CLINICAL APPRAISAL OF COMBINED VITAMIN B_6 IN ANTICANCER THERAPY FOR CANCER PATIENTS

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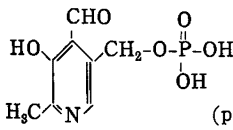
Antitumor agents, at present time, do not warrant satisfactory expectations regarding the therapeutic effect upon the patients with malignant tumor, although they show remarkable effects upon many experimental tumors in animals, These effects of antitumor agents upon the experimental tumors are partly due to the administration dose. The administration dose of antitumor agents for tumor-bearing animals is prodigious quantities for human and cannot be prescribed to the patients with malignant tumor by reason of their side effects that are hardly avoidable.

In recent years, it was reported that a kind of antitumor agents, antimetabolite[6,12,37,46] was administered together with its antidote to tumor-bearing patients with result that was followed by no evil reaction. It is considered possible that, if the side effects of the drugs slleviate slightly, it might be of value in the treatment of a given patient with neoplastic disease.

The chief purpose of this study was to investigate the methods of diminishing the side effects of antitumor drugs.

Vitamin B_6, pyridoxal-phosphate that participates to both amino acid metabolism and heme synthesis was administered th the patients with cancer who have been injected antitumor agents, for the purpose of alleviating the side effects of the agents.

The molecular structure of pyridoxal-phosphate is shown below.



(pyridoxal -5′-phosphate)

MATERIALS AND METHODS

Animal Experiment Male, *Donryu* rats*1, weighing 130 to 150 g were used. The animals were fed the normal pellet*2 or vitamin B_6 free diet*3, with water ad libitum.

The animals were inoculated subcutaneously with ascitic fluid of AH 130 (one of the ascitic hepatoma), which was obtained from rats seventh day after inoculation and containing approximately 10^7 cells.

The animals were divided into seven groups of 10 rats each (Tabulation 1).

The normal pellet was given the first to the fifth group. The first group served as the control. The

Tabulation 1

1 st group	control	given the pellet
2 nd group	AH 130 was inoculated subcutaneously	given the pellet
3 rd group	AH 130 was inoculated subcutaneously + Mitomycin C	given the pellet
4 th group	AH 130 was inoculated subcutaneously + Vitamin B_6	given the pellet
5 th group	AH 130 was inoculated subcutaneously + Mitomycin C + Vitamin B_6	given the pellet
6 th group	AH 130 was inoculated subcutaneously	given Vitamin B_6 free diet
7 th group	AH 130 was inoculated subcutaneously + Desoxypyridoxine	given Vitamin B_6 free diet

*1 The animals were supplied from Central Laboratories far Experimental Animals in Tokyo.

*2 The normal pellet was CE-2 type provided from Central Laboratories for Experimetal Animals in Tokyo.

*3 Vitamin B_6 free diet was composed as follows

Saccharose	70 g
Casein (vitamin free)	20 g
Maccallum salt	4 g
Corn oil	4 g
Cod liver oil	0.5 g
Choline chloride	200 mg
Inositol	15 mg
Nicotinic acid	4 mg
p-Aminobenzoic acid	20 mg
Pteroylglutamic acid	0.1 mg
Ca. pantothenate	10 mg
Vitamin B_1	0.8 mg
Vitamin B_2	0.8 mg

second group was subcutaneously inoculated AH 130, without treatment. The third group, inoculated AH 130, was administered intraperitoneally the antitumor agent, Mitomycin C. From eighth day after inoculation, each rat was given 0.584 mg of Mitomycin C per kg of body weight, three times, every other day. The fourth group, inoculated AH 130, was injected every day 1.0 mg of vitamin B_6 from the day of inoculation. The fifth group, inoculated AH 130, was given both Mitomycin C and vitamin B_6 as described above.

Both the sixth and seventh groups were employed after 4 weeks of feeding with vitamin B_6 free diet. The sixth group was inoculated AH 130 with vitamin B_6 free diet. The last group with vitamin B_6 free diet was given both inoculation of AH 130 and administration of desoxypyridoxine hydrochloride, antagonist of vitamin B_6, which was obtained through the courtesy of Chugai Pharmaceutical Co. Ltd. Desoxypyridoxine hydrochloride, dissolved in physiological saline, was administered intraperitoneally every day at a dose level of 20 mg/kg body weight.

Blood oxamination(red and white blood cell count, hemoglobin, and plasma protein), liver function, and body and tumor weight of these animals were measured during or after these treatments.

Liver function was determined by measuring both plasma transminases levels (SIGMA-FRANKEL'S method[43]) and plasma alkaline phosphatase level (BODANSKY'S method[5]).

Clinical Cases After the animal experiments, vitamin B_6 (10~30 mg every day) was administered to 35 patients with malignant tumor who had been treated with the antitumor agents and ^{60}Co irradiation. Total dose of vitamin B_6 were 250 to 1,900 mg and its mean value were about 800 mg. The antitumor agents administered are as follows: Mitomycin C, Chromomycin A_3, Thio TEPA (triethylenethiophosphoramide), and Cyclophosphamide. The patients of the control group, who had not been given vitamin B_6, were 20 gastric cancer patients who had been administered the antitumor agents approximately equal in administration dose to the above-mentioned patients.

The same tests used in the animal experiment were carried out through this treatment.

The coefficient of correlation was expressed by the following formula[26]:

$$t=\frac{x-y}{\sqrt{n_1S_1^2+n_2S_2^2}}\sqrt{\frac{n_1n_2(n_1+n_2-2)}{n_1+n_2}}$$

Where x is the mean value in the first group; y is the mean value in the second group; S_1 is the standard deviation in the first group; and S_2 is the standard deviation in the second group.

RESULTS

Influences of Vitamin B_6 on Blood It is clear from Table 1 that there exist the sharp decreases of hemoglobin, erythrocyte, and plasma protein values in vitamin B_6 deficient groups as compared with the third and the fifth groups treated with Mitomycin C. The same may be said of the second group vs the groups treated with Mitomycin C.

Table 1 Comparison of mean value of hematologic examination in each group

	Hemoglobin g/100 ml	Erythrocyte $\times 10^4$	Leukocyte	Plasma protein g/100 ml
The control group	11.8±1.9	604±82	7100±1420	7.2±0.6
The tumor group nontreated	5.7±0.7	310±96	7480±1600	4.7±1.2
The tumor group treated with MMC	8.3±1.3*1	433±75*1	3600±720*2	5.7±0.5*1
The tumor group treated with V. B_6	5.4±0.8*3	301±84	7700±1260*4	5.0±0.7
The tumor group given MMC and V. B_6	8.6±0.7*1	440±65*1	5900±890	6.5±0.7*1
The tumor group given V. B_6 free diet	5.0±0.6	281±83	6700±910*5	4.4±1.0
The tumor group given both desoxypyridoxine and V. B_6 free diet	4.5±0.5	247±92	5100±1020	4.1±0.8

*1 Significant difference with regards to 6 th or 7 th group.
*2 The "t" value between 3 rd and 5 th group is 6.026.
*3 The "t" value between 4 th and 7 th group is 4.082.
*4 The "t" value between 4 th and 7 th group is 4.808.
*5 The "t" value between 6 th and 7 th group is 3.509.

Table 2 Influence of combined vitamin B_6 in antitumor treatment on enzyme level in plasma

	Plasma GOT	Plasma GPT	Plasma AlP
The control group	163.0±29.4	35.6±1.2	8.5±2.2
The tumor group nontreated	647.0±182.0	45.0±5.2	21.4±3.0
The tumor group treated with MMC	217.0±22.0*	40.0±4.0	12.0±3.3
The tumor group treated with V. B_6	585.0±164.8**†	44.5±4.8**†	20.7±4.4†
The tumor group treated with MMC and V. B_6	174.0±19.0	36.0±3.7	11.1±1.6
The tumor group given V. B_6 free diet	713.0±160.0	50.6±4.4	27.3±5.1
The tumor group given both desoxypyridoxine and V. B_6 free diet	622.0±102.5	47.0±3.9	24.0±2.7

* The "t" value between 3rd and 5th group is 4.435.

** The "t" value between nces with regards to the 6th group.

† No significant differences with gerards to the 7th group.

Table 3 Influence of combined vitamin B_6 in antitumor treatment on hematologic test and serum enzyme level

Groups		Operable control group (Gastric cancer)		Operable group given V. B_6 (Gastric cancer)		Inoperable group given V. B_6 (Stomach, lung cancer etc.)	
Number of patients		20		27		8	
Treatment	Procedure	Billroth No. 2 ope.		Billroth No. 2 ope. or Subtotal gastrectomy		Exploratory operation	
	Antitumor therapies (Total dose)	MMC* 30~44 mg Thio TEPA 90~105 mg CAP 2700~4300 mg Chr A_3 8~14 mg (i. v.)		MMC* 38~44 mg Thio TEPA 90~150 mg CPA 2800~3700 mg Chr A_3 7.5~20 mg (i. v.)		MMC 8~60 mg (i. v., i. a., i. pl.)* Thio TEPA 15~45 mg (i. v., i. pl.) CPA 1000~2000 mg (i. v.) ^{60}Co 2100~5400 r. ^{198}Au 50 mc (i. pl.)	
	Vitamin B_6			Daily dose 10~30 mg Durst. ther. 21~44 days (Mean value : 32.4 days) Total dose 250~1900 mg (Mean value : 794 mg)		Daily dose 10~30 mg Durat. ther. 24~64 days (Mean value : 34 days) Total dose 250~1750 mg (Mean value : 850 mg)	
		Bef. ther.	Aft. ther.	Bef. ther.	Aft. ther.	Bef. ther.	Aft. ther.
Hemoglobin (g/100 ml)		11.1±1.5	10.9±1.2	11.7±1.2	11.2±0.8	10.3±0.8	10.4±1.0
Erythrocyte ($\times 10^4$)		343±59	346±42	358±38	348±31	316±21	307±22
Leukocyte		5620±653	2690±717**	5400±831	3720±898**	5520±777	3075±783
Serum protein (g/100 ml)		7.1±0.44	6.96±0.41	7.06±0.42	6.99±0.31	6.4±0.7	6.7±0.5
Serum GOT		26.8±5.9	29.3±7.8	27.0±9.4	26.9±7.2	47.5±14.3	33.1±14.3
Serum GPT		20.2±6.6	27.1±10.4	19.7±6.9	25.4±5.4	34.0±8.5	27.5±8.0
Serum AlP		3.9±1.0	4.2±0.9	4.2±1.0	3.8±0.9	5.4±2.2	3.9±1.6

* In these columns, the following abbreviations are used : i. v., intravenously, i. p., intraperitonealy, i. pl., intrapleurally, i. a., intraarterialy, MMC, Mitomycin C, CPA, Cyclophosphamide, and Chr A_3, chromomycin A_3. Dosage of telecobalt are expressed here as tumor dose.

** The "t" value between operable control group and operable group given vitamin B_6 is 4.310.

Making a comparison between the 2 groups administered Mitomycin C, a marked increase in leukocyte was recognized in the fifth group treated with vitamin B_6.

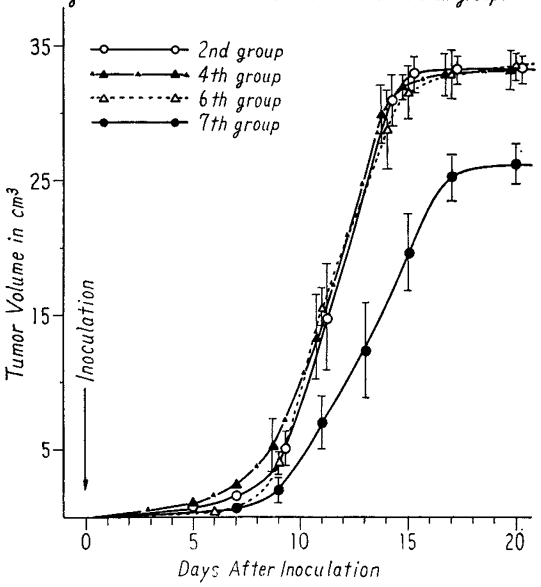
Little difference in the 4 hematologic tests were shown between the fourth group administered vitamin B_6 and the sixth group fed vitamin B_6 free diet, whereas considerable differences in hemoglobin, leukocyte, and plasma protein values were shown between the fourth group and the seventh group with both vitamin B_6 deficient diet and desoxypyridoxine. There was no difference in the hematologic tests except for leukocyte count between the 2 groups fed vitamin B_6 free diet.

Influences of Vitamin B_6 on Liver Function Test. As indicated in Table 2, liver function of the third and the fifth group treated with Mitomycin C remained more nearly normal than that of the other groups except for the control group.

Liver function between the third group given Mitomycin C and the fifth group given both Mitomycin C and vitamin B_6 made iittle difference with the exception of plasma GOT level. Liver disorder of the fourth group injected vitamin B_6 was about the same as that of the sixth or the seventh group fed vitamin B_6 free diet.

Influences of Vitamin B_6 on Tumor Growth. This experiment was designed to analyze the relationship between administration of vitamin B_6 and tumor growth. Fig. 1 represents the time course of the volume of tumor. As shown in Fig. 1 there was but a negligible difference between the second and the fourth groups.

Fig. 1 *Time course of the volume of tumor in each group.*



On the other hand, the last group was inhibited tumor growth throughout 10 days after subcutaneous inoculation. Nevertheless, the sixth group did not inhibit tumor growth as indicated in Fig. 1.

Influences of Vitamin B_6 on Body Weight. There was little difference in body weight either between the second and the fourth groups or between the third and the fifth groups.

Based on the above-mentioned experiments, two possibilities existed that the administration of vitamin B_6 did not accelerate the tumor growth and was favorable to the general condition of the tumor-host treated with antitumor agents with special reference to diminishing the side effects of antitumor agents.

Based on available evidences, vitamin B_6 has been administred to the patients with malignant tumor to secure the alleviation of the side effects of antitumor therapies.

Clinical application. As shown in Table 3, the drop of leukocyte count is slightly inhibited in the operable group administered vitamin B_6 as compared with that of the control group; whereas little difference in the other 3 hematologic tests is inhibited between the 2 operable groups.

No patient of the operable groups received blood transfusion. Since almost all the inoperable gastric cancer cases were more or less given the blood transfusion, they were excepted from studies of hematologic examination.

Influence of Vitamin B_6 on Liver Function. Table 3 shows inappreciable difference in liver function between the operable group administered vitamin B_6 and the operable control group.

In the inoperable cases the group given combined-treatment with vitamin B_6 revealed the better result in each serum enzyme than those of the operable groups. Nevertheless, the total number of the inoperable cases is small and no broad conclusions should be drawn.

DISCUSSION

There are patients whose bone marrows are so sensitive that even very small doses of many of the antitumor agents will produce leukopenia. It is obvious that, if this damaging effect to bone marrow could be counteracted, finer efficacy will be produced by mass administration of the antitumor agents.

For the purpose of averting this side effect, there have been numerous trials, *i.e.* regional perfusion[9,10,17,30] combined treatment with antidote[1,12,20,37,46] or bone marrow transplantation[4,22,29,32,33,47] and revelation of short-lived agents[18,42,50].

On the other hand, protein metabolism of tumor bearer turns into the catabolic phase[35,39] as time goes on. Antitumor agents frequently make cancer patient's state worse, as is often attributed to the disturbance of protein metabolism. For the attainment of the aim of alleviating the negative effect of antitumor agents on protein metabolism in cancer patients, combined administration with both antitumor agents and vitamin B_6, coenzyme in amino acid metabolism and heme synthesis, should be proper[15].

While, anemia from vitamin B_6 deficiency has been reported in such species as chick[31], rat[28], pig[7], dog[34], and monkey[36]. This hematologic alterations have been characterized by a hypochromic, microcytic anemia with high plasma iron that is unresponsive to the usual hematopoietic agents. As vitamin B_6 is known to serve as a coenzyme in the heme synthesis[27,40] from glycin or succinate in the red cells, it is understandable that this anemia brings with high plasma iron.

It appears from several studies[2,3,13,19,44] that this anemia in a human being brings also with a moderate leukopenia and moreover the administration of vitamin B_6 to this anemia is followed by restoration of normal hemoglobin, erythrocyte, and leukocyte values and rapid decrease of plasma iron.

It have been pointed out[25] that vitamin B_6 deficiency also exists in tumor-bearing animal.

At this point, it is very important for this article whether growth of cancer stimulates by vitamin B_6 or not.

When vitamin B_6 was intraperitoneally administered to rats inoculated subcutaneously AH 130, growth of the tumor did not differ much from that of control rats. Difference of tumor growth between rats fed vitamin B_6 free diet and control rats was so slight as to be almost negligible. These data are in accord with those of BOUTWELL's report[6].

When Desoxypyridoxine was administered to rats rendered vitamin B_6 deficient diet, it produced the effect in retarding the growth of rat tumors, but could not avoid tumor-death, This differs somewhat from STOERK's report[45]: mouse lymphosarcoma was brought to regression by the administration of desoxypyridoxine when the animal was maintained on a low vitamin B_6 intake.

Although vitamin B_6 poverty is sure to cocur in tumor-bearing animal, hemoglobin and erythrocyte values in the fourth group injected vitamin B_6 do not differ from those in the vitamin B_6 deficient groups (6 th and 7 th groups), except for hemoglobin in the seventh group. This phenomenon has a signification that anemia from cancer is dominant over that from vitamin B_6 deficiency. It is clear from the fact that there is little difference in the 4 hematologic tests between the second and the fourth group.

However, thinking that anemia originated in cancer has partly its source in hemorrhage, the administration of vitamin B_6 to the tumor bearer, that has always vitamin B_6 deficiency, should be maintained by KORNBERG's report[28]: a latent erythropoietic inadequacy indicated by an impairment in the rate of red blood cell regeneration after hemorrhage has been demanstrated in pyridoxine-deficient rats.

Anemia in either the sixth or the seventh group is overlapping of anemia originated from both cancer and vitamin B_6 deficiency; and a moderate fall of leukocyte in either the seventh vs the fourth group or the seventh vs the sixth group might be one of the partial symptoms in vitamin B_6 deficient anemia.

It appears from the reports of the present author[14] and others[23,38,41] that liver disorder is certain to occur in tumor bearer, and that administration of vitamin B_6 to liver disorder is followed by fall

of plasma enzymes levels.

In spite of the above-mentioned facts, liver disorder of the second group did not differ from that of the fourth group given vitamin B_6 as shown in Table 2. As appeared by these data, there is very slight doubt that administration of vitamin B_6 can not inhibit the serious liver disorder originated in malignant tumor.

This conception has also been answered by either indistinguishable plasma enzymes levels between the fourth and the seventh groups or distinguishable plasma GOT level between the third and the fifth groups.

As mentioned above, vitamin B_6 proved beneficial to tumor-bearing animal treated with cancer chemotherapy.

The author can not pass in silence the reason why vitamin B_6 might counteract the side effect of leukopenia from antitumor agent. No one has given an intelligent reason why vitamin B_6 responsive anemia accompanies with leukopenia and the leukopenia is quite restored by the administration of vitamin B_6.

The reasons for explaining prevention of the leukopenia by vitamin B_6 on the basis of the aforecited data are as follow : (1) favorable return of the systemic metabolism by lightening both anemia and liver disorder; (2) improvement in protein metabolism.

On the other hand, antitumor action of Mitomycin C has been inactivated in liver. In the latest general meeting of the Japan Society of Chemotherapy, FUJITA *et al*[16]. reported that Mitomycin C was inactivated in about 30 minutes in the phosphate buffer solution added both vitamin B_6 and liver homogenate and was hard to be inactivated in the liver homogenate solution without vitamin B_6.

This increased inactivation of Mitomycin C in liver might play a part in proof of considerable effect of vitamin B_6 on leukopenia by Mitomycin C.

It has been definitely shown by the authors[24,49] that effective concentration of Mitomycin C in blood do not continue more than 30 minutes after intravenous injection of Mitomycin C, 0.2 mg/kg. Accordingly, the administration of vitamin B_6 has no direct effect on antitumor action of Mitomycin C.

Four one reason or another, it might be supposed that, on that point recovering from leukopenia, vitamin B_6 dose not act directly neither as antidote to antitumor agent nor as stimulant to bone marrow, but acts indirectly.

Clinical results (as recovery from leukopenia) shown in Table 3 are slightly inferior to those of combined treatment either with antidote[37,46] or with transplantation of bone marrow[4,22,29,32,33,47]. However, the exact analysis of clinical results would mean little by reasons as follows: (1) the individual difference of general condition when patient was admitted; (2) the unrestricted diets; (3) the different invasion of operation; and (4) the others therapies except for blood transfusion. Vitamin B_6 could be recognized its services of improvement in liver function, protein metabolism, and exhausted reticuloendothelial system[21,48].

From these results, administration of vitamin B_6 would be one of the most available therapies warding off the side effects of the antitumor agents.

SUMMARY

Vitamin B_6 that is essential to both amino acid metabolism and heme synthesis was used for the purpose of alleviating the side effects of antitumor agents.

(1) Thirty-five cancer patients were treated with the combined therapy with antitumor treatment and vitamin B_6. It had a wholesome effect on leukopenia from antitumor treatment.

(2) The similar therapy to the tumor-bearing rats had more effect than clinical patients in arresting leukopenia and failed to bring about the considerable effect on liver disorder and anemia originated in ascitic hepatoma.

(3) It was clearly recognized that the growth of subcutaneous tumor in rat was not stimulated by administration of vitamin B_6 and was halted by the injection of desoxypyridoxine into rat fed vitamin B_6 free diet. While, the administration of vitamin B_6 did not weaken the effect of Mitomycin C on subcutaneous tumor in rat.

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