The Metabolism of Folinic Acid (Leucovorin) Following Oral and Parenteral Administration

Hirokuni TAGUCHI

Department of Internal Medicine, Kochi Medical School, Okohcho Kohasu, Nankoku City, Kochi 781-51, Japan
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Summary Serum and urinary distributions of following oral and parenteral administration of leucovorin (3–15 mg) were examined in normal adult volunteers microbiologically using Lactobacillus casei, Streptococcus faecalis and Pediococcus cerevisiae as test organisms. By the parenteral route, nearly one-third of the folate in the serum and urine was in the form of folic acid and the remainder as 5-methyltetrahydrofolic acid. Almost all the folate in serum and urine was in the form of 5-methyltetrahydrofolic acid after oral administration. Peak serum folate was observed 3 hr after oral administration, later than that seen after parenteral administration (30 min). Elevation of serum folate was achieved by the increase of the methyl form of folate following repeated administration of leucovorin orally and parenterally. As the form of folate actually rescuing normal cells in a high-dose methotrexate regimen was thought to be methyl, use of the oral route as a principal means of administration of leucovorin in a rescue program was looked into.

Key Words folic acid, leucovorin, 5-methyltetrahydrofolic acid, oral and parenteral administration, methotrexate, high-dose regimen, rescue

Since the first description of the effectiveness of anti-folates in acute lymphoblastic leukemia in 1948 (1), low doses of MTX have been widely used for the chemotherapy of a variety of malignancies such as chorioncarcinoma, acute lymphoblastic leukemia, and breast and intrathecal carcinoma. Recent reports of the successful use of high doses of MTX with LV rescue in the adjuvant treatment of osteogenic sarcoma (2) have greatly expanded the potential of this agent and prompted further clinical investigations. The effectiveness of high doses of MTX had been reported in malignant lymphoma (3), epidermoid carcinoma of the head and neck (4) and small cell carcinoma of the lung (5). Extensive clinical experience using high doses MTX with a variety of neoplasms has recently been reported from
two groups (6, 7). Although the pharmacokinetics of MTX in these high-dose regimens has been extensively studied (8), little investigation on the pharmacology of large amounts of LV has been done. In almost all protocols of high-dose MTX treatment, the amount of LV and the time interval of its administration have been planned without a knowledge of the pharmacokinetics of this vitamin (9). Serum distribution and urinary excretion of LV following oral and parenteral administrations were studied in the present paper in order to determine the pharmacokinetics of LV during a rescue program of a high-dose MTX regimen.

MATERIALS AND METHODS

LV was obtained from Lederle (Japan), Ltd. LV (3 mg, intramuscularly; 15 mg, intravenously; 15 mg, orally) was administered to four healthy volunteers. The subjects were taking normal diets and were not receiving any other form of medication. All subjects had normal serum folate levels at the start of the experiment. Serum samples were taken for folic acid assay before and 30, 60, 180, 360, 720 min and 24 hr after the administration of LV. Urine was collected over 24 hr after the administration of LV. The effects of four intermittent injections and three intermittent oral administrations of LV were studied in a healthy volunteer. Assay of serum and urinary folate was performed microbiologically using Lactobacillus casei, ATCC 7469, Streptococcus faecalis, ATCC 8043 and Pediococcus cerevisiae, ATCC 8081 as test organisms. The aseptic addition method of Herbert (10) was employed after some modifications (11). Bacto folic acid casei medium (Difco) was used (12). Pteroylglutamic acid was used as a reference material for the standard curve of the assay using Lactobacillus casei and Streptococcus faecalis. D, L-Leucovorin calcium salt (Lederle, Japan) was used as a reference for Pediococcus cerevisiae assay and results were expressed as half of the assayed values in order to make them equivalent to those of L-leucovorin. The response of these three microorganisms to various folate compounds is shown in Table 1. Lactobacillus casei was used to assay all samples from four subjects in every experiment. In each experiment, either Streptococcus faecalis or Pediococcus cerevisiae was used to assay samples from four subjects and another organism was used to confirm the results in two subjects.

Table 1. Response of three microorganisms to folate compounds.

|                     | Folic acid (Pteroylglutamic acid) | 5-Methyl-tetrahydrofolate | Folinic acid (Leucovorin) |
|---------------------|----------------------------------|---------------------------|--------------------------|
| Lactobacillus casei | +                                | +                         | +                        |
| Pediococcus cerevisiae | −                                | −                         | +                        |
| Streptococcus faecalis | +                                | −                         | +                        |

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RESULTS

(1) **Effect of intramuscular injection of 3 mg of LV** (Fig. 1)
A peak serum folate level of 75.3 ng/ml was obtained 30 min after the injection of LV, of which 1/3 was considered to be in the form of FTHF and the remainder, of MTHF, judging from the response of the three microorganisms. LV was rapidly converted to MTHF and disappeared from blood 6 hr after the injection. The amount of urinary excretion over the 24 hr after the injection of LV was 117.7 µg. As in the serum, nearly 1/3 of this amount was in the form of FTHF and the remainder, of MTHF.

(2) **Effect of intravenous injection of 15 mg of LV** (Fig. 2)
Peak level was 435 ng/ml at 30 min after the injection. About 40% was FTHF and the remainder MTHF. The level of FTHF rapidly declined during the 6 hr following intramuscular injection. Excretion into the urine over the 24 hr after injection was 1,042 µg, about 30% being FTHF and the remainder MTHF.

(3) **Effect of oral administration of 15 mg of LV** (Fig. 3)
The peak serum folate level was obtained 3 hr after the administration, the level being lower than that attained by intravenous injection of the same amount of LV.

![Fig. 1. Effect of intramuscular injection of 3 mg of leucovorin.](image1)

![Fig. 2. Effect of intravenous injection of 15 mg of leucovorin.](image2)
The elevation of serum folate levels was achieved almost exclusively by MTHF. Also, almost all the folate excreted in the urine was MTHF.

(4) Effect of four intermittent injections of 15 mg of LV (Fig. 4)

Peak serum folate levels became higher each time after 3 injections of LV every 3 hr but FTHF levels on the 2nd and 3rd injections were not elevated as much as total folate levels. The final injection was made 6 hr after the 3rd one. The level of

Fig. 3. Effect of oral administration of 15 mg of leucovorin.

Fig. 4. Effect of 4 intermittent intravenous injections of leucovorin.

Fig. 5. Effect of 3 intermittent oral administrations of leucovorin.
FTHF was less than 0.2 ng/ml just before this injection although the total serum folate level remained high (265 ng/ml). The peak level was lower than that with the 3rd injection but no difference was seen in the peak level of FTHF compared to that seen with the previous three injections. The serum folate level was high (43.5 ng/ml) even 36 hr after the final injection.

(5) Effect of three intermittent oral administrations of 15 mg of LV (Fig. 5)

As in the single oral administration of LV, elevation of serum folate levels was achieved by MTHF. No accumulation of MTHF was seen by giving LV every 3 hr. A high serum folate level (21 ng/ml) was maintained even 24 hr after the final administration.

DISCUSSION

Few studies have been made on the metabolism of massive doses of LV in the high-dose MTX regimen, as Nixon pointed out in his recent review (9). Data available on the pharmacokinetics of LV only concern the physiological levels of this vitamin (13, 14).

The present study revealed the pharmacodynamics of LV after the respective administrations of 3 mg intramuscularly, 15 mg intravenously and 15 mg orally. Serum levels of folate after several intermittent administrations of LV intravenously and orally were also assayed. A massive dose of LV was almost completely converted into MTHF when administered orally, as was the case with physiological dose (15). Although about 30–40% of serum folate was in the form of FTHF when administered parenterally, this form rapidly disappeared and almost all of the serum folate after 3 hr was MTHF. Intermittent administrations of 15 mg of LV resulted in higher peak serum folate levels on each occasion but FTHF levels was not elevated as much as total folate levels. These results suggest that MTHF may play a principal role in the rescue program of high-dose MTX therapy.

The present study was performed on normal volunteers not receiving MTX. In high-dose MTX therapy, high serum levels of MTX are maintained when LV is given. A question arises on whether the metabolism of LV is influenced by the presence of MTX in the blood or not. Mehta et al. (16, 17) developed methods for assaying FTHF and MTHF microbiologically in the presence of MTX. They assayed serum levels of FTHF and MTHF in patients with meningeal carcinomatosis receiving intra-Ommaya MTX and intravenous LV and in patients with osteogenic sarcoma receiving high-dose MTX followed by oral LV rescue (18). It was found that following intravenous infusion of LV in the presence of MTX, about 60% of the folate in serum was in the form of MTHF and that oral administration of 10–12 mg of LV tablets also elevated serum levels up to 250 ng/ml mainly in the form of MTHF, showing no inhibition of conversion of LV to MTHF during absorption in the presence of MTX. The results are quite compatible with ours obtained in the absence of MTX. Therefore it is assumed that all data obtained in
the present study are applicable to the consideration of the distribution of serum folate in the high-dose MTX regimen.

Among various theories on the rationale for the selective effectiveness of the high-dose MTX regimen with LV rescue (19), the one proposed by Halpern et al. (20) based on their study using a tissue culture system is very interesting. They found that when a variety of neoplastic cell types characterized by a deficiency of vitamin B₁₂-dependent MTHF methyltransferase and normal adult cells was grown in media containing MTX and with either MTHF or FTHF, not only was the selective toxicity of MTX demonstrated but the advantage of using MTHF in place of FTHF also revealed. They concluded that MTHF should be given as a tool of rescue in high-dose MTX therapy because it selectively rescues normal cells leaving tumor cells destroyed by MTX. On the other hand, LV rescues normal and neoplastic cells equally well.

Consideration of the results of the present study showing that most of the serum folate was in the form of MTHF after oral administration and that about 2/3 of serum folate was also MTHF after parenteral administration, leads to the postulation that the type of folate actually rescuing normal cells in the high-dose MTX regimen may be MTHF. Assuming that MTHF may be more important theoretically in high-dose MTX treatment, what is the best way to achieve sufficient levels of MTHF to rescue only normal cells? As mentioned before, the amount of LV in almost all protocols of high-dose MTX regimens was settled without knowing the metabolism of pharmacological doses of LV. Theoretically, the amount of LV to protect normal cells and achieve the best tumorcidal effect of MTX should be minimum. If the amount of LV is high, tumor cells as well as normal cells will be rescued. Although it is conceivable that the amount of LV required to protect normal cells will increase as the amount of MTX increases, no study has been made on the quantitative relationship between MTX and LV. The only way to determine the minimum amount of LV in high-dose MTX regimens is to explore the previous protocols employing minimum dosage of LV.

The protocol used by Frei et al. (7) is very promising for this purpose. The amount of MTX in their protocol (3–7.5 g/m²) is rather high compared to other protocols. They gave 10 mg/m² of LV intravenously 24 hr after MTX followed by the same amount orally 12 times every 6 hr. As shown in the present study, MTHF levels attained by repeated oral administration of LV (15 mg) remained constant and relatively low (80–90 ng/ml) compared to those attained by intravenous administration of the same amount of LV (300 ng/ml in the first injection, 1,000 ng/ml in the 2nd and 1,500 ng/ml in the 3rd). Therefore it is very interesting that the protocol of Frei et al. using mainly the oral route of LV administration was successfully carried out. This fact strongly suggests that a relatively low level of MTHF (less than 100 ng/ml judging from the present study) is sufficient to rescue normal cells in their protocol. Almost all the investigations so far made have involved the use of larger amounts of LV for the smaller dose of MTX than the protocol of Frei et al. and also, most of them have favored the parenteral route over
the oral one. This means that the amount of LV used in those protocols has been excessive. Even the amount employed by Frei et al. may have been surplus to requirements. Further experimental and clinical studies are required to settle the proper amount of LV to rescue normal cells in high-dose MTX regimens.

As MTHF is thought to play the main role in rescuing only normal cells in high-dose MTX treatment, the trial with LV given orally as the main route of rescue should be explored more extensively. The oral route is more reasonable because this results in elevation of the serum MTHF level only and the attainment of a sufficient level of MTHF to avoid the side effects of high doses of MTX. Another benefit of using the oral route is the relief of the load on patients and medical staff.

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