In Vivo Effect of Nucleotide and Nucleosides (OG-VI) Solution and Dietary Nucleotide Deficiency on Tumor Proliferation With Coadministration of 5-Fluorouracil (5 FU)

Usami, Makoto ; Zheng, Jianghua ; Haji, Seiji ; Kotani, George ; Sen, Hiroyoshi ; Kasahara, Hiroshi ; Tabuchi, Yoshiki

(Citation)
Bulletin of health sciences Kobe,14:73-80

(Issue Date)
1998-12-26

(Resource Type)
departmental bulletin paper

(Version)
Version of Record

(JaLCDOI)
https://doi.org/10.24546/00181752

(URL)
https://hdl.handle.net/20.500.14094/00181752

In Vivo Effect of Nucleotide and Nucleosides (OG–VI) Solution and Dietary Nucleotide Deficiency on Tumor Proliferation With Coadministration of 5–Fluorouracil (5 FU)

Makoto Usami[1], Jianghua Zheng[2], Seiji Haji[2], George Kotani[2], Hiroyoshi Sen[2], Hiroshi Kasahara[2], and Yoshiki Tabuchi[1]

Aim: To evaluate the effect of nucleotide and nucleosides mixture solution administration on tumor cell proliferation.
Methods: Tumor growth of Yoshida sarcoma, inoculated subcutaneously, was evaluated in male Donryu rats under a nucleotide deficient diet with or without intragastric administration of 5-FU (20 mg/kg/day, 7 days) and intraperitoneal administration of OG–VI solution (a mixture of inosine, 5'–GMP, cytidine, uridine, and thymidine in a 4 : 4 : 4 : 3 : 1 molar ratio, 2.8–14 ml/kg/day, 7 days).
Results: Tumor weight was 0.50 ± 0.39, 0.13 ± 0.13, and 0.00 ± 0.00 g, in 0, 2.8, and 14 ml/kg/day OG–VI with 5-FU administration, respectively with statistical significance ($p < 0.01$). Tumor size in dietary nucleotide deficiency rats with OG–VI and 5-FU was 0.13 ± 0.13, against 0.64 ± 0.53 g in a normal diet ($p < 0.01$). These results were obtained without major complications. The effect of OG–VI mixture was stronger than the effect of each component of OG–VI alone ($p < 0.05$). Enhancing effect of OG–VI on 5-FU action in tumor proliferation is apparent. Also this pilot study suspects that dietary nucleotide deficiency enhanced the effect of OG–VI.
Conclusions: OG–VI is a potential biochemical modulator of 5-FU and dietary nucleotide influences the effect.

Key Words
OG–VI,
Dietary nucleotides,
Tumor growth,
5-FU,
Biochemical modulation.

Introduction

Introduction

Purine and pyrimidine are essential for cellular proliferation[1,2,3]. But, they are synthesized from glutamine in de novo synthesis pathway and are not considered as essential nutrients. Patients with gastrointestinal disorders including bowel obstruction require bowel rest and are treated with total parenteral nutrition (TPN) or elemental diet (ED) to maintain sufficient nutrition. TPN and ED consist of synthesized macronutrients and micronutrients, however they do not contain purine and pyrimidine. Recently, a solution containing a mixture of nucleosides and nucleotide (OG–VI), consisting of inosine, cytidine, guanosine 5'–monophosphate (5'–GMP), uridine, and thymidine, has been developed as a nutritional supplement (Table 1)[4]. OG–VI is known to increase hepatic regeneration and improve

Faculty of Health Sciences[1] and First Department of Surgery[2], Kobe University School of Medicine, Kobe, Japan

Table 1. Constituent of OG–VI 1

	(W/V%)	n mol/l	molar ratio
inosine	0.80	29.83	4
5'-GMP	1.22	30.01	4
cytidine	0.73	29.96	4
uridine	0.55	22.52	3
thymidine	0.18	7.43	1
total nucleic acids	3.35		

protein metabolism after partial hepatectomy in normal and cirrhotic rats[5, 6], and increase DNA synthesis in primary cultured hepatocytes[7]. From these results, OG–VI may be a useful therapeutic device for periopertive nutritional management in abdominal surgery. However, the augmentation of DNA synthesis in hepatoma cell culture by OG–VI was pointed out in some concentration range[7], and it arouses a great anxiety that it may enhance the proliferation of tumor cell.

Our results in cell culture indicate that OG–VI itself does not have an enhancing effect on proliferation of human gastric cancer cells[8]. Purines and pyrimidines in the culture medium are utilized by human gastric cancer cells and their consumption rates were modified by the addition of 5'-fluorouracil (5-FU). 5-FU is an important component of chemotherapeutic regimens used in the treatment of common malignancies, including breast cancer and gastrointestinal carcinomas. Also we showed that OG–VI or its components affect 5-FU metabolism and increase 5-FU action on cellular proliferation as a biochemical modulator of 5-FU[8].

The effect of protein depletion on 5-FU metabolism has been reported[9]. Protein depletion increased toxicity of 5-FU associated with a significantly decreased rate of hepatic metabolism and clearance of 5-FU. However, the effect of dietary nucleotide deficiency on 5-FU metabolism has not been reported.

The purpose of this study is to evaluate the effect of repeated intraperitoneal OG–VI administration and/or dietary nucleotide deficiency on tumor growth and their modulation on 5-FU in an in vivo study.

Materials and Methods

Male Donryu rats, 8 to 9 weeks of age, obtained from Japan SLC, Shizuoka, were kept in wire–bottomed cages at a temperature controlled, 12–h light–dark cycle. Yoshida sarcoma (YS), a gift from SRL Hachioji Lab., Tokyo, were maintained by intraperitoneal implantation. Rats were anesthetized lightly with ether and 1 x 10^6 YS cells were inoculated subcutaneously. One day after transplantation the rats were divided into each experimental groups. Drug was administered in the morning. Dosage of OG–VI in rats is considered 2.8-14 ml/kg/day according to the data of Ogoshi[4].

Experiment 1

Rats fed a nucleotide free (NF) diet ad libitum (AIN–B, Oriental Co., Japan), with intragastric administration of 5-FU or saline (20 mg/kg/day, dissolved in saline as 10 mg/ml, Kyowa Hakko Kogyo Co., Japan) and intraperitoneal administration of OG–VI solution (2.8–14 ml/kg/day) or saline for 7 days. OG–VI, composed of 30 mM inosine, 30 mM 5'–GMP, 30 mM cytidine, 23 mM uridine and 7.4 mM thymidine and each component were kind gifts from Otsuka Pharmaceutical Co., Tokushima, Japan. 2.8 ml/kg/day of its each component was administered intraperitoneally , uracil (24.61% in saline, uracil : 5-FU = 4 : 1 mol, the same constituents as in UFT, commonly used biochemical modulator[10]) was intragastrically administered.

Experiment 2

Rats fed NF diet or standard diet ad libitum (CE 2, Nihon Crea, Japan) were compared with daily 5-FU (all experiments were performed with 20 mg/kg/day administration except indicated) or saline in combination with 2.8 ml/kg/day OG–VI or saline administration.

After 7 days, rats were laparotomized for blood and organ sampling under pentobarbital anesthesia at the 8 th experimental day. Blood biochemical tests by serum multiple analyzer (TBA 80 S multianalyzer, Toshiba) and blood counts (Sysmex K–1000, Toa Medical Electronics) were performed. The tumor was separated surgically from subcutaneous tissue and the weight was measured. Body weight and organs (liver, spleen, kidney) weight were measured

Each result was taken as the mean value of a series of 5–12 experiments carried out and expressed as mean ± standard deviation (SD). Statistical analysis was based upon Student's t test and Mann–Whitney U test. A p–value less than 0.05 was considered significant.

Results

Rats in the experiment 1 tolerated well except uracil group. OG–VI did not enhance the tumor growth in all dosage examined and did not change organ weights or results of blood biochemical tests (Fig 1). 5-FU decreased cell growth. Tumor size in the 5-FU group was 1.20 ± 0.93 g compared with 2.77 ± 1.26 g in the control group ($p < 0.05$).

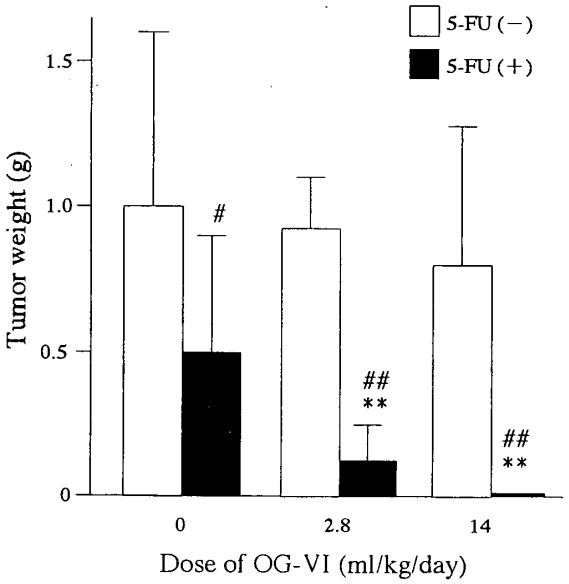

Figure 1. Effect of OG–VI on tumor proliferation with 5-FU.
n = 12, mean ± SD, * $p < 0.01$ vs 5–FU (+) and OG–VI (–), # $p < 0.05$, ## $p < 0.01$ vs 5–FU (–)

Coadministration of each component of OG–VI equivalent to total purine and pyrimidine in 2.8 ml/kg/day OG–VI decreased tumor growth (Fig 2). Thymidine, uridine, inosine and 5'–GMP except cytidine decreased tumor growth. Tumor

weight in the OG–VI group was 0.09 ± 0.07 g compared with 1.20 ± 0.93 g in the 5-FU group ($p < 0.05$). OG–VI remarkably suppressed tumor growth in comparison with its components. Body weight, organ weights and results in blood biochemical tests in the OG–VI group did not show any statistical changes compared with 5-FU alone. Rats in the uracil group showed severe diarrhea, decreased body weight and spleen weight, then four out of six died before sacrifice.

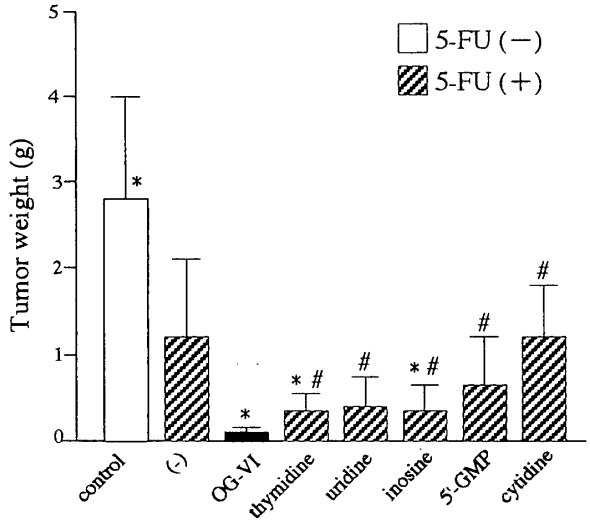

Figure 2. Effect of OG–VI and its components on tumor proliferation with 5-FU. n = 6–8, mean ± SD, * $p < 0.05$ vs 5–FU (+), # $p < 0.05$ vs 5-FU + OG–VI.

Tumor size in the 2.8 ml/kg/day OG–VI with 5-FU group was 0.13 ± 0.13 g in comparison with 0.91 ± 0.18 g without 5-FU. Increased dosage of OG–VI, from 2.8 ml/kg/day to 14 ml/kg/day completely diminished subcutaneous tumor, 0.0 ± 0.0 g with 5-FU vs. 0.80 ± 0.43 g without 5-FU ($p < 0.01$). And all rats tolerated these treatment. However, decreased albumin and increased uric acid blood levels indicated adverse effect of suppressed protein metabolism and increased nucleotide degradation with tumor cell distraction in higher OG–VI administration with 5-FU.

Dietary nucleotide deficiency decreased tumor weights without 5-FU coadministration (Table 2). Also, antitumor effect of 5-FU was facilitated with or without OG–VI under a NF diet ($p < 0.01$). Apparent adverse effect was not observed under a NF diet (Table 3).

Discussion

The present in vivo pilot study demonstrated that OG–VI itself does not affect tumor growth, however, it enhances 5-FU action of tumor suppression without increasing adverse effect. This biochemical modulation effect of OG–VI is stronger than each component of OG–VI. The

Table 2. Effect of Dietary Nucleotide on Tumor Weight (g) with Coadministration of 5–FU and OG–VI

		NF (n = 12)	C (n = 6)
5-FU (−)		1.00 ± 0.60*	3.25 ± 1.42
5-FU (+)	OG-VI 0#	0.50 ± 0.93*	1.44 ± 0.94
	OG-VI 2.8	0.13 ± 0.13*	0.64 ± 0.53

NF: nucleotide free diet, C: control diet, 5-FU: 5-fluorouracil, mean ± SD,

*$p < 0.01$ vs. C, # OG-VI dosage, ml/kg/day

Table 3. Body Weight and Blood Level Changes under Ne Diet with Coadministration of 5-FU and OG-VI

			NF (n =12)	C (n =6)
Body W. (% change)	5-FU (−)		0.95 ± 0.06 **	1.10 ± 0.05
	5-FU (+)	OG-VI 0 §	0.77 ± 0.04 *	0.84 ± 0.08 ##
		OG-VI 2.8	0.77 ± 0.05	0.78 ± 0.08
Albumin (g/dl)	5-FU (−)		3.23 ± 0.46 **	2.32 ± 0.21
	5-FU (+)	OG-VI 0	2.60 ± 0.23 ##	2.90 ± 0.44 #
		OG-VI 2.8	2.52 ± 0.30 ##	2.62 ± 0.31
Uric Acid (mg/dl)	5-FU (−)		1.15 ± 0.48	1.30 ± 0.47
	5-FU (+)	OG-VI 0	1.53 ± 0.37 #	1.25 ± 0.40
		OG-VI 2.8	1.70 ± 0.80	1.27 ± 0.51
RBC (×10000/ml)	5-FU (−)		749 ± 49	721 ± 33
	5-FU (+)	OG-VI 0	807 ± 64 #	840 ± 58
		OG-VI 2.8	777 ± 67	851 ± 139
WBC (×100/ml)	5-FU (−)		180 ± 42	232 ± 62
	5-FU (+)	OG-VI 0	124 ± 43 § §	103 ± 61 ##
		OG-VI 2.8	125 ± 39 § §	67 ± 26 ##

NF: nucleotide free diet, C: control diet, 5-FU: 5-fluorouracil, mean ± SD, ** $p < 0.01$ vs. C, * $p < 0.05$ vs. C, ## $p < 0.01$ vs. 5-FU (−), # $p < 0.05$ vs. 5-FU (−), § OG-VI dosage, ml/kg/day, § § data from 10 mg/kg/day 5-FU administration

effect of OG-VI with coadministration of 5-FU to tumor growth is prominent in a nucleotide deficient diet.

This in vivo result showing no enhancement of tumor growth by OG-VI in the dosage between 2.8 and 14 ml/kg/day range agrees with our previous in vitro study indicating that OG-VI does not increase cellular proliferation with the broad concentration range but inhibits growth in higher concentration[8]. These results of OG-VI on cellular proliferation are controvertial in different disease condition. It is also reported by us that preoperative OG-VI administration before partial hepatectomy facilitates hepatic regeneration in vivo and DNA and RNA synthesis of hepatocytes in vitro[6,7]. Two times repeated preoperative administration of 10 ml/kg/day of OG-VI increases blood pyrimidine level and pool size before operation and increases ATP level at 5 hour after operation, then improves hepatic regeneration after 24 hours[6]. However, postoperative administration does not affect hepatic regeneration with TPN nor hepatic ATP content in fasting rabbit[6,11]. Many important discrepancies exist between tumor bearing rats and hepatectomized rats. After partial hepatectomy, purine and pyrimidine metabolism changes are physiological and well organized pleiotypic response occurring in short duration. Also, the administration

of OG–VI before operation and to feeding rats is important after hepatectomy. In malignant disorder, uncontrolled constant tumor growth with abnormal purine and pyrimidine metabolism is characteristic. The data from two different experimental models indicates that OG–VI is effective to increase purine and pyrimidine pool size, but requires some suitable condition in DNA and RNA synthesis. Further investigation is required to explain the discrepancy of OG–VI effect in no tumor proliferation in comparison with facilitation in hepatic regeneration.

The in vivo results revealed that OG–VI by itself did not influence tumor growth, but it enhanced the effect of 5-FU on tumor growth showing 86% reduction in tumor weight without major complication. The in vivo result of this biochemical modulation is in agreement with our in vitro observation[8]. In vitro results of purine and pyrimidine utilization in cell culture indicate that the addition of 5-FU increases the consumption of uridine and cytidine, decreases the consumption of inosine and 5'–GMP, and addition of OG–VI changes 5-FU metabolism. The anti–tumor effect under the combination of 0.1 μg/ml of 5-FU with OG–VI is equal to that of 1.0 μg/ml of 5-FU. Both in vivo and in vitro results suggest the possibility of decreasing 5-FU dosage and consequently decreasing the side effect of 5-FU without changing the anti–tumor effect of 5-FU in combination with OG–VI administration.

The result of in vivo comparison among OG–VI and its components on tumor growth showed the advantage of the mixture solution in biochemical modulation of 5-FU. In vitro report shows comparable effect of thymidine and OG–VI in cellular proliferation[8], but this in vivo result indicates statistically significant suppression of tumor growth by OG–VI compared with thymidine.

The mechanism of biochemical modulation by the addition of different purines and pyrimidines differs[12, 13]. Inosine, 5'–GMP and thymidine increase the cytotoxicity of 5-FU[2, 3], but uridine decreases the cytotoxicity[14]. Thymidine inhibits thymidine kinase activity, ribonucleotide reductase activity, the production of 5–fluorodeoxyuridine 5' monophosphate, and facilitates 5-FU incorporation into RNA in tumor cells. Inosine increases 5-FU incorporation into RNA and their metabolism. Cytidine competes against fluorouridine phosphorylation. 5'–GMP has higher water solubility than guanosine and enhances 5-FU selectivity in tumor cells. Our in vitro results indicate enhanced de novo and salvage synthesis of DNA and RNA, then enhanced anticancer effect of 5-FU[8]. In comparison with those cytotoxic effect of biochemical modulation, uridine has "rescue" effect of increasing lethal dose of 5-FU as much as twice[12].

More prominent in vivo effect indicates that the combination of purine and pyrimidine in the OG–VI mixture solution is well balanced than its components alone in an in vivo 5-FU metabolism.

The components of OG–VI, especially thymidine, suppress hapatic dehydrouracil dehydrogenase activity, which is 5-FU degradation enzyme in the liver, and might increase 5-FU levels in vivo. The effect of OG–VI in modulation of cellular immunity observed in infection or endotoxin treatment experiments may support the in vivo result in tumor immunity[14, 15].

The result showing that dietary nucleotide deficiency enhances the effect of OG

–VI with coadministration of 5-FU suspects changes in the bioavailability and efficacy of 5-FU following the dietary nucleotide level. It is reported that the amounts of utilizable purine and pyrimidine are increased with increase in the dose of dietary nucleic acids[17]. This difference in dietary nucleotide may be related to the circadian rhythm of 5-FU metabolism enzyme activity in relation to the activity of orotate phosphoribosyl transferase, pyrimidine nucleoside phosphorylases and dihydrouracil dehydrogenase in the liver[18].

Further in vivo study is required to evaluate the effect of combined administration of 5-FU with OG–VI on cellular growth in different cancer cells and its toxicity. For this hypothesis, tissue or blood 5-FU levels and enzyme activity in 5-FU metabolism, including hepatic dehydrouracil dehydrogenase, should be measured.

References

1. Bootsma D, Budke L, Vos O. Studies on synchronous division of tissue culture cells initiated by excess thymidine. Exptl Cell Res 33 : 301–309, 1964
2. Engelbrecht C, Ljungquist I, Lewan L, et al. Modulation of 5–fluorouracil metabolism by thymidine : In vivo and vitro studies on RNA–directed effects in rat liver and hepatoma. Biochem Pharmacol 33 : 745–750, 1984
3. Iigo M, Ando N, Hoshi A, Kuretani K. Effect of pyrimidines, purines and their nucleosides on antitumor activity of 5–fluorouracil against L–1210 leukemia. J Pharmacobiodyn 5 : 515–520, 1982
4. Ogoshi S, Iwasa M, Yonezawa T, Tamiya T. Effect of nucleotide and nucleoside mixture on rats given total parenteral nutrition after 70% hepatectomy, J Parental Enteral Nutr 9 : 339–342, 1985
5. Ogoshi S, Iwasa M, Mizobuchi S. Effect of nucleoside and nucleotide mixture on protein metabolism in rats given total parenteral nutrition after 70% hepatectomy. (In) Nutritional Support in Organ Failure. Tanaka T & Okada A (Ed) Amsterdam, Elsevier. pp 309–317, 1990
6. Usami M, Ohyanagi H, Furuchi K, et al. The effect of a nucleotide–nucleoside solution on hepatic regeneration after partial hepatectomy in rats. Nutr 12 : 797–803, 1992
7. Ohyanagi H, Nishimatu S, Kanbara K, et al. Effect of nucleosides and a nucleotide on DNA and RNA syntheses by the salvage and de novo pathway in primary monolayer cultures of hepatocytes and hepatoma cells. J Parenter Enteral Nutr 13 : 51–58, 1989
8. Wang J, Usami M. Yasuda I, et al. Effect of nucleosides and a nucleotide mixture on proliferation of human gastric cancer cells (KATOⅢ). Kobe J Med Sci 40 : 65–75, 1994
9. Davis LE, Lenkinski RE, Shinkwin MA et al. The effect of dietary protein depletion on hepatic 5–fluorouracil metabolism. Cancer 72 : 3715–3722, 1993
10. Fujii S, Kitano S, Ikenaka K, et al. Effect of coadministration of uracil or cytosine on the antitumor activity of clinical doses of 1–(2–tetrahydrofuryl)–5–fluorouracil and level of 5–fluorouracil in rodents. Gann 70 : 209–214, 1979
11. Yamaguchi T, Takeda Y, Shimahara Y, et al. A mixture of nucleosides a nucleotide alters hepatic energy metabolism 34 hours after hepatectomy in rabbits. J Nutr 122 : 340–, 1992
12. Inaba M. Potentiation of antitumor activity of 5–fluorouracil by biochemical modulation (in Japanese). Jpn J Cancer Chemother 15 : 380–391, 1988
13. Parker WB, Cheng YC. Metabolism and mechanism of 5–fluorouracil. Pharmacol.

Ther. 48 : 381–395, 1990
14. Nord LD, Stolfi RL, Martin DS, Biochemical modulation of 5–fluorouracil with leucovorin or delayed uridine rescue. Biochem Pharamcol 43 : 2543–2549, 1992
15. Hirai A, Usami M, Haji S, et al. Changes of polymorphonuclear leukocyte (PMN) functions under dietary nucleic avid deficiency and effects of administration of nucleotide and nucleosides mixture solution in mice. (In) Purine and Pyrimidine Metabolism in Man. Griesmacher et al A (Ed) New York, Plenum Press. 1998 (in press)
16. Adjei AA, Matsumoto Y, Kina T, et al. Protection of BALB/c mice against Methicillinresistant Staphylococcus aureus infection by intraperitoneal administration of nucleosidenucleotide mixture. Tohoku J Exp Med 169 : 179–186, 1993
17. Sonoda T, Tatibana M. Metabolic fate of pyrimidines and purines in dietary nucleic acids ingested by mice. Biochim Biophys Acta 521 : 67–73, 1978
18. Naguib FNM, Soong SJ, El Kouni MH. Circadian rhythm of orotate phosphoribosyltransferase, pyrimidine nucleoside phosphorylases and dihydrouracil dehydrogenase in mouse liver. Possible relevance to chemotherapy with 5–fluoropyrimidines. Biochemical Pharmacology 45 : 667–673, 1993