

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

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

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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.<sup>1,2</sup> During the invasion of malignant tumor cells, chemotactic motilities assumed to be controlled by the autocrine motility factor,<sup>3</sup> and/or cell adhesion to various glycoproteins or glycosaminoglycans<sup>4</sup> are involved. Further degradation of surrounding tissue and blood vessels continues by secreting different classes of enzymes such as matrix metalloproteases (MMPs) and heparanases.<sup>5,6</sup> 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.<sup>7</sup>

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<sup>8</sup> 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.<sup>9</sup> 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.<sup>10</sup> 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<sup>11</sup> in DMF generated 9-(2'-hydroxyethyl)adenine 2'- triphosphate (3).



Scheme 1

Figure 1. Inhibition ratios on HT-1080 tumor cell invasion using MG<sup>16</sup>





9-(2'-Hydroxyethyl)purine derivatives were evaluated for HT-1080 tumor cell invasion using MG.<sup>12</sup> 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  $\mu$ M), 53% (100  $\mu$ M)]. 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<sup>6</sup>-methyl derivative (2b) also inhibited the 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.<sup>6</sup> There are many reports on the relation between MMP-9 secretion and the metastatic ability of cancer cells.<sup>13</sup> 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  $\mu$ M and 100  $\mu$ M of **2a**, 32% and 57% of inhibition ratios were observed on type IV collagen degradation by MMP-9,<sup>14</sup> 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, 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<sup>15</sup> 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.

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brd). UV (H<sub>2</sub>O)  $\lambda_{max}$  260 nm.

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- 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  $^{\circ}$ C in 5% CO<sub>2</sub> atmosphere with 9-(2'-hydroxyethyl)purine derivatives of concentrations at 10  $\mu$ M and 100  $\mu$ M in upper compartment of the Transwell chamber.
- 17. FITC-labeled type IV collagens and MMP-9 were incubated at 37 °C. 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<sub>mixture</sub> FI<sub>blank</sub>)/(FI<sub>total</sub> FI<sub>blank</sub>)] x 100.

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