Antimicrobial and antiviral activity of xylosyl-methylthio-adenosine, a naturally occurring analogue of methylthio-adenosine from *Doris verrucosa*[1]

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Abstract. Xylosyl-methylthio-adenosine, a naturally occurring analogue of 5'-deoxy-5'-methylthio-adenosine, has been postulated to play a protective role during egg development in the mollusc *Doris verrucosa*. However, in vitro tests showed that this analogue is devoid of activity against fungi, bacteria and viruses.
Key words. Xylosyl-methylthio-adenosine; methylthio-adenosine; xylofuranosyl-adenine.

5'-deoxy-5'-methylthio-adenosine (MTA) is a compound generated from S-adenosyl-methionine during the synthesis of the polyamines spermidine and spermine. Investigations on the chemical defense mechanisms of Mediterranean opisthobranch molluscs led to the finding, in the nudibranch *Doris verrucosa*, of a naturally occurring analogue of MTA: xylosyl-methylthio-adenosine (xylo-MTA)[2]. Early studies on the metabolism of xylo-MTA in this mollusc indicated that the analogue does not interfere with MTA catabolism nor with the MTA regulatory effects on polyamine biosynthesis. Further, in vivo experiments with radiolabeled precursors showed that xylo-MTA originates from MTA through isomerization at C-3'. It is a product with a slow turnover rate and its intracellular content is two orders of magnitude higher than that of MTA[3]. Since xylo-MTA appears to concentrate in the egg mass of *Doris verrucosa*, it has been postulated that this analogue may play a protective role during the development of eggs of this mollusc.

For this reason we deemed it useful to investigate the antibacterial and antimycotic properties of xylo-MTA in comparison to those of both related compounds: MTA and 9-(B-D-xylofuranosyl)adenine (xylo-A). In addition, since xylo-nucleosides have been reported to interfere with virus multiplication in vitro[4], tests were carried out also to investigate the antiviral properties of both MTA and xylo-MTA.

Materials and methods

In order to obtain xylo-MTA in a sufficient quantity for biological assays, we developed a short synthetic procedure starting from commercially available MTA[5]. A more elaborate synthesis of xylo-MTA has already been reported[6]. Cells, bacteria, fungi and viruses were the same as those used in previous studies[7,8]. The maximum non-toxic doses of MTA, xylo-MTA and xylo-A were determined in Vero and C8166 cell cultures (10^5 cells per ml) which were incubated at 37 °C in the absence or in the presence of various drug concentrations. Cell numbers were determined after 72 h with a Coulter counter. The antimycotic assays were carried out in Sabouraud-dextrose broth, pH 5.6, and the antibacterial tests in nutrient broth containing 5% NaCl, pH 7.2. The initial inoculum of fungi was 10^4 cells, that of bacteria was 10^3 cells. Minimum inhibitory concentrations were determined after incubation at 37 °C for 18 h (bacteria) or 24 h (fungi). The inhibitory effect on DNA viruses was evaluated in plaque-reduction tests performed on Vero cell monolayers according to the procedure of Collins and Bauer[9]. The anti-HIV activity was evaluated in yield reduction tests performed in C8166 cell cultures. Briefly, 10^6 cells per ml were infected for 2 h with HIV-1 at a multiplicity of infection of 0.1. After extensive washings, the cells were resuspended at 10^5 per ml, in the absence or in the presence of various drug concentrations, and incubated at 37 °C for 72 h. The infectious HIV-1 yield in the supernatants was determined by the standard limiting dilution method (dilution 1:2, four replica wells per dilution) in C8166 cultures seeded at 10^5 cells per ml. Virus titers were determined after 96 h by scoring of syncytia and expressed as cell-culture-infectious-doses fifty ($CCID_{50}$) according to the Reed and Muench method[10].

Results

Data in table 1 show that, in the case of both fibroblast- and of lymphoblast-like cells, xylo-MTA was less toxic

Table 1. Cytotoxic, antibacterial and antimycotic activity of MTA, xylo-MTA and xylo-A

Compound	aMNTD		bMIC				
	μM						
	Vero	C8166	*S. aureus*	*B. subtilis*	*E. coli*	*C. diphteriae*	*C. albicans*
MTA	16	20	33	33	33	33	33
xylo-MTA	100	190	200	200	200	200	200
xylo-A	7	5	18	18	18	18	18

aMNTD (Maximum Non Toxic Dose): concentration of a compound that allowed exponential cell growth for three cell cycles. bMIC (Minimum Inhibitory Concentration): minimum concentration of a compound required to completely inhibit microorganism growth as judged by visual observation.

Table 2. Antiviral activity of MTA, xylo-MTA and xylo-A

Compound	aED_{90}		bED_{50}	
	μM			
	HIV-1	HSV-1	VV	ASFV
MTA	>20	>16	>16	>16
xylo-MTA	>190	>100	>100	>100
xylo-A	>5	0.7	0.4	0.1

aED_{90} (Effective Dose 90): concentration of a compound required to reduce by 90% the HIV-1 yield. Virus yield in untreated controls was 2.7×10^5 CCID50 per mL. bED_{50} (Effective Dose 50): concentration of a compound required to reduce the number of plaques by 50%. Number of plaques in untreated cultures was: 130 (HSV-1), 140 (VV), 145 (ASFV).

than MTA and xylo-A. None of the compounds showed a selective activity against fungi and bacteria, their MICs being always greater than the respective MNTDs for Vero and C8166 cells. When tested for antiviral activity, neither xylo-MTA nor MTA inhibited the multiplication of human immuno deficiency virus (HIV-1) or that of the other viruses at concentrations non-toxic for uninfected cells. By contrast, xylo-A was totally inactive against HIV-1 but showed a potent and selective activity against herpes simplex type 1 (HSV-1), vaccinia (VV) and African swine fever virus (ASFV). Selectivity indices (ratio MNTD Vero/ED50) were 10, 18 and 70, respectively. The lower cytotoxicity of xylo-MTA with respect to MTA could explain the conversion of MTA into a less toxic metabolite which can be safely accumulated in the egg mass of the mollusc.

However, the evidence presented here does not support the idea of a protective role of xylo-MTA as such.

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Oriental orchid (*Cymbidium pumilum*) attracts drones of the Japanese honeybee (*Apis cerana japonica*) as pollinators

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Abstract. The discovery that drones of the Japanese honeybee (*Apis cerana japonica*) pollinate the oriental orchid (*Cymbidium pumilum*) is reported. Drones are attracted to the orchid flower aroma mainly during their mating flights in April through May. Some drones cluster on the flower racemes and others insert their heads deep into the flowers. Drones with pollinia on their scutellum visit other orchids, which facilitates pollination. Individual workers and swarming colonies are also strongly attracted by the flower aroma, but the allopatric western honeybee (*Apis mellifera*) is not attracted.
Key words. Drone; male honeybee; pollination; *Cymbidium pumilum*; *Apis cerana japonica.*

The pollination strategy by which some groups of orchids attract particular bees or wasps by mimicking their sex attractants is well known as a fascinating example of insect-plant coevolution [1]. A similar relationship may have developed between the oriental orchid (*Cymbidium pumilum* Rolfe) and the oriental honeybee (*Apis cerana*). We knew of the contribution of workers of *A. cerana japonica* as pollinators of this orchid before 1986, but in the spring of 1988 we also noticed that many drones were attracted to the flowers of a potted orchid placed near an apiary of *A. cerana japonica* at Tamagawa University, Tokyo. Around noon, before the time of normal mating flights, some drones had already been attracted and were flying around or landing intermittently on the flowers or other parts of the plant. Some of them inserted their heads and thoraxes deep into the flowers between the column and lip (fig. 1). During the efforts of these drones to escape from being trapped, by using their middle legs,