STRUCTURAL REQUIREMENTS FOR FORMYCIN ACTIVITY

Sir:

The formycin family is a series of purine nucleoside analogs containing unusual C-riboside linkages. They are formycin A (FMA), formycin B (FMB) and oxoformycin B (OFMB), the analogs corresponding to adenosine, inosine and xanthosine respectively[1,2]. FMA has antitumor activity[3,4] and is believed to exert its major effect when it is phosphorylated in tumor cells to its mono-, di- and triphosphates[5]. Among them, FMA-triphosphate is proposed to be the most active principle interferring with various aspects of purine nucleotide metabolism[6,7]. Adenosine kinase should play a key role in this "lethal synthetic process", since an FMA-resistant strain of EHRLICH ascites tumor lacks this enzyme[8,9]. In various organisms including tumor bearing mice, a part of FMA is converted to FMB, catalized by adenosine deaminase[10~12], and further to OFMB[2]. They are less active and entirely inactive to tumors, respectively[2,10]. Because of these metbolic conversions, the narrow antibiotic spectrum of FMA could be ascribed to biochemical characteristics of target organisms, namely, lack of adenosine kinase and/or high activity of adenosine deaminase. The antibiotic spectrum of FMA is broadened by simultaneous addition of coformycin[5], a potent inhibitor of adenosine deaminase. FMB is as active as FMA only against *Xanthomonas oryzae* and some viruses[13]. Only with these organisms, no synergism between FMA and coformycin was observed[14], and hence, it is suggested that there is another mode of action of the formycin on these particular organisms. Both FMA and FMB, probably without being phosphorylated, cause blockage in the entry of normal nucleosides into the cell of *Xanthomonas oryzae* at concentrations near their effective doses[15]. No such biochemical effect is observed with tumor cells nor with other bacteria. The mode of antiviral action remains to be studied.

In order to obtain better chemotherapeutic agents, various derivatives of the formycins were examined for their biological activities. In this communication, we will present the results and will discuss possible correlations between chemical structure and biological activity.

Antitumor activity:

It is reported that FMA was as good a substrate as adenosine for both adenosine kinase (I) from human tumor cells[16] and

Table 1. Biological activities of formycin derivatives

Compounds	Inhibitory effect on		
	YOSHIDA sarcoma *in vitro*[18]	*Xanthomonas oryzae* (CPM)	Influenza virus (CAM)[20]
7-Substituted-3-ribosyl-pyrazolo[4,3-d]pyrimidine			
-H	+(S−)	−	−
-Cl	+(S+)	++	−
-I			−
-SH	++(S−)	+	+
$-SCH_3$	+(S−)	−	+
$-NH\cdot CH_3$	−(S+)	+	−
$-N\cdot(CH_3)_2$	−	−	−
-NH·OH	+++(S+)	+	
Ribose moiety modified			
FMA-5′-P	+++	+++	
FMB-5′-P	−	+++	−
FMA-2′(3′)-P	++	+	
FMB-2′(3′)-P	−	+	−
2′,3′-isopropyridene-FMB	−	−	−
5′-deoxy-5′-amino-FMA	−	−	−
5′-tosyl-FMA	−	−	−
$NaIO_4$-degradation product of FMA[19]	−	−	−
7-NH_2-3-methylpyrazolo[4,3-d]pyrimidine	+	−	−
Formycin A	+++(S+)	+++	+++
Formycin B	+(S−)	+++	+++
Oxoformycin B	−	−	−

Abbreviations are FMA for formycin A, FMB for formycin B, OFMB for oxoformycin B, CPM for cylinder plate method, CAM for cholioallantoic membrane method[20], and S+ (or −) for positive (or negative) synergism with coformycin. Activity expressions are +++, as active as FMA; ++, *x*/10 FMA; +, *x*/100 FMA; and −, undetectable.

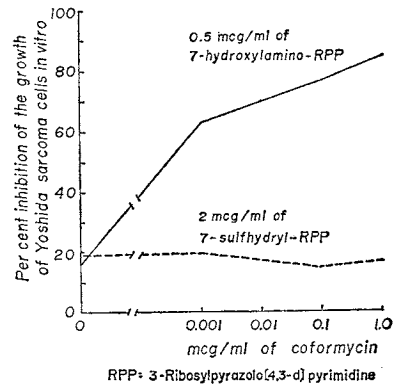
This table includes a few published results.

adenosine deaminase (II) from *Aspergillus oryzae*[17]. These observations suggest that both enzymes do not distinguish the C-riboside from the normal N-riboside. Among 6-substituted adenosines, 6-methylaminopurine riboside, for instance, is known to be a much better substrate for (I)[16] and a much poorer substrate for (II)[17] than adenosine itself. Since (I) catalyzes the obligatory step for FMA activity and, in contrast, (II) is responsible for the catabolism of FMA, we expected that similar modification on FMA would yield more potent antitumor agents. The results, as shown in the left column of Table 1, were against our expectation. Above all, 7-N-methylated FMA which had been the most hoped-for one was entirely inactive by itself, though it showed a slight synergistic effect with coformycin. Several analogs, which could serve (II) as substrates, showed more or less synergism with coformycin. Among the derivatives tested, hydroxylamino derivative which had marked synergism with coformycin appears promising. In contrast, no such synergism was observed with 7-SH-substituted formycin, though it had a moderate effect by itself. This observation seems reasonable because sulfhydryl is an isostere of hydroxyl group. The synergistic effect of these derivatives with coformycin is indicated more clearly in Fig. 1. Any modification on the ribose moiety except phosphorylation resulted in marked or total loss of activity. The phosphoryl group on the ribose moiety would readily be split off by phosphatase before permeating the cells, because the phosphorylated derivatives were only as active as FMA.

Inhibitory effects on *Xanthomonas oryzae* and Influenza A_1 virus:

Structure-activity relationships in these two systems, given in the middle and in the right column of Table 1 respectively, were almost identical except 7-Cl- and 5'-P-derivatives which were effective against *Xanthomonas oryzae* but not against the virus. Phosphorylation of the ribose moiety, namely "the lethal synthesis", was unlikely to be involved as a prerequisite process in these biological activities, however, 5'-OH as well as 3'-OH (or 2'-OH) should be essential because any substitution at these positions caused loss of activity.

Fig. 1. Synergism between coformycin and formycin derivatives



RPP: 3-Ribosylpyrazolo[4,3-d] pyrimidine

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