

## **PRODUCT MONOGRAPH**

### **Pr LEUCOVORIN CALCIUM INJECTION**

(Calcium Folate Injection BP)

Folinic acid 10 mg/mL

Sterile Solution

Folic Acid Derivative

Sandoz Canada Inc.  
145 Jules-Léger  
Boucherville (QC)  
J4B 7K8

Date of Preparation:  
March 22, 2013

Submission Control No: 137634

## **Prescribing Information**

### **Pr Leucovorin Calcium Injection**

(Calcium Folate Injection BP)

Folinic acid 10 mg/mL

Sterile Solution

Therapeutic Classification

Folic Acid Derivative

**CAUTION: DO NOT ADMINISTER CALCIUM FOLINATE INJECTION INTRATHECALLY.**

## **ACTION AND CLINICAL PHARMACOLOGY**

Calcium folinate is the calcium salt of folinic acid (citrovorum factor). It is a mixture of the diastereoisomers of the 5-formyl derivative of tetrahydrofolic acid. The (-)-L stereoisomer is the biologically active component of the mixture. It is a metabolite of folic acid and an essential coenzyme for nucleic acid synthesis. Folinic acid acts as an antidote to folic acid antagonists, such as methotrexate, which block the conversion of folic acid to tetra-hydrofolate by binding to the enzyme dihydrofolate reductase.

Folinic acid is readily converted to other reduced folic acid derivatives such as tetrahydrofolate. Folinic acid does not require reduction by dihydrofolate reductases, therefore, it is not affected by blockage of this enzyme by folic acid antagonists (dihydrofolate reductase inhibitors). This allows purine and thymidine synthesis, and thus DNA, RNA, and protein synthesis to occur.

Folinic acid probably acts by competing with methotrexate for the same transport processes into the cell thus limiting the action of methotrexate on normal cells.

Folinic acid rescues bone marrow and gastrointestinal cells from methotrexate toxicity, but does not appear to have an effect on pre-existing methotrexate nephrotoxicity.

Folinic acid enhances the cytotoxicity of fluoropyrimidines such as 5-fluorouracil (5-FU) by the metabolites, methylene tetrahydrofolate and fluorodeoxyuridine monophosphate. It forms a stable ternary complex with thymidylate synthase, and thereby, decreases intracellular levels of that enzyme and the product thymidylate. Cellular necrosis occurs as a result of thymine starvation.

Folinic acid has an onset of action of 20-30 minutes with peak levels occurring at 1.7 hours.

Similar serum levels are produced after oral and intravenous administration, approximately 12% greater than after intramuscular use. Onset of action is 10-20 minutes after intramuscular administration with peak levels occurring at 0.7 hours. Folinic acid has a 3-6 hour duration of action. Absorption from the deltoid is 8% higher than from the gluteal muscle after intramuscular injection.

## **INDICATIONS AND CLINICAL USE**

Calcium Folate Injection is indicated:

- (1) to diminish the toxicity and counteract the effect of impaired METHOTREXATE elimination, and of accidental overdosages of folic acid antagonists
- (2) to treat the megaloblastic anemias due to folate deficiency, as in sprue, nutritional deficiency, megaloblastic anemias of pregnancy and infancy.
- (3) for pre-treatment followed by 5-fluorouracil to prolong survival in the palliative treatment of patients with advanced colorectal cancer.  
(See CONTRAINDICATIONS)

## **CONTRAINDICATIONS**

Calcium Folate Injection is not indicated for use in the treatment of folic acid deficiency. Calcium Folate Injection should never be used for the treatment of pernicious anemia or other megaloblastic anemias secondary to a deficiency of vitamin B<sub>12</sub>. As with folic acid, its use can result in an apparent response of the hematopoietic system, but neurological damage may occur or progress if already present.

## **WARNINGS**

### **General**

Calcium Folate Injection should be administered as promptly as possible in the treatment of accidental overdose of folic acid antagonists. As the time interval between antifolate administration [e.g., Methotrexate (MTX)] and Calcium Folate Injection increases, the effectiveness of folinic acid in counteracting the toxicity decreases. It is essential to monitor serum methotrexate concentrations in order to determine the optimal dose and duration of treatment with Calcium Folate Injection.

Delayed excretion of MTX may be caused by a third space fluid accumulation (i.e., ascites, pleural effusion), renal insufficiency, or inadequate hydration. In the above circumstances, higher doses of Calcium Folate Injection or prolonged administration may be required.

### **Interaction with Fluorouracil**

There have been occasional reports of treatment-related deaths in patients on calcium folinate and fluorouracil combination therapy. Diarrhea or stomatitis/mucositis are generally the first indication that severe and potentially life-threatening toxicity could develop. Patients who experience these symptoms while receiving any therapy regimen incorporating Calcium Folate Injection plus fluorouracil should be carefully monitored. Further therapy should be withheld until these symptoms resolve.

Calcium Folate Injection enhances the toxicity of fluorouracil. When these drugs are administered concurrently to reduce the severity of advanced colorectal cancer, the dosage of fluorouracil must be reduced. Although the toxicities observed in patients treated with combination of Calcium Folate Injection plus fluorouracil are qualitatively similar to those observed in patients treated with fluorouracil alone, gastrointestinal toxicities (particularly stomatitis and diarrhea) are observed more commonly and may be more severe in patients receiving the combination (see PRECAUTIONS).

Therapy with Calcium Folate Injection /fluorouracil must be withheld or discontinued in patients who have symptoms of gastrointestinal toxicity of any severity, until those symptoms have resolved. Patients with diarrhea must be carefully monitored until the diarrhea has resolved, as rapid clinical deterioration leading to death can occur. Elderly or debilitated patients are at greater risk for severe toxicity with this therapy.

Seizures and/or syncope have been reported rarely in cancer patients receiving folinic acid, usually in association with fluoropyrimidine administration, and most commonly in those with CNS metastases or other predisposing factors, however, a causal relationship has not been established.<sup>24</sup>

### **PRECAUTIONS**

Because of the  $\text{Ca}^{++}$  content of calcium folinate solutions, no more than 160 mg of Calcium Folate Injection should be administered intravenously per minute.

Since Calcium Folate Injection may enhance the toxicity of fluorouracil, combination therapy with these drugs for advanced colorectal cancer should be administered under the supervision of a physician experienced in the use of antimetabolite cancer chemotherapy. Particular care should be taken in the treatment of elderly or debilitated colorectal cancer patients, as these patients may be at increased risk of severe toxicity. Deaths from severe enterocolitis, diarrhea and dehydration have been reported in elderly patients receiving Calcium Folate Injection and fluorouracil. Concomitant granulocytopenia and fever were present in some, but not all of the patients.

### **DRUG INTERACTIONS**

Large amounts of folic acid may counteract the antiepileptic effect of phenobarbital, phenytoin

and primidone, and increase the frequency of seizures in susceptible children.

Preliminary animal and human studies have shown that small quantities of systemically administered folinic acid enter the cerebrospinal fluid (CSF) primarily as 5-methyl-tetrahydrofolate and, in humans, remain 1-3 orders of magnitude lower than the usual methotrexate concentration following intrathecal administration. However, high doses of Calcium Folate Injection may reduce the efficacy of intrathecally administered methotrexate. Calcium Folate Injection has no effect on other toxicities of methotrexate, such as the nephrotoxicity resulting from drug precipitation in the kidney.

Calcium Folate Injection may enhance the toxicity of fluorouracil (see Warnings).

**Pregnancy-Teratogenic Effects:**

Animal reproduction studies with Calcium Folate Injection have not been conducted. It is not known whether Calcium Folate Injection can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. Calcium Folate Injection should be given to a pregnant woman only if the need is clearly demonstrated and the benefits have been weighed against the possible risks.

**Nursing Mothers:**

There has been evidence that folinic acid is excreted in human breast milk, therefore, caution should be exercised when administering Calcium Folate Injection to nursing mothers.

**Pediatric Use:** See Drug Interactions.

**ADVERSE EFFECTS**

Occasional allergic reactions have been reported following oral and parenteral administration of folic acid; pyrexia has occurred after injections. In combination therapy the toxicity of 5-FU is enhanced by Calcium Folate Injection. The most common manifestations are leukopenia, mucositis, stomatitis, and/or diarrhea which may be dose limiting. Clinical trials with this drug combination demonstrated that these toxicities were reversible with appropriate modification of 5-FU administration.

## REPORTING SUSPECTED SIDE EFFECTS

You can report any suspected adverse reactions associated with the use of health products to the Canada Vigilance Program by one of the following 3 ways:

- Report online at [www.healthcanada.gc.ca/medeffect](http://www.healthcanada.gc.ca/medeffect)
- Call toll-free at 1-866-234-2345
- Complete a Canada Vigilance Reporting Form and:
  - Fax toll-free to 1-866-678-6789, or
  - Mail to: Canada Vigilance Program  
Health Canada  
Postal Locator 0701E  
Ottawa, Ontario  
K1A 0K9

Postage paid labels, Canada Vigilance Reporting Form and the adverse reaction reporting guidelines are available on the MedEffect™ Canada Web site at [www.healthcanada.gc.ca/medeffect](http://www.healthcanada.gc.ca/medeffect).

NOTE: Should you require information related to the management of side effects, contact your health professional. The Canada Vigilance Program does not provide medical advice.

## SYMPTOMS AND TREATMENT OF OVERDOSAGE

Folic acid is a water soluble vitamin converted in the body by the action of folate reductase to folinic acid which is rapidly eliminated in the urine.

Folic acid has low acute and chronic toxicity in man. No adverse effects have been noted in adults after the ingestion of 400 mg/day for 5 months or 10 mg/day for 5 years.

Excessive amounts of Calcium Folate Injection may nullify the chemotherapeutic effect of folic acid antagonists.

**For management of a suspected drug overdose, contact your regional Poison Control Centre immediately.**

## DOSAGE AND ADMINISTRATION

Intravenous administration is employed when Calcium Folate Injection is used for folinic acid pre-treatment followed by 5-fluorouracil (5-FU) in the treatment of patients with advanced colorectal cancer. Because of the  $\text{Ca}^{++}$  contents of calcium folinate solutions, no more than 160 mg of Calcium Folate Injection should be administered per minute.

Dose:

a) Impaired Methotrexate Elimination or Inadvertent Overdosage

For the treatment of accidental overdosage of folic acid antagonists, it is generally given in amounts equal to the weight of the antagonist used.

Rescue with Calcium Folate Injection should begin as soon as possible after an inadvertent overdosage and within 24-36 hours of methotrexate administration when there is delayed excretion. (See Warnings). Calcium Folate Injection  $10 \text{ mg/m}^2$  should be administered intravenously, or intramuscularly every 6 hours until the serum methotrexate level is less than  $5 \times 10^{-8} \text{ M}$ . In the presence of gastrointestinal toxicity, nausea or vomiting due to methotrexate, Calcium Folate Injection should be administered. Because absorption is saturable, doses greater than 25 mg should be given intravenously.

Serum creatinine and methotrexate levels should be determined at 24 hour intervals. If the 24 hour serum creatinine has increased 50% over baseline or if the 24 hour methotrexate level is greater than  $5 \times 10^{-6} \text{ M}$  or the 48 hour level is greater than  $9 \times 10^{-7} \text{ M}$ , the dose of Calcium Folate Injection should be increased to  $100 \text{ mg/m}^2$  i.v. every 3 hours until the methotrexate level is less than  $5 \times 10^{-8} \text{ M}$ .

Hydration (3 L/day) and urinary alkalization with  $\text{NaHCO}_3$  should be employed concomitantly. The bicarbonate dose should be adjusted to maintain the urine pH at 7.0 or greater.

b) Megaloblastic Anemia Due to Folic Acid Deficiency

Doses of 15 mg daily by mouth or not greater than 1 mg intravenously have been suggested for the treatment of megaloblastic anemia. There is no evidence that doses greater than 1 mg daily have greater efficacy than those of 1 mg. The loss of folates in the urine becomes roughly logarithmic when the amount administered exceeds 1 mg.

c) Advanced Colorectal Cancer

Calcium Folate Injection is administered at  $200 \text{ mg/m}^2$  by slow intravenous injection immediately prior to dosing with  $370 \text{ mg/m}^2$  5-FU (fluorouracil) by slow intravenous injection, for five consecutive days. This 5 day treatment course may be repeated at 4 week (28 day) intervals, provided that the patient has completely recovered from the toxic effects of the prior treatment course.

In subsequent treatment courses, the dosage of fluorouracil should be adjusted based on patient tolerance of the prior treatment course. The daily dosage of fluorouracil should be reduced by 20% for patients who experienced moderate hematologic or gastrointestinal toxicity in the prior treatment course, and by 30% for patients who experienced severe toxicity in the prior treatment course. Fluorouracil dosage may be increased by 10% for patients who experienced no toxicity in the prior treatment course. Dosages of Calcium Folate Injection are not adjusted for toxicity.

Laboratory Tests During Treatment with Folinic acid-5-Fluorouracil Combination:

Myelosuppression is most often the dose-limiting toxicity factor, and stomatitis, mucositis, diarrhea, nausea and abdominal pain are often seen. Myelosuppression, predominantly leukopenia may occur.

Patients should be monitored routinely with complete blood counts, liver function tests, neutrophil and platelet nadir. Tumour measurements and CEA counts may be performed monthly.

The 5-FU dose may be increased by 30 mg/m<sup>2</sup>/day (from an initial dose of 370 mg/m<sup>2</sup>/d) if the leukocyte nadir is > 1.5 x 10<sup>9</sup>/L and the platelet nadir is >50 x 10<sup>9</sup>/L.

Generally, the dose of 5-FU (when given with folinic acid) may be increased by 10% per treatment cycle in the absence of toxicity from the preceding cycle of therapy.

### **Administration**

Calcium Folate Injection is used for intravenous or intramuscular injection.

Rotate site when giving the drug by intramuscular administration. Absorption from the deltoid muscle is better than from the gluteal muscle.

For direct intravenous injection and intermittent infusion the rate should be < 160 mg/minute because of the calcium content.

Parenteral drug products should be inspected for particulate matter and discolouration prior to administration, whenever solution and container permit.



## PHARMACEUTICAL INFORMATION

### Drug Substance

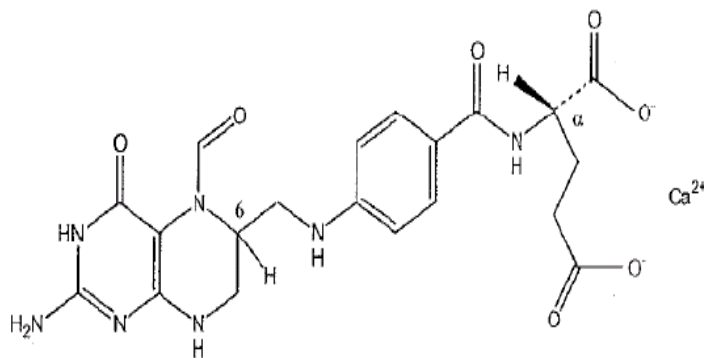
**Proper name:** Calcium folinate (folic acid derivative) This drug substance is also known as calcium folinate, citrovorum factor, or the calcium salt of 5-formyl-5,6,7,8-tetrahydrofolic acid.

**Chemical name:** (R,S)-N-{4-[[2-amino-5-formyl-1,4,5,6,7,8-hexahydro-4-oxo-6-pteridiny]methyl]amino]-benzoyl}-L-glutamate, calcium salt

**Molecular formula:**  $C_{20}H_{21}CaN_7O_7$

**Molecular Weight:** 511.5 g/mol (salt)  
471.5 g/mol (base)

**Structural formula:**



**Physicochemical properties:** Calcium folinate is a yellowish white or yellow, odourless, powder. It is very soluble in water and practically insoluble in alcohol. It decomposes above 250°C.

**Composition:** Calcium Folate Injection is a sterile solution of folinic acid (as the calcium salt), supplied as 10 mg/mL, in Water for Injection with Sodium Chloride 7.70 mg/mL, added for isotonicity. **Contains no preservatives.** Sodium Hydroxide or Hydrochloric Acid may be used for pH adjustment.

## **STABILITY AND. STORAGE RECOMMENDATIONS**

Vials of 10 mg/mL should be stored at refrigerated temperatures (2-8°C). Calcium Folate Injection is light sensitive and should be protected from light. The liquid formulation should be used immediately once removed from refrigeration. Discard any unused portion.

### **Parenteral Products:**

Parenteral Products:

Calcium Folate Injection, 10 mg/mL, may be further diluted for intravenous infusion to concentrations of 0.06 mg/mL to 1.0 mg/mL with one of the following solutions:

Dextrose 5% in water  
Dextrose 10% in water  
Dextrose 10% in saline  
Ringer's Injection  
Lactated Ringer's Injection  
Physiological saline (0.9% sodium chloride)

The vial contents diluted with Ringer's Injection, Lactated Ringer's injection, and Physiological saline are stable for up to 24 hours at room temperature. The vial contents diluted with Dextrose 5% in water are stable for up to 12 hours at room temperature. The vial contents diluted with Dextrose 10% in water and Dextrose 10% in saline are stable for up to 6 hours at room temperature.

Parenteral products should be inspected for particulate matter and discoloration whenever solution and container permit.

## **AVAILABILITY OF DOSAGE FORMS**

Leucovorin Calcium Injection is available as a 10 mg/mL sterile, unpreserved, isotonic solution of folinic acid as the calcium salt, in single use Pharmacy Bulk Vials for IV administration only in the following doses and volumes:

500 mg/ 50 mL supplied in amber vials of 50 mL

1000 mg/ 100 mL supplied in amber vials of 100 mL

The Pharmacy Bulk Vial is supplied to hospitals with a recognized intravenous admixture program only.

## PHARMACOLOGY

### (i) Human Pharmacology

The pharmacokinetics of folinic acid was evaluated after intravenous, intramuscular and oral administration of a 25 mg dose of calcium folinate to healthy male subjects in a randomized crossover study.

Serum total reduced folates (as measured by *Lactobacillus casei* assay) reached a mean peak of 1259 ng/mL (range-1625) at 10 minutes and 436 ng/mL (range 240-725) at 52 minutes after intravenous and intramuscular administration respectively. This initial rise in total reduced folates was primarily due to the parent compound 5-formyl-tetrahydrofolate (measured by *Streptococcus faecalis* assay) which rose to 1206 ng/mL at 10 minutes and 360 ng/mL at 28 minutes after intravenous and intramuscular administration, respectively. A sharp drop in parent compound followed and coincided with the appearance of the metabolite (also active) 5-methyl-tetrahydrofolate which became the predominant circulating form of the drug (intravenous administration). The mean peak of 5-methyl tetrahydrofolate was 258 ng/mL and occurred at 1.3 hours. The level of the metabolite 5-methyl-tetrahydrofolate increased subsequently over time until at 1.5 hours it represented 50% of the circulating total folates (intramuscular administration). The terminal half-life of total reduced folates was 6.2 hours with parenteral administration. There was no statistically significant difference between i.m. and i.v. administration in the AUC for total reduced folates, 5-formyl-tetrahydrofolate, or 5-methyl-tetrahydrofolate.

Folinic acid distributes to all tissues, readily penetrates the blood brain barrier and actively concentrates in the cerebrospinal fluid. Folinic acid (5-formyl-tetrahydrofolate) is rapidly and extensively metabolized to other tetrahydrofolate derivatives, the major metabolite being 5-methyl-tetrahydrofolate. Approximately 80-90% of the dose is excreted in the urine. Elimination half-lives of parent drug and active metabolite are 32 and 227 minutes respectively.

After oral administration of calcium folinate reconstituted with aromatic elixir, the mean peak concentration of serum total folates occurred at 2.3 hours and was 393 ng/mL (range 160-550). The terminal half-life was 5.7 hours. The major metabolite 5-methyl-tetrahydrofolate to which folinic acid is converted-in the intestinal mucosa. The mean peak of the parent compound and 5-methyl-tetrahydrofolate was 51 ng/mL and 367 ng/mL at 1.2 and 2.4 hours, respectively. The AUC (bioavailability) of total reduced folates after oral administration was 92% of the AUC after intravenous administration.

Following oral administration, folinic acid is rapidly absorbed and enters the general body pool of reduced folates. Oral absorption of folinic acid is saturable at doses above 25 mg. The apparent bioavailability of folinic acid was 97% for 25 mg, 75% for 50 mg and 37% for 100 mg.

The serum half-life of citrovorum factor (or 5-formyl-tetrahydrofolate) was 35-45 minutes following both oral and i.m. administration. The serum half-life of 5-methyl-tetrahydrofolate was about 2 1/4 hours. 5-methyl-tetrahydrofolate is excreted via the kidneys in a manner proportional to its serum concentration.

Folinic acid can enhance the therapeutic and toxic effects of fluoropyrimidines, such as fluorouracil, used in cancer therapy. Fluorouracil is metabolized to fluorodeoxyuridylic acid, which binds to and inhibits the enzyme thymidylate synthase (an enzyme important in DNA repair and replication). Folinic acid is readily converted to another reduced folate, 5,10-methylene-tetrahydrofolate, which acts to stabilize the binding of fluorodeoxyuridylic acid to thymidylate synthase and thereby enhance the inhibition of this enzyme.

A folic acid deficiency is produced during therapy with the folic acid antagonists, such as aminopterin and amethopterin (Methotrexate), used as antineoplastic agents and with the chemotherapeutic agent pyrimethamine. These agents competitively inhibit the conversion of folic acid to folinic acid. Their affinity for folate reductase is so much greater than that of folic acid that not even large doses of folic acid will correct the drug-induced deficiency. In the event of a severe toxic reaction, the already reduced form, folinic acid, can be given, since it can be used directly to form new coenzyme.

A clinical study had shown that when pregnant women received folic and folinic acid to determine placental transfer, the new born infants had normal weight and did not show any anomalies. Allergic sensitization has been reported after oral and parenteral administration of folic acid. Such sensitization may also occur with calcium folinate since it is a folic acid derivative. However, in general, calcium folinate is remarkably free of side effects. Methotrexate does not seem to affect the absorption of folate.

#### (i) Animal Pharmacology

A study was carried out to determine the differences in pharmacokinetic parameters of the diastereoisomers of citrovorum factor in dogs. The results indicated that d and I isomers have different pharmacokinetic behaviours with respect to the postdistribution plasma decay rates. The I isomer had a half-life of  $47 \pm 4$  (SE) minutes compared to the half life of  $143 \pm 15$  minutes for the d isomer. Plasma clearance of the I isomer was about 2.5 times its urinary clearance, which indicates that nonrenal mechanisms play a major role in the disposition of the I isomer. The apparent volume of distribution was approximately 58% for both isomers. In animal studies, toxic manifestations from folinic acid were not observed.

## REFERENCES

1. Bertrand M, Doroshaw JH, Multhauf P, et al. High dose continuous infusion folinic acid and bolus 5-Fluorouracil in patients with advanced colorectal cancer: a phase II study. *J Clin Oncol* 1986; 4:1058-1061.
2. Borrie P and Clark PA. Megaloblastic anaemia during methotrexate treatment of psoriasis. *Br Med J* 1966; 1:1339.
3. Budd GT, Fleming TR, Bukowski RM, et al. 5-Fluorouracil and folinic acid in the treatment of metastatic colorectal cancer: a randomized comparison. A southwest oncology group study. *J Clin Oncol* 1987; 5:272-277.
4. Chabner BA. Methotrexate. *Pharmacologic Principles of cancer treatment* 1982. pg. 229-255. Published by W.B. Saunders Company.
5. Chanarin I, Mollin DL and Anderson BB. Folic acid deficiency and the megaloblastic anaemias. *Proc Royal Societ Med* 1958; 51:757-763.
6. Creaven PJ. 5-fluorouracil and folinic acid: summary of clinical experience. 1988, pg. 303-311.
7. Delap RJ. The effect of leucovorin on the therapeutic index of flourouracil in cancer patients. *Yale J Bio Med* 1988; 61:23-34.
8. Erlichman C, Fine S, Wong Alfred and Elhakin T. A randomized trial of fluorouracil and folinic acid in patients with metastatic colorectal carcinoma. *J Clin Oncol* 1988; 6:469-475.
9. Evans WE, Crom WR, Yelowich JC. Methotrexate. In: Evans WE, Schentag JJ, Jusko WJ, eds. *Applied Pharmacokinetics*. Spokane: Applied Therapeutics Inc; 1986:1009-1056.
10. Flynn LM, Williams VB, O'Dell BL, and Hogan AG. Medium for assay of vitamins with lactic acid bacteria. *Anal Chem* 1951; 23:180-186.
11. Hines JD, Zakem MH, Adelstein DJ, et al. Bioavailability of high dose oral leucovorin. *NCI Monographs* 1987; 5:57-60.
12. Izak G, Rachmilewitz M, Zan S and Grossowicz N. The effect of small doses of folic acid in nutritional megaloblastic anemia. *Am J Clin Nutr.* 1963;13:369-377.
13. Laufman LR, Krezeczowski KA, Roach R and Segal M. Leucovorin plus 5-fluorouracil: an effective treatment of metastatic colon cancer. *J Clin Oncol* 1987; 5:1395-1400.

14. Marini G, Zaniboni A, Gorni F, et al. Clinical experience with 5-fluorouracil (5-FU) and high dose folinic acid in solid tumours. *Drugs Exptl Clin Res* 1987;6:373-376.
15. Machover D, Goldschmidt E, Chollet P, et al. Treatment of advanced colorectal and gastric adenocarcinomas with 5-fluorouracil and high dose folenic acid. *J Clin Oncol* 1986; 4:685-696.
16. Mane JV, Vives - Corrons JL and Rozman C. Congenital folate-dependent megaloblastic anemia of unknown aetiology. *Lancet* 1977:262-263.
17. McGuire BW, Sia LL, Haynes JD, et al. Absorption kinetics of orally administered leucovorin calcium. *NCI Monographs* 1987; 5:47-56.
18. Mehta BM, and Hutchison DJ. A microbiological disc assay for 5-methyltetrahydrofolate in the presence of methotrexate. *Cancer Treat Report* 1977; 61:1657-1663.
19. Mehta BM, Gisolfi AL, Hutchison DJ, et al. Serum distribution of citrovorum factor and 5-methyltetrahydrofolate following oral and im administration of calcium leucovorin in normal adults. *Cancer Treat Reports* 1978; 62:345-350.
20. Nixon PF and Bertino JR. Effective absorption and utilization of oral for myltetrahydrofolate in man. *NEJM* 1972; 286:175-179.
21. O'Connell MJ, Klaassen DJ, Everson LK, et al. Clinical studies of biochemical modulation of 5-fluorouracil by leucovorin in patients with advanced colorectal cancer by the north central cancer treatment group and Mayo Clinic. *NCI Monographys*, 1987; 5:185-188.
22. O'Connell MJ. A controlled clinical trial including folinic acid at two distinct dose levels in combination with 5-fluorouracil (5-FU) for the treatment of advanced colorectal cancer: experience of the Mayo Clinic and north central cancer treatment group. 1988 pg.173-184.
23. Petrelli N, Hewera L, Rustum Y, et al. A prospective randomized trial of 5-fluorouracil versus 5-fluorouracil and high dose leucovorin versus 5-fluorouracil and methotrexte in previously untreated patients with advanced colorectal carcinoma. *J Clin Oncol* 1987; 5:1559-1565.
24. Physicians' Desk Reference. 1998, 52<sup>th</sup> edition. Medical Economics Company, Montvale, N.J. 07645-1742, USA, page 1271.
25. Pinedo HM, Chabner BA eds. *Cancer chemotherapy/7: The EORT C Cancer Chemotherapy Annual*. New York: Elsevier Science Publishing Co. Inc;1985:8-11.
26. Pratt RF and Cooper BA. Folates in plasma and bile of man after feeding folic acid - 3H

- and 5-formyltetrahydrofolate (folinic acid). J Clin Invest 1971;50:455-462.
27. Roman J, Westervelt F, Atuk N, and Wheby M. The occurrence of megaloblastic anemia with normal serum folate during antipurine therapy. pg. 94.
  28. Scott - 1957. Folic acid in megaloblastic anaemia of pregnancy. Br. Med J 1957;270-272.
  29. Stokes PL, Melikian V, Leeming RL, et al. Folate metabolism in scurvy. Am J Clin Nutr.1975; 28:126-129.
  30. Straw JA, Azapary D and Wynn WT. Pharmacokinetics of the diastereoisomers of leucovorin after intravenous and oral administration to normal subjects. Cancer Res 1984; 44:3114-3119.
  31. Straw JA, Newman EM, and Doroshaw JH. Pharmacokinetics of leucovorin (D,L-5-Formyltetrahydrofolate) after intravenous injection and constant intravenous infusion. NCI Monographs 1987; 5:41-45).
  32. Unglaub WG and Goldsmith GA. Folic acid and vitamin B12 in medical practice. JAMA 1956; 161:623-627.
  33. Whitehead VM, Freedman MH, Rivard GE and Townsend SR. Response to folinic acid in B12 -deficiency anaemia. Lancet 1971:552-554.
  34. Wilke H, Schmoll HJ, Preusser P. et al. Folinic acid (CF) 15-fluorouracil (Fura) combination in advanced gastrointestinal carcinomas.1988:233-243.
  35. Wilmanns W, Saver H, and Schalhorn A. Biochemical control of high dose methotrexate/leucovorin rescue therapy. Cancer research 1980; 74:42-49.
  36. Product Monograph, LEUCOVORIN CALCIUM INJECTION, USP Control No.: 058177, Date of Revision: December 21, 1998, Novopharm Limited Canada, Toronto Canada.