CLINICAL APPRAISAL OF COMBINED VITAMIN B₆ IN ANTICANCER THERAPY FOR CANCER PATIENTS

SHIGERU FUJIMOTO, M.D.
From the First Surgical Department, School of Medicine, Chiba University
(Received May 13, 1965)

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, antimetabolite¹,¹²,¹⁷,¹⁸) 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 alleviate 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 B₆, pyridoxal-phosphate that participates to both amino acid metabolism and heme synthesis was administered to 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.

\[
\text{CHO} \quad \text{O} \\
\text{HO} \quad \text{CH}_{3}-\text{O-P-OH} \\
\text{H}_2\text{C}^\text{N} \quad \text{OH} \\
\text{(pyridoxal -5'-phosphate)}
\]

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 B₆ 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 10⁷ 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

<table>
<thead>
<tr>
<th>Group</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>control</td>
</tr>
<tr>
<td>2nd</td>
<td>AH 130 was inoculated subcutaneously</td>
</tr>
<tr>
<td>3rd</td>
<td>AH 130 was inoculated subcutaneously + Mitomycin C</td>
</tr>
<tr>
<td>4th</td>
<td>AH 130 was inoculated subcutaneously + Vitamin B₆</td>
</tr>
<tr>
<td>5th</td>
<td>AH 130 was inoculated subcutaneously + Vitamin B₆</td>
</tr>
<tr>
<td>6th</td>
<td>AH 130 was inoculated subcutaneously</td>
</tr>
<tr>
<td>7th</td>
<td>AH 130 was inoculated subcutaneously + Desoxypyridoxine</td>
</tr>
</tbody>
</table>

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

*³ Vitamin B₆ 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 B₁: 0.8 mg
  - Vitamin B₂: 0.8 mg

*¹ The animals were supplied from Central Laboratories for Experimental Animals in Tokyo.
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 B₆ from the day of inoculation. The fifth group, inoculated AH 130, was given both Mitomycin C and vitamin B₆ as described above.

Both the sixth and seventh groups were employed after 4 weeks of feeding with vitamin B₆ free diet. The sixth group was inoculated AH 130 with vitamin B₆ free diet. The last group with vitamin B₆ free diet was given both inoculation of AH 130 and administration of desoxypyridoxine hydrochloride, antagonist of vitamin B₆, 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 transaminases levels (SIGMA-FRANKEL's method⁴³) and plasma alkaline phosphatase level (BODANSKY's method⁴). Clinical Cases After the animal experiments, vitamin B₆ (10~30 mg every day) was administered to 35 patients with malignant tumor who had been treated with the antitumor agents and ⁶⁰Co irradiation. Total dose of vitamin B₆ were 250 to 1,900 mg and its mean value were about 800 mg. The antitumor agents administered are as follows: Mitomycin C, Chromomycin A₃, Thio TEPA (triethylene-thiophosphoramide), and Cyclophosphamide. The patients of the control group, who had not been given vitamin B₆, 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 formula²⁶:

\[ t = \frac{x - y}{\sqrt{s_1^2/n_1 + s_2^2/n_2} \sqrt{n_1n_2(n_1+n_2-2)}} \]

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 B₆ on Blood It is clear from Table 1 that there exist the sharp decreases of hemoglobin, erythrocyte, and plasma protein values in vitamin B₆ 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

<table>
<thead>
<tr>
<th></th>
<th>Hemoglobin g/100 ml</th>
<th>Erythrocyte ×10⁴</th>
<th>Leukocyte</th>
<th>Plasma protein g/100 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>The control group</td>
<td>11.8±1.9</td>
<td>604±82</td>
<td>7100±1420</td>
<td>7.2±0.6</td>
</tr>
<tr>
<td>The tumor group nontreated</td>
<td>5.7±0.7</td>
<td>310±96</td>
<td>7480±1600</td>
<td>4.7±1.2</td>
</tr>
<tr>
<td>The tumor group treated with MMC</td>
<td>8.3±1.3*¹</td>
<td>433±75*¹</td>
<td>3600±720*²</td>
<td>5.7±0.5*¹</td>
</tr>
<tr>
<td>The tumor group treated with V. B₆</td>
<td>5.4±0.8*³</td>
<td>301±84</td>
<td>7700±1260*⁴</td>
<td>5.0±0.7</td>
</tr>
<tr>
<td>The tumor group given MMC and V. B₆</td>
<td>8.6±0.7*¹</td>
<td>440±65*¹</td>
<td>5900±890</td>
<td>6.5±0.7*¹</td>
</tr>
<tr>
<td>The tumor group given V. B₆ free diet</td>
<td>5.0±0.6</td>
<td>281±83</td>
<td>6700±910*⁵</td>
<td>4.4±1.0</td>
</tr>
<tr>
<td>The tumor group given both desoxypyridoxine and V. B₆ free diet</td>
<td>4.5±0.5</td>
<td>247±92</td>
<td>5100±1020</td>
<td>4.1±0.8</td>
</tr>
</tbody>
</table>

*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 B₆ in antitumor treatment on enzyme level in plasma

<table>
<thead>
<tr>
<th>Groups</th>
<th>Plasma GOT</th>
<th>Plasma GPT</th>
<th>Plasma AIP</th>
</tr>
</thead>
<tbody>
<tr>
<td>The control group</td>
<td>163.0±29.4</td>
<td>35.6±1.2</td>
<td>8.5±2.2</td>
</tr>
<tr>
<td>The tumor group nontreated</td>
<td>647.0±182.0</td>
<td>45.0±5.2</td>
<td>21.4±3.0</td>
</tr>
<tr>
<td>The tumor group treated with MMC</td>
<td>217.0±22.0*</td>
<td>40.0±4.0</td>
<td>12.0±3.3</td>
</tr>
<tr>
<td>The tumor group treated with V.B₆</td>
<td>585.0±164.8**</td>
<td>44.5±4.8**</td>
<td>20.7±4.4†</td>
</tr>
<tr>
<td>The tumor group treated with MMC and V.B₆</td>
<td>174.0±19.0</td>
<td>36.0±3.7</td>
<td>11.1±1.6</td>
</tr>
<tr>
<td>The tumor group given V.B₆ free diet</td>
<td>713.0±160.0</td>
<td>50.6±4.4</td>
<td>27.3±5.1</td>
</tr>
<tr>
<td>The tumor group given both desoxypyridoxine and V.B₆ free diet</td>
<td>622.0±102.5</td>
<td>47.0±3.9</td>
<td>24.0±2.7</td>
</tr>
</tbody>
</table>

* 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 B₆ in antitumor treatment on hematologic test and serum enzyme level

<table>
<thead>
<tr>
<th>Groups</th>
<th>Operable control group (Gastric cancer)</th>
<th>Operable group given V.B₆ (Gastric cancer)</th>
<th>Inoperable group given V.B₆ (Stomach, lung cancer etc.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>20</td>
<td>27</td>
<td>8</td>
</tr>
<tr>
<td>Procedure</td>
<td>Billroth No. 2 ope.</td>
<td>Billroth No. 2 ope. or Subtotal gastrectomy</td>
<td>Exploratory operation</td>
</tr>
<tr>
<td>Antitumor therapies (Total dose)</td>
<td>MMC* 30~44 mg</td>
<td>MMC* 38~44 mg</td>
<td>MMC 8~60 mg (i.v., i.a., i.pl.)*</td>
</tr>
<tr>
<td></td>
<td>Thio TEPA 90~105 mg (i.v.)</td>
<td>Thio TEPA 90~150 mg (i.v.)</td>
<td>Thio TEPA 15~45 mg (i.v., i.pl.)</td>
</tr>
<tr>
<td></td>
<td>CAP 2700~4300 mg</td>
<td>CPA 2800~3700 mg</td>
<td>CPA 1000~2000 mg (i.v.)</td>
</tr>
<tr>
<td></td>
<td>Chr A₃ 8~14 mg</td>
<td>Chr A₃ 7.5~20 mg</td>
<td>RaCo 2100~5400 r.</td>
</tr>
<tr>
<td>Vitamin B₆</td>
<td>Daily dose 10<del>30 mg Durst. ther. 21</del>44 days (Mean value : 32, 4 days)</td>
<td>Daily dose 10<del>30 mg Durst. ther. 24</del>64 days (Mean value : 34 days)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total dose 250~1900 mg (Mean value : 794 mg)</td>
<td>Total dose 250~1750 mg (Mean value : 850 mg)</td>
<td></td>
</tr>
<tr>
<td>Bef. ther.</td>
<td>11.1±1.5</td>
<td>10.9±1.2</td>
<td>11.3±0.8</td>
</tr>
<tr>
<td></td>
<td>343±59</td>
<td>346±42</td>
<td>316±21</td>
</tr>
<tr>
<td></td>
<td>5620±653</td>
<td>2690±717**</td>
<td>307±22</td>
</tr>
<tr>
<td></td>
<td>7.1±0.44</td>
<td>6.96±0.41</td>
<td>6.4±0.7</td>
</tr>
<tr>
<td></td>
<td>26.8±5.9</td>
<td>29.3±7.8</td>
<td>47.5±14.3</td>
</tr>
<tr>
<td></td>
<td>20.2±6.6</td>
<td>27.1±10.4</td>
<td>33.1±14.3</td>
</tr>
<tr>
<td></td>
<td>3.9±1.0</td>
<td>4.2±0.9</td>
<td>3.9±1.6</td>
</tr>
<tr>
<td>Erythrocyte (×10⁶)</td>
<td>11.7±1.2, 358±38</td>
<td>348±31</td>
<td>316±21</td>
</tr>
<tr>
<td></td>
<td>5600±831</td>
<td>3720±898**</td>
<td>3075±783</td>
</tr>
<tr>
<td>Hemoglobin (g/100 ml)</td>
<td>10.9±1.2</td>
<td>348±31</td>
<td>307±22</td>
</tr>
<tr>
<td></td>
<td>5600±831</td>
<td>3720±898**</td>
<td>3075±783</td>
</tr>
<tr>
<td></td>
<td>7.06±0.42</td>
<td>6.99±0.31</td>
<td>6.7±0.5</td>
</tr>
<tr>
<td></td>
<td>26.9±7.2</td>
<td>27.0±9.4</td>
<td>47.5±14.3</td>
</tr>
<tr>
<td></td>
<td>27.5±8.0</td>
<td>29.3±7.8</td>
<td>33.1±14.3</td>
</tr>
<tr>
<td></td>
<td>4.2±1.0</td>
<td>3.8±0.9</td>
<td>3.9±1.6</td>
</tr>
</tbody>
</table>

* 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 A₃, chromomycin A₃. Dosage of telecobalt are expressed here as tumor dose.
** The “t” value between operable control group and operable group given vitamin B₆ 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 B6.

Little difference in the 4 hematologic tests were shown between the fourth group administered vitamin B6 and the sixth group fed vitamin B6 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 B6 deficient diet and desoxypyridoxine. There was no difference in the hematologic tests except for leukocyte count between the 2 groups fed vitamin B6 free diet.

Influences of Vitamin B6 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 B6 made little difference with the exception of plasma GOT level. Liver disorder of the fourth group injected vitamin B6 was about the same as that of the sixth or the seventh group fed vitamin B6 free diet.

Influences of Vitamin B6 on Tumor Growth. This experiment was designed to analyze the relationship between administration of vitamin B6 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.

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 B6 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 B6 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 B6 has been administered 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 B6 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 B6 on Liver Function. Table 3 shows inappreciable difference in liver function between the operable group administered vitamin B6 and the operable control group.

In the inoperable cases the group given combined-treatment with vitamin B6 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, combined treatment with antidote, or bone marrow transplantation and revelation of short-lived agents. On the other hand, protein metabolism of tumor bearer turns into the catabolic phase 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 B₆, coenzyme in amino acid metabolism and heme synthesis, should be proper.

While, anemia from vitamin B₆ deficiency has been reported in such species as chick, rat, pig, dog, and monkey. 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 B₆ is known to serve as a coenzyme in the heme synthesis from glycine or succinate in the red cells, it is understandable that this anemia brings with high plasma iron.

It appears from several studies that anemia in a human being brings also with a moderate leukopenia and moreover the administration of vitamin B₆ 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 that vitamin B₆ deficiency also exists in tumor-bearing animal.

At this point, it is very important for this article whether growth of cancer stimulates by vitamin B₆ or not.

When vitamin B₆ 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 B₆ free diet and control rats was so slight as to be almost negligible. These data are in accord with those of BOUTWELL’s report.

When Desoxyxpyridoxine was administered to rats rendered vitamin B₆ 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: mouse lymphosarcoma was brought to regression by the administration of desoxyxpyridoxine when the animal was maintained on a low vitamin B₆ intake.

Although vitamin B₆ poverty is sure to occur in tumor-bearing animal, hemoglobin and erythrocyte values in the fourth group injected vitamin B₆ do not differ from those in the vitamin B₆ deficient groups (6th and 7th groups), except for hemoglobin in the seventh group. This phenomenon has a signification that anemia from cancer is dominant over that from vitamin B₆ 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 B₆ to the tumor bearer, that has always vitamin B₆ deficiency, should be maintained by KORNBERG’s report: a latent erythropoietic inadequacy indicated by an impairment in the rate of red blood cell regeneration after hemorrhage has been demonstrated in pyridoxine-deficient rats.

Anemia in either the sixth or the seventh group is overlapping of anemia originated from both cancer and vitamin B₆ 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 B₆ deficient anemia.

It appears from the reports of the present author and others that liver disorder is certain to occur in tumor bearer, and that administration of vitamin B₆ 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 B₆ as shown in Table 2. As appeared by these data, there is very slight doubt that administration of vitamin B₆ 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 B₆ proved beneficial to tumor-bearing animal treated with cancer chemotherapy.

The author can not pass in silence the reason why vitamin B₆ might counteract the side effect of leukopenia from antitumor agent. No one has given an intelligent reason why vitamin B₆ responsive anemia accompanies with leukopenia and the leukopenia is quite restored by the administration of vitamin B₆.

The reasons for explaining prevention of the leukopenia by vitamin B₆ on the basis of the aforesaid 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. reported that Mitomycin C was inactivated in about 30 minutes in the phosphate buffer solution added both vitamin B₆ and liver homogenate and was hard to be inactivated in the liver homogenate solution without vitamin B₆.

This increased inactivation of Mitomycin C in liver might play a part in proof of considerable effect on antitumor action of Mitomycin C.

Four one reason or another, it might be supposed that, on that point recovering from leukopenia, vitamin B₆ 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 or with transplantation of bone marrow. 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 B₆ could be recognized its services of improvement in liver function, protein metabolism, and exhausted reticuloendothelial system.

From these results, administration of vitamin B₆ would be one of the most available therapies warding off the side effects of the antitumor agents.

SUMMARY

Vitamin B₆ 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 B₆. 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 B₆ and was halted by the injection of desoxyypridoxine into rat fed vitamin B₆ free diet. While, the administration of vitamin B₆ did not weaken the effect of Mitomycin C on subcutaneous tumor in rat.

REFERENCES

1) BEEHLER, C. C., and MALETTE, W. C.: Portal venous nitrogen mustard infusion: The effect...
26) JOHNSON, P. O.: Statistical Method in Re-


