitro by the prodrug indicates that the prodrug can penetrate resistant cells, and be activated within the cells; thus (b) and (c) apparently are not primarily responsible for failure of the prodrugs. An indirect, but strong, argument can be built that (d) and (e) are the factors important for the failure of the prodrugs. Some extracellular degradation of drug would be expected, and to the extent that this occurred selective toxicity to host cells would be favored. A fact relevant to this argument is that in cell cultures drug-resistant cells have been found to be much less sensitive to the prodrug than are the parent cell lines from which the resistant lines were derived. For example, 22-fold more bis(MPR)P was required to inhibit MP-resistant (HPRT-deficient) HEp-2 cells than was required to inhibit the parent cell line,[23] and the 50% inhibitory concentrations of the dihexanoyl derivative [bis(dihexanoyl-MPR)P] for MP-resistant L1210 cells and CHO cells were 2-3 orders of magnitude greater than those for the parent cell line[26,27]. The selective toxicity of the prodrugs to the sensitive cells is most likely a consequence of breakdown of the prodrug, either extracellularly or intracellularly, to products that can be converted to toxic metabolites in sensitive but not resistant cells. These findings may be applied to the situation in vivo by the consideration that host cells would be

metabolically similar to sensitive tumor cells in that both have the capacity to anabolize bases and nucleosides to nucleotides. Hence, one would predict that, since sensitive tumor cells both in vivo and in vitro are more sensitive to the prodrugs than are resistant cells, then at least some host cells would be more sensitive than the resistant cells to the prodrug. Such differences in sensitivity would preclude giving the prodrug at a concentration sufficient to inhibit the resistant cells. Or, to look at the question from a somewhat different viewpoint, if one considers that in chemotherapy of sensitive tumors effective drugs must be used at doses close to those toxic to the host, it is obvious that it is not feasible to increase the amount of prodrug to the concentration required to inhibit the resistant cells.

Although the results with prodrugs of nucleotide analogs have been discouraging, one cannot exclude the possibility that a prodrug can be developed that will be effective against resistant cells in vivo. Conceivable effective prodrugs would be those that can be selectively taken up by resistant cells or those that are not subject to extracellular degradation but are still converted to toxic nucleotides in resistant cells. A peripheral consideration is the possibility that some "masked" nucleotides may have activity as such, and indeed there is evidence that the mechanism of toxicity of bis(dibutyryl MPR)P and bis(dihexanoyl MPR)P is not the same as that of MP[27]. However, in such an event, inhibition of resistant cells by a masked nucleotide would not be a circumvention of resistance in the sense described here but would rather be an inhibition of resistant cells by another drug and another mechanism.

Finally, it may be noted, that, although prodrugs of analog nucleotides may not be promising as a means of circumventing resistance, they may be of use in introducing into cells nucleotide analogs that cannot be formed intracellularly by metabolism of the corresponding base or nucleoside, such as, for example, phosphates of analogs of inosine and guanosine which cannot be produced intracellularly from the nucleoside analogs because of the absence or low activity of inosine and guanosine kinases.

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