Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 7, pp. 833-836, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)00116-9

INHIBITORY EFFECT ON HT-1080 TUMOR CELL INVASION *IN VITRO* USING 9-(2'-HYDROXYETHYL)ADENINE 2'-PHOSPHATES

Yukio Kitade,*[1] Masa-atsu Hayashi,[1] Chizuko Yatome,[1]

Masamitsu Chajima,[2] and Hisamitsu Nagase*[2]

[1]Laboratory of Molecular Biochemistry, Department of Chemistry, Faculty of Engineering, Gifu University, Yanagido 1-1, Gifu 501-11, Japan, [2]Department of Public Health, Gifu Pharmaceutical University, Mitahora 5-6-1, Gifu 502, Japan

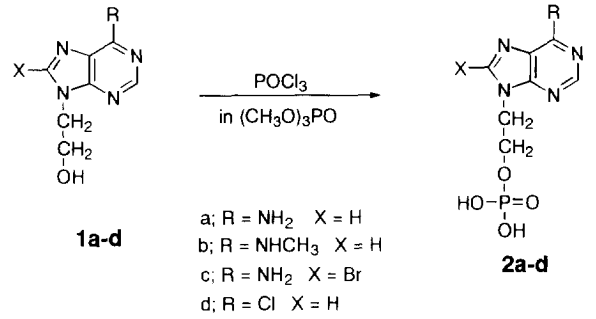
Abstract: Several 9-(2'-hydroxyethyl)purine 2'-phosphates (**2a-c** and **3**) showed a moderate inhibitory effect of tumor cell invasion using Matrigel. These 2'-phosphates (**2a-c** and **3**) also inhibited the activity on type IV collagen degradation by matrix metalloprotease-9. © 1997 Elsevier Science Ltd.

Tumor cell invasion to surrounding tissue or penetration into blood vessels is a critical stage of cancer metastasis.[1,2] During the invasion of malignant tumor cells, chemotactic motilities assumed to be controlled by the autocrine motility factor,[3] and/or cell adhesion to various glycoproteins or glycosaminoglycans[4] are involved. Further degradation of surrounding tissue and blood vessels continues by secreting different classes of enzymes such as matrix metalloproteases (MMPs) and heparanases.[5-6] MMPs are a family of homologous enzymes and can degrade components of the cellular matrix. Inhibition of MMPs is expected to limit the formation of metastasis by preventing degradation of the basement membrane and/or inhibiting angiogenesis.[7]

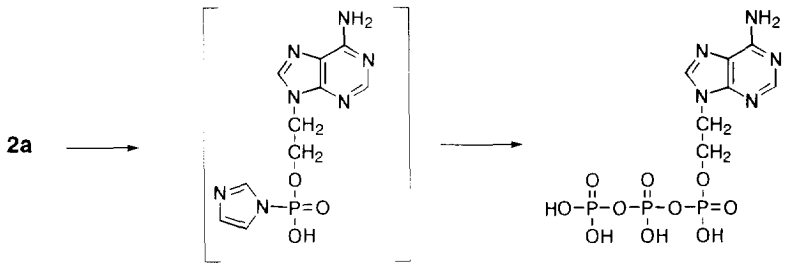
For the purpose of finding a new type of anti-invasion drug, several compounds were evaluated for their inhibitory effects on tumor cell invasion using reconstituted-basement membrane Matrigel (MG). We have recently found that 9-(2'-hydroxyethyl)purine 2'-phosphates possessed an inhibitory effect on HT-1080 tumor cell[8] invasion *in vitro* and also prevented the type IV collagen degradation by matrix metalloprotease 9 (MMP-9). This paper describes the first finding of purine derivatives possessing an inhibitory effect on tumor cell invasion.

9-(2'-Hydroxyethyl)adenine (**1a**) was easily obtained by the reaction of adenine with ethylene carbonate.[9] Reaction of **1a** with methyl iodide in *N,N*-dimethylacetamide, followed by refluxing in a sodium hydroxide solution, gave the corresponding N^6-methyl derivative (**1b**). Bromination of **1a** afforded 8-bromo-9-(2'-hydroxyethyl)adenine (**1c**). The coupling reaction of 6-chloropurine with bromoethanol in the presence of potassium carbonate gave 6-chloro-9-(2'-hydroxyethyl)purine (**1d**). The structures of these compounds (**1a-d**) were identified by their elemental analyses and spectral data.[10] Phosphorylation of 9-(2'-hydroxyethyl)purine derivatives (**1a-d**) with phosphorus oxychloride in trimethyl phosphate resulted into the formation of the corresponding 2'-monophosphates (**2a-d**), respectively. Reaction of **2a** with imidazole in the presence of triphenylphosphine and 2,2'-dipyridyldisulfide gave a 2'-phosphoroimidazolidate intermediate. Further treatment with tri-*n*-butylammonium pyrophosphate[11] in DMF generated 9-(2'-hydroxyethyl)adenine 2'-

triphosphate (**3**).



1a-d **2a-d**



3

Scheme 1

Figure 1. Inhibition ratios on HT-1080 tumor cell invasion using MG[16]

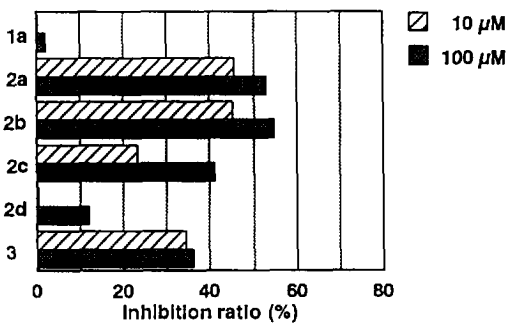
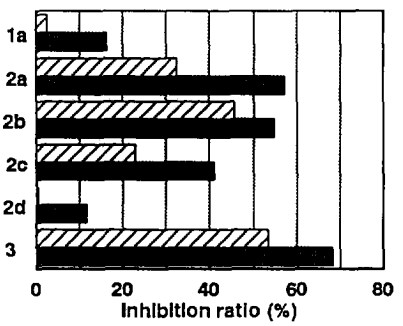


Figure 2. Inhibition ratios on type IV collagen degradation by MMP-9[17]



9-(2'-Hydroxyethyl)purine derivatives were evaluated for HT-1080 tumor cell invasion using MG.[12] The inhibitory effect on HT-1080 tumor cell invasion is summarized in Figure 1. Although 9-(2'-hydroxyethyl)purines (**1**) did not show any inhibitory activity on HT-1080 tumor cell invasion, its 2'-monophosphate derivative, 9-(2'-hydroxyethyl)purine 2'-monophosphate (**2a**), inhibited HT-1080 tumor cell invasion [inhibition ratio: 46% (10 μM), 53% (100 μM)]. Further introduction of pyrophosphate on the 2'-monophosphate residue yielding 2'-triphosphate derivative (**3**) decreased the inhibitory effect of **2a**. This observation could indicate that **2a** itself possesses anti-invasion properties without the expected metabolic conversion of **2a** into **3**. The N^6-methyl derivative (**2b**) also inhibited the invasion at the same level as **2a**. Bromination at the 8-position of the purine ring reduced inhibitory properties. The anti-invasion activity of **2d** dramatically disappeared when the amino group at the 6-position of **2a** was replaced with a chlorine atom. Both an amino-group at the 6-position and a phosphate group at the 2'-hydroxy position are requisite for anti-invasion properties.

It is well-known that HT-1080 tumor cells secrete MMP-2 and MMP-9.[6] There are many reports on the relation between MMP-9 secretion and the metastatic ability of cancer cells.[13] Therefore, these 9-(2'-hydroxyethyl)purine derivatives were also evaluated for their inhibitory effect on type IV collagen degradation by MMP-9 (see Figure 2). At concentrations 10 μM and 100 μM of **2a**, 32% and 57% of inhibition ratios were observed on type IV collagen degradation by MMP-9,[14] respectively. Compounds **2b** and **2c** also inhibited the effect on the type IV degradation by MMP-9. However, **1a** and **2d** did not show any inhibitory effect on type IV collagen degradation. In sharp contrast to the inhibitory activity on HT-1080 tumor cell invasion, the inhibitory effect of the triphosphate (**3**) was remarkably stronger than that of the monophosphate (**2a**) for the inhibitory effect on type IV collagen degradation by MMP-9. The inhibitory effect on tumor cell invasion using MG is almost parallel to the inhibitory activity on type IV collagen degradation by MMP-9. Compound **2a** did not show any activity of migration[15] or adhesion and possesed no cytotoxicity on HT-1080 cell.

To our knowledge, this is the first report providing the inhibitory effects on tumor cell invasion using 9-(2'-hydroxyethyl)adenine 2'-phosphates (**2a-c** and **3**). Our current results suggest positive implications for the development of an anti-metastasis drug in cancer chemotherapy.

References and Notes

1. Fidler, I. J. *Cancer Res.* **1990**, *50*, 6130-6138.
2. Yagel, S.; Khokha, R.; Denhardt, D. T.; Kerbel, R. S.; Parhar, R. S.; Lala, P. K. *J. Natl. Cancer Inst.* **1989**, *81*, 768-775.
3. Liotta, L. A.; Mandler, R.; Murano, G.; Katz, D. A.; Gordon, R. K.; Chiang, P. K.; Schiffmann, E. *Proc. Natl. Acad. Sci. USA* **1986**, *83*, 3302-3306.
4. Terranova, V. P.; Liotta, L. A.; Russo, R. G.; Martin, G. R. *Cancer Res.* **1982**, *42*, 2265-2269.
5. Nakajima, M.; Chop, A. M. *Cancer Biol.* **1991**, *2*, 115-127.
6. Stetler-Stevenson, W. G. *Cancer Matastasis Rev.* **1990**, *9*, 289-303.
7. Moses, M. A.; Sudhalter, J.; Langer, R. *Science*, **1990**, *248*, 1408-1410.
8. Human fibrosarcoma HT-1080 cells were kindly provided by Japanese Cancer Research Resources Bank.
9. Ustyuzhanin, G. E.; Kolomeitseva, V. V.; Tikhomirova-Sidorova, N. S. *Khim. Geterotsikl. Soedin.* **1978**, *5*, 684-689.
10. Selected data for **2a**: ^{1}H NMR (D_2O, 400 MHz) δ 8.12 (1H, s), 8.10 (1H, s), 4.34 (2H, brs), 4.04 (2H,

brd). UV (H_2O) λ_{max} 260 nm.

11. Kitade, Y.; Nakata, Y.; Hirota, K.; Maki, Y.; Pabuccuoglu, A.; Torrence, P. F. *Nucleic Acids Research*, **1991**, *19*, 4103-4108.
12. a) Collier, I. E.; Wilhelm, S. M.; Eisen, A. Z.; Marmer, B. L.; Grant, G. A.; Seltzer, J. L.; Kronberger, A.; He, C. S.; Bauer, E. A.; Goldberg, G. I. *J. Biol. Chem.* **1988**, *263*, 6579-6587. b) Nagase, H.; Haga, A.; Kito, H.; Sasaki, K.; Sato, T. *Cancer Research, Therapy, Control*, **1995**, *4*, 301-307.
13. a) Yamagata, S.; Tanaka, R.; Ito, Y.; Shimizu, S. *Biochem. Biophys. Res. Commun.* **1989**, *158*, 228-234. b) Kato, Y.; Nakayama, Y.; Umeda, M.; Miyazaki, K. *J. Biol. Chem.* **1992**, *267*, 11424-11430.
14. a) Ward R. V.; Atkinson, S. J.; Slocombe, P. M.; Docherty, A. J.; Reynolds, J. J.; Murphy, G. *Biochem. Biophy. Acta* **1991**, *1079*, 242-246. b) Chin, J. R.; Murphy, G.; Werb, Z. *J. Biol. Chem.* **1985**, *260*, 12367-12376.
15. a) Saiki, I.; Murata, J.; Watanabe, K.; Fujii, H.; Abe, F.; Azuma, I. *Jpn. J. Cancer Res.* **1989**, *80*, 873-878. b) Saiki, I.; Murata, J.; Nakajima, M.; Tokura, S.; Azuma, I. *Cancer Res.* **1990**, *50*, 3631-3637.
16. The effect of 9-(2'-hydroxyethyl)purine derivatives on tumor cell invasion to reconstituted basement membrane MG was measured by using HT-1080 tumor cell line. The tumor cells were incubated for 120 min at 37 ℃ in 5% CO_2 atmosphere with 9-(2'-hydroxyethyl)purine derivatives of concentrations at 10 μM and 100 μM in upper compartment of the Transwell chamber.
17. FITC-labeled type IV collagens and MMP-9 were incubated at 37 ℃. To suspended enzymatic reaction, 5 μl of o-phenanthroline in 50% ethanol were added to the mixture. The released fluorescent isothiocyanate in supernatant of the mixture separated by centrifugation (10,000 g, 15 min) was measured by spectrophotometer with excitation and emission wavelengths of 495 nm and 520 nm, respectively. Inhibition ratio (%) was expressed as follows:$[1-(FI_{mixture}-FI_{blank})/(FI_{total}-FI_{blank})] \times 100$.

(*Received in Japan* 20 *December* 1996; *accepted* 20 *February* 1997)