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EFFECT OF NUCLEOSIDES AND A NUCLEOTIDE MIXTURE ON PROLIFERATION OF HUMAN GASTRIC CANCER CELLS (KATO III)

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INDEXING WORDS

purine nucleoside; pyrimidine nucleoside; metabolism; cancer cell proliferation; 5FU; biochemical modulation

SYNOPSIS

The effect of the nucleosides and a nucleotide mixture (OG-VI), consisting of inosine, guanosine 5'-monophosphate (5'-GMP), cytidine, uridine, thymidine (TdR) (4:4:4:3:1 in molar ratio), and TdR co-administration on proliferation of KATO III human gastric cancer cells in culture was evaluated. Consumption of purine and pyrimidine by cancer cells and changes in cell number with OG-VI or TdR were compared with the control culture medium (Williams E) after 72 hour-culture. Addition of OG-VI or TdR did not enhance the cellular proliferation, but inhibited growth when given in higher concentrations (0.3-3 mM inosine, 0.3-3 mM 5'-GMP, 0.22-2.2 mM uridine, 74-740 μM TdR). Consumption rate of TdR in the medium was less in the TdR group, 33.7%, than in the OG-VI group, 72.2% ($p<0.05$). This suggests that TdR metabolism is modulated by other nucleosides and nucleotide included in OG-VI. Under the coadministration of 5-fluorouracil (FUra), addition of OG-VI or TdR suppressed

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cellular proliferation ($p<0.05$). The inhibition rate of cellular proliferation in the OG-VI group was slightly higher than the TdR group, but there was no statistically significant difference between the two groups. The combination of FUra with OG-VI or TdR enhances the antitumor effect of FUra. It is concluded that the OG-VI does not enhance the tumor cell proliferation and it is a potential biochemical modulator of FUra matabolism in human cancer cells.

Key Words: Nucleosides and a nucleotide mixture, Proliferation, Human gastric cancer cell, Biochemical modulation, 5-Fluorouracil.

INTRODUCTION

Recently, a solution containing a mixture of nucleosides and nucleotide (OG-VI), consisting of 0.83% (w/v) inosine, 0.73% cytidine, 1.22% guanosine 5'-monophosphate (5'-GMP), 0.55% uridine, and 0.18% thymidine (TdR), has been developed as a nutritional supplement [14]. Administration of OG-VI with total parenteral nutrition (TPN) improved protein metabolism after partial hepatectomy in rats [15] and reduced liver injury after galactosamine injection in rats [16]. Addition of OG-VI increased DNA synthesis in primary cultured hepatocytes[17] and increased hepatic regeneration after partial hepatectomy in rats [22]. From these results, OG-VI may be a useful therapeutic device for periopertive nutritional management in general surgery. However, the augmentation of DNA synthesis in hepatoma cell culture by OG-VI was pointed out in some concentration range[5.17], and it arouses a great anxiety that it may enhance the proliferation of tumor cell. In order to clarify the direct effect of OG-VI on the proliferation of cancer cells, the proliferation of human gastric cancer cell was evaluated in vitro.

5' Fluorouracil (FUra), the fluorinated analogue of uracil, has been used in chemotherapy for various tumors and certain chemicals affect metabolism of FUra [2.8.18]. TdR and cytidine, pyrimidines, and inosine and GMP, purines, of OG-VI have been found to be biochemical modulators which change the metabolism of FUra and increase the effectiveness of FUra in cancer chemotherapy [20.21]. However, uridine decreases the effect of FUra [7.13]. Thus it is hypothesized that OG-VI, a mixture of these materials, is also a biochemical modulator of FUra.

In this study, we investigated the effects of the nucleosides and a nucleotide mixture on the proliferation of human gastric cancer cells by evaluating the consumption of purine and pyrimidine in the culture medium, and by analyzing cellular proliferation through counting cell numbers and MTT assay.

MATERIALS AND METHODS

Cell, medium and major reagents. KATO III human gastric cancer cells (Research Institute, Gunma, Japan) was used in all experiments. Six milliliter of 2x10^4 cells/ml suspension was inoculated into a 60 mm diameter tissue culture dish (Becton Dickinson Labware, Lincoln Park, NJ). Williams-E medium (Flow Laboratories, Scotland), with 100 U/ml of penicillin G (Meiji Seika Kaisya, Tokyo, Japan), 100 μg/ml of kanamycine (Meiji) and 10% fetal calf serum (GIBCO, Grand Island, NY), was sterilized through the use of a 0.22 μm filter (Millipore, Yonezawa, Japan).

OG-VI and TdR were kind gifts from Otsuka Pharmaceutical Company, Tokushima, Japan. FUra (Kyowa Hakko Kogyo Co., Tokyo, Japan) and other chemicals were obtained from local commercial sources. OG-VI composed of 30 mM inosine, 30 mM 5'-GMP, 30 mM cytidine, 23 mM uridine and 7.4 mM TdR [17]. OG-VI or TdR were added at various concentration, with or without FUra at concentrations ranging from 0.01 to 1.0 μg/ml. The cells were incubated at 37 °C in 5% CO_2 and 100% humidified atmosphere for 72 hours.

Consumption of purine and pyrimidine by KATO III. The concentration of purine and pyrimidine in the medium before and after the introduction and incubation of KATO III cells were measured by high-performance liquid chromatography (HPLC) [17]. A TSK gel ODS-80 TM column (Shimazu, Tokyo, Japan) were used in the LC-6AD HPLC system (Shimazu). The consumption rate of purine and pyrimidine was calculated as follows: (Conc. pre - Conc. post)/Conc. pre x 100(%).

Cell count. The viability of the cells was judged by dye exclusion test using 0.15% trypan blue and the number of cells was counted using a Burker-Turk counting chamber under a light microscope before and after incubation. Cell number in each experimental group was expressed as percent change from the control group.

MTT [3-(4,3-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide] calorimetric assay procedure. The cellular proliferation was evaluated using the MTT assay. High correlation between the result of MTT assay and thymidine uptake study has been reported [12].

Statistical analysis. Each result is taken as the mean value of a series of 6-10 experiments carried out. Values were expressed as mean ± standard deviation (SD). Statistical analysis were based upon Student's*t* test.

RESULTS

The consumption of purine and pyrimidine in the culture medium.

The consumption rate of purine (inosine and 5'-GMP) was greater than that of pyrimidine (cytidine, uridine and TdR) without FUra (Table 1). The concentration of

Table I. Percent consumption rate of OG-VI constituents with or without FUra by human gastric cancer cells (KATO III)

	FUra (-)	FUra (+)
Inosine	66.7 ± 9.8	33.5 ± 3.0*
5'-GMP	97.5 ± 2.8	58.3 ± 13.6*
Cytidine	11.0 ± 12.2	16.8 ± 9.8
Uridine	0	13.7 ± 9.0
TdR	16.7 ± 17.9	33.7 ± 8.4*

Comparison with FUra (n=3) vs without FUra (n=3). Values are mean ± SD, * $p<0.05$, unpaired t test. The concentration of FUra was 0.1 μg /ml, and that of a mixture solution of nucleosides and a nucleotide was 1/10 of OG-VI

Table II. Percent consumption rate of TdR with or without FUra by human gastric cancer cells (KATO III)

	FUra (-)	FUra (+)
TdR	54.4 ± 11.9	72.2 ± 6.9*

Comparison with FUra (n=6) vs without FUra (n=6). Values are mean ± SD. * $p<0.05$ (unpaired t test). The concentration of TdR was 740 μM.

xanthine and hypoxanthine in the medium increased after the incubation. By the addition of OG-VI or TdR, consumption of purine and pyrimidine was greater in the medium with higher concentration (Fig. 1, 2).

With the addition of 0.1 μg/ml of FUra, the consumption rates of purine and pyrimidine in OG-VI differed from those without FUra. The consumption rate of purine decreased, but that of pyrimidine increased (Table 1). The consumption rate was 54.4% in the TdR group vs. 16.7% in the OG-VI group ($p<0.05$) (Table 1, 2). Further more, the consumption rate of TdR in the TdR group with FUra was up by 72.2%, which was

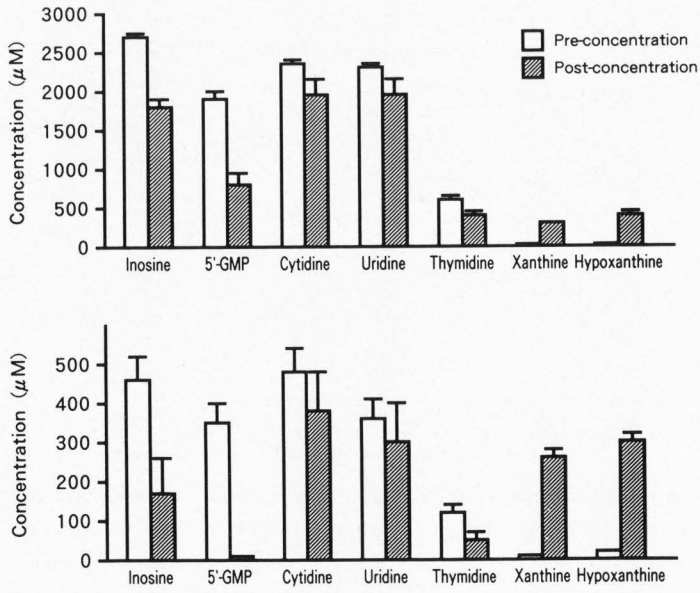

Fig. 1 Changes of purine and pyrimidine concentration in the medium, before and 3 days after incubation. The upper figure is the result of 1/10 (n=6) and the lower figure is the result of 1/100 concentration (n=6) of OG-VI. Values are mean ± SD.

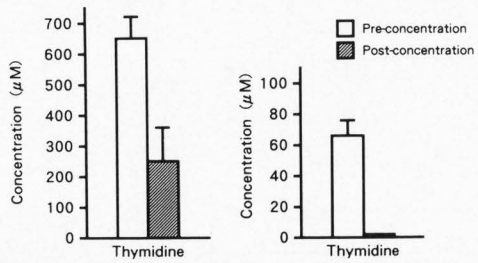

Fig. 2 Changes of TdR concentration in the medium, before and 3 days after incubation. The left figure is the result with 740 μM of TdR (n=6), and the right figure is the result with 74 μM of TdR (n=6). Values are mean ± SD. $P<0.05$ by unpaired t test.

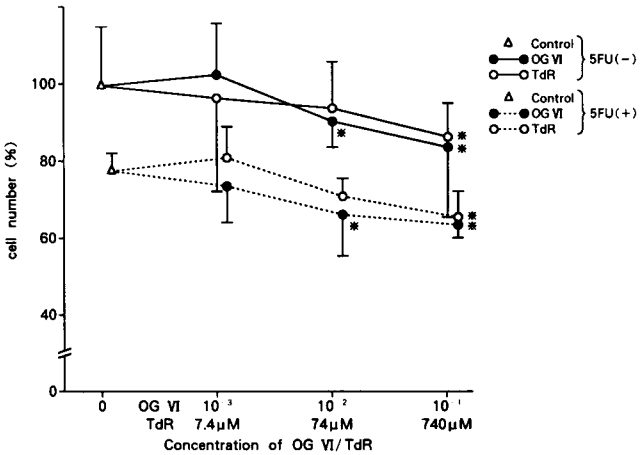

Fig. 3 Percent changes in KATO III cell number for the 3 day-culture with or without 0.1 μg /ml FUra in different concentrations of OG-VI and TdR. Abscissa is the concentration of OG-VI and TdR. Ordinate is the percent change in cell numbers as compared to the control group. Values are mean ± SD. *: P<0.05 by unpaired t test. N=6-10 in each point.

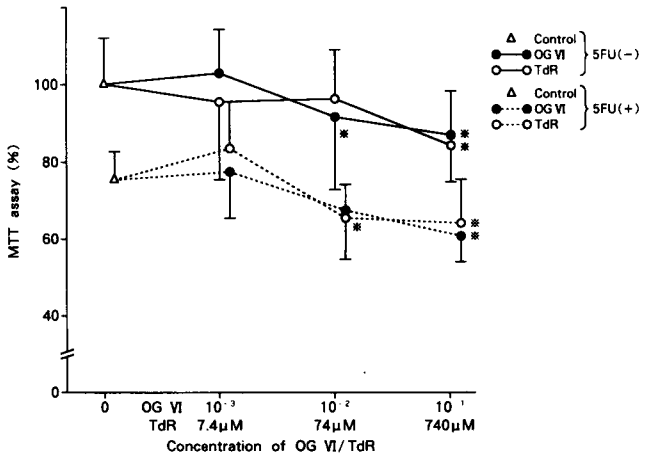

Fig. 4 Percent changes of MTT assay of KATO III cells for the 3 day-culture with or without 0.1 μg/ml of FUra in different concentration of OG-VI and TdR. Abscissa is the concentration of OG-VI and TdR. Ordinate is the percent changes of MTT assay as compared to the control group. Values are mean ± SD. *: P<0.05 by unpaired t test. N=6-10 in each point.

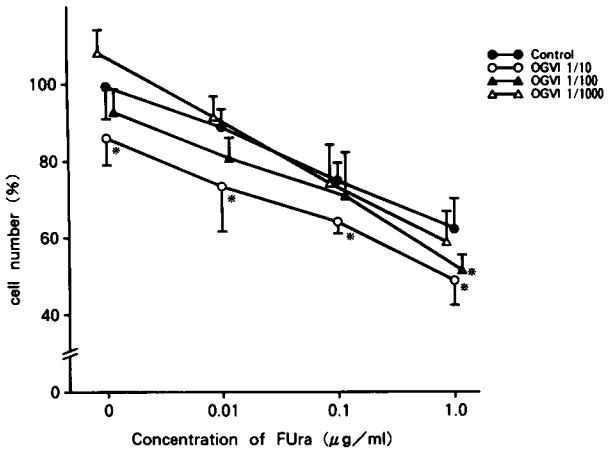

Fig. 5 Percent changes in KATO III cell number for the 3 day-culture in three concentrations of OG-VI with 0.01, 0.1, 1.0 μg/ml of FUra respectively. Abscissa is the concentration of FUra. Ordinate is the percent change in cell number as compared to the control group. Values are mean ± SD. *: P<0.05 by unpaired t test. N=6 in each point.

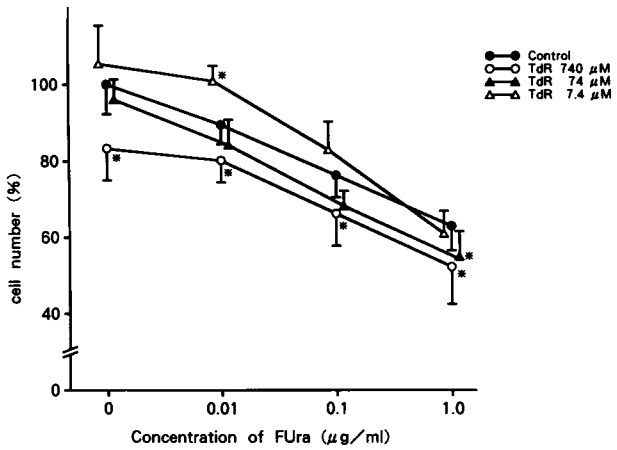

Fig. 6 Percent changes in KATO III cell number for the 3 day-culture in three concentration of TdR with 0.01, 0.1, 1.0 μg/ml of FUra respectively. Abscissa is the concentration of FUra. Ordinate is the percent change in cell number as compared to the control group. Values are mean ± SD. *: P<0.05 by unpaired t test. N=6 in each point.

statistically significant (Table 2). Consumption rate in the OG group was also increased ($p<0.05$). As to the purine metabolism, the production of xanthine and hypoxanthine was less in the TdR group, 15.2 ± 3.3 μM and 2.2 ± 1.3 μM under 740 μM of TdR, respectively, than those in the OG-VI group.

Cell proliferation.

Addition of OG-VI did not enhance cellular proliferation in all concentration ranges examined (Fig. 3). Furthermore, OG-VI decreased cell growth to 84% of the control in higher concentration range at 1-10% of OG-VI solution with statistical significance ($p<0.05$). TdR exhibited the same tendency toward inhibition of cellular proliferation, but the inhibitory effect was observed only at the highest concentration, 740 μM (Fig. 3, 4). The result of MTT assay was almost the same as that of cell number count (Fig. 4).

FUra decreased cell growth in a concentration dependent fashion (Fig. 5). Addition of OG-VI enhanced the inhibitory effect of FUra in all FUra concentration ranges ($p<0.05$). TdR showed the same inhibitory tendency as OG-VI in the cellular growth with FUra (Fig. 6, $p<0.05$).

DISCUSSION

The effect of a mixture of nucleosides and a nucleotide on the proliferation of human cancer cells was evaluated in cell culture.

No enhancement of proliferation was observed by the addition of OG-VI within the broad concentration ranges (1/10-1/1000). When higher concentrations of OG-VI or TdR was added, the proliferation of KATO III cell was inhibited further. This result is in agreement with the report that TdR produces cytokinetic arrest of cellular progression through S phase at mM concentration [1]. Moreover, when the concentration of OG-VI in the culture medium was in the range of 1/10-1/100 dilution of the original concentration, there was an obvious FUra augmentation effect in the inhibition of KATO III cell proliferation. There was a statistically significant difference in cell count observed between the culture with or without OG-VI

The augmented inhibition of cellular growth by OG-VI and TdR with FUra was observed, and the antitumor effect under the combination of 0.1 μg/ml of FUra with OG-VI and TdR is equal to that of 1.0 μg/ml of FUra. This result suggests the possibility of decreasing the dosage and consequently the side effect of FUra without changing the antitumor effect of FUra by the combined administration of OG-VI and FUra. Although the OG-VI group showed a slightly stronger effect than the TdR group in the inhibition of cancer cell proliferation, there was no statistically significant difference between the two groups. In our in vitro study, a mixture of nucleosides and a nucleotide did not show any additional effect on the TdR's ability to modulate cellular proliferation.

The consumption of purine and pyrimidine in the medium may imply competitive utilization of them by cancer cells. The consumption rate of TdR was more in the TdR group (54.4%) than in the OG-VI group (16.7%). This result indicates that TdR group was used in place of other pyrimidines. The amount of purine catabolized in the TdR group was less than the OG-VI group. This suggests that nutrients in OG-VI are better balanced than TdR alone for the purine and pyrimidine metabolism in cancer cells.

FUra decreased the usage of purine and increased the usage of pyrimidine. The consumption rate of TdR increased by the addition of FUra in both the OG-VI and the TdR group. The mechanism of increased TdR utilization by FUra is considered as follows: FUra is converted to 5-fluoro-2'-deoxyuridine-5'-monophosphate (FdUMP), which binds to thymidylate synthetase and inhibits de novo synthesis of deoxy-thymidine monophosphate (dTMP) [4.21]. In place of that, the salvage synthesis of dTMP from TdR increases [17]. And then the consumption of TdR in the medium increases.

In the OG-VI group, the amount of pyrimidine utilization increased and the amount of purine utilization decreased by the addition of FUra. The mechanism for increased pyrimidine consumption is considered as follows: Increased TdR utilization has been explained already. Phosphoribosyl pyrophosphate amidotransferase (PRPP) is utilized in the metabolism of FUra to fluorouridin monophosphate (FUMP) and this decreases the level of metabolically available PRPP [10,11]. Since PRPP plays an important role in the metabolism of orotic acid to orotidine monophosphate (OMP) in the de novo pyrimidine synthesis, decreased PRPP level decreases the de novo synthesis of OMP and thus increases the salvage synthesis pathway of uridine and cytidine. Therefore, the addition of FUra increases the consumption of uridine and cytidine.

The decreased amount of purine consumption observed is postulated to be the result of decreased cellular activity secondary to the metabolic disturbances of pyrimidine caused by the addition of FUra.

Differences were observed in the consumption of TdR between the OG-VI group and the TdR group. This suggests that the metabolic change brought about by OG-VI with FUra differs from that by TdR with FUra, even though there were no difference in cellular proliferation. The mechanism of biochemical modulation by the addition of different materials differ. Inosine, GMP and TdR increase cytotoxicity of FUra[3.6], but uridine decreases cytotoxicity [13]. In this study, the concentration of uridine in the medium did not change between pre- and post-incubation. This suggests that uridine is a potential inhibitor of dihydropyrimidine dehydrogenase (DPD), the catabolic enzyme of pyrimidine, but does not affect the DNA and RNA synthesis of cancer cells [6].

This result of biochemical modulation is in agreement with those of Spiegelman et al [19]. They reported that TdR pretreatment can cause a three to five fold increase in the amount of FUra incorporated at equivalent RNA synthesis. High-dose FUra can

produce a powerful antitumor effect by at least two mechanisms, the inhibition of thymidylate synthesis and the incorporation of FUra into RNA. This concept supports the direct effect of TdR as a biochemical modulator of FUra on cancer cells.

It is possible that patients suffering from cancer can benefit from the combined administration of FUra with OG-VI or TdR for alleviating the side effect of FUra. Moreover, compared to TdR, it can be postulated that OG-VI shows a different effect in the inhibition of tumor cell proliferation and in the survival benefit, because cellular immunity modulation was observed in experiments when animals were given yeast RNA diet [23] and the mechanism of modulation in purine and pyrimidine metabolism by OG-VI differed from that by TdR. In vivo study is required to evaluate the effect that combined administration of FUra and OG-VI has on cellular growth and toxicity.

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