Acute toxicity of folic acid in mice

M. Parchure, R.Y. Ambaye, V.S. Lalitha and S.V. Gokhale[1,2]

Chemotherapy Division and Cell and Developmental Pathology Division, Cancer Research Institute, Tata Memorial Centre, Parel, Bombay-400012 (India), 26 September 1983

Summary. The toxicity of folic acid (PGA) was studied in different inbred strains of mice. LD_{50} values of PGA by the i.p. route showed a unique toxicity pattern. In some strains, convulsions, ataxia and weakness were observed. Histopathological study in strains S/RVCri, BDF_1, DBA/2 and DBA/2fNCri showed acute renal tubular necrosis.
Key words. Folic acid toxicity; renal necrosis.

Folic acid (Pteroylglutamic acid-PGA) has been recognized as an essential nutrient in humans for more than 40 years[3,4]. Its role in hemopoesis and proliferation of normal and abnormal cells is well known. In the metabolic interrelationships, various steps involving folic acid have been well defined[5]. Folic acid has been used in the treatment of human macrocytic anaemias at doses ranging from 50 µg–15 mg daily by either the oral or the parenteral route, and it has been described a generally not toxic for man[6].

During our studies on murine leukemias, we combined folic acid in large doses with anticancer drugs[7]. In these studies, it was noted that folic acid at doses above 100 mg/kg/day brought about the death of a certain number of animals. The Registry of toxic effects of chemical substances (US Dept. of Health and Human Services, NIOSH Cincinnati, Ohio, 1979) gives 2 LD_{50} values for folic acid, namely 100 mg/kg for i.p. application[8] and 239 mg/kg for i.v. injection[9]. However, detailed studies in mice have not been conducted and they are not mentioned in the standard texts of pharmacology or physiology[10,11]. It was, therefore, decided to carry out an acute toxicity study of PGA in mice. The results are reported below.

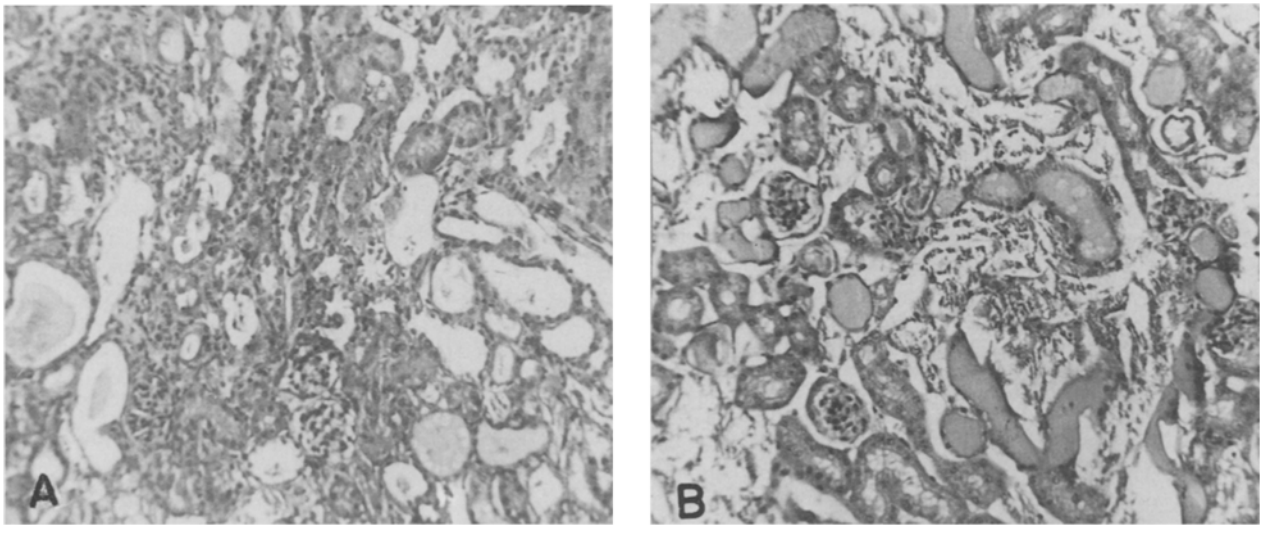
Materials and methods. PGA was obtained in powder form from BDH Laboratories, England. Mice belonging to different inbred strains[12] were obtained from the Animal Colony of the Cancer Research Institute, Bombay (table). Both male and female mice, weighing 18–22 g, were used for toxicity studies.

The animals were maintained on the colony diet (Composition: Cracked wheat (70%), cracked Bengal gram (20%), yeast powder (40%), fish-meal (5%), shark liver oil (0.25%) and seasame oil (0.75%)) and water ad libitum, before and during the period of observation. All the animals were randomly distributed in groups of 10 mice each. PGA was dissolved in 2% $NaHCO_3$ just prior to use. It was injected i.p. at various doses (50 ~ 400 mg/kg) (table). Control animals received an appropriate 2% $NaHCO_3$ volume i.p. The animals were observed for 7 days and their abnormal movements and deaths were recorded. The LD_{50} values were computed by the method of Miller and Tainter[13]. Some animals were specially treated with PGA at different doses and after 72 h, their organs including liver, kidneys, skeletal muscle, brain and spinal cord were removed after perfusion with Ringer lactate followed by 10% neutral buffered formalin, and subjected to histopathological examination. LD_{50} values were not determined in DBA/2 mice. However, 2 groups of 5 mice each, of this strain, were treated with PGA (75 mg/kg and 150 mg/kg) and their organs were subjected to histopathological examination.

Results. As shown in the table, the LD_{50} values of PGA differ considerably between the strains, with C57BL/Cri being the most susceptible one, while S/RVCri mice are most resistant to the lethal effects of PGA. Observation of the animals, prior to death, showed that DBA/2fNCri mice had convulsions on the 2nd day after PGA injection and usually succumbed on the 3rd or 4th day. Animals that survived after the 4th day usually recovered. Mice belonging to other strains exhibited ataxia and muscular weakness for 24–48 h, prior to death. Terminal coma

LD_{50} values of folic acid in various strains of mice by i.p. route

Strain	Sex	LD_{50} (mg/kg) ± SE
C57BL/Cri	Male	100 ± 12.86
C57BL/Cri	Female	85 ± 10.00
AKR/RdBCri	Male	260 ± 18.21
AKR/RdBCri	Female	180 ± 14.54
S/RVCri	Male	330 + 20.11
S/RVCri	Female	225 ± 9.50
DBA/2fNCri	Male	175 ± 32.80
BDF_1	Male	180 ± 14.63
ICRC/HiCri	Female	225 ± 11.74
S/RVCri-ba	Female	225 ± 10.94



Renal tubular necrosis in FA-treated S/RBCri mice (Dose: 300 mg/kg, IP) *A* Control; *B* Treated, hematoxylin and eosin stain. ×130.

lasted for only a few hours. Histopathological examination of PGA-treated animals showed that a large number of these animals had acute renal tubular necrosis (fig.). In only one DBA/2 mouse, there was shrinkage of a few Purkinje cells in the cerebellum.

Discussion. PGA, as a water-soluble vitamin supplement, is expected to have a very high LD_{50} value, unlike drugs. It was surprising, therefore, to note a fairly low LD_{50} value in some of the murine strains. Histopathological examination has revealed acute renal tubular necrosis (fig.) as one of the causes of death. PGA has a low solubility in cold water but it dissolves in dilute solutions of alkali hydroxides and carbonates[11]. Vitamin B_{12} and folic acid appear to require binding to polypeptides as a precondition for storage. Levels above the serum and tissue-binding capacity tend to be excreted rather than retained[14]. Ther renal tubular necrosis observed by us might have been due to precipitation in the acidic urine within the tubules. This could result in renal shut-down and death after several hours or days. However, since the drug was given on a mg/kg basis, the large differences between various strains were surprising, especially because they were housed under identical experimental conditions and were given the same diet. Preterminal convulsions in only 2 strains and ataxia in the others suggest a specific susceptibility of certain strains possibly due to a difference in the metabolic conversion of the drug by the animal.

It was interesting to note that C57BL/6 mice which are used for transplantation of Lewis lung carcinoma (LL) and B16 melanoma (B16) have a relatively low LD_{50} value. LL responds well to methotrexate[15]. This suggests a possible biochemical uniqueness in the metabolic transformation of PGA by this specific strain.

The behavioral changes such as hyperkinesis and ataxia, convulsions and also coma suggested neural involvement. It is known that folic acid at a dose of 15 mg per day for 1–4 weeks to patients exacerbates epilepsy[6] and 5-methyltetrahydrofolate has been reported to be neurotoxic[16,17]. We did not detect any structural changes in the CNS of the mice treated with folic acid. It is possible that definite histological alterations may become manifest only after chronic repeated administration of folic acid. The acute changes in CNS function which were detected by us may have resulted from acute uremia resulting from renal tubular necrosis. No firm opinion regarding the CNS can be given on the basis of the present study.

In conclusion, it may be said that the administration of folic acid to different strains of mice by the i.p. route has shown a unique pattern of toxicity. The most common observation in the PGA-treated mice is renal tubular necrosis.

1 To whom correspondence and reprint requests should be sent (at the Chemotherapy Division)
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3 Wills, L., Clutterbuck, P.W., and Evans, P.D.F., Biochem. J. *31* (1937) 2136.
4 Mitchell, H.K., Snell, E.E., and Williams, R.J., J. Am. chem. Soc. *63* (1941) 2284.
5 Erbe, R.W., New Engl. J. Med. *293* (1975) 753.
6 Hillman, R.S., in: The pharmacological basis of therapeutics, 6th edn, p.1331. Eds A.G. Gilman, L.S. Goodman and A. Gilman. Macmillan Publishing Co. Inc, New York 1980.
7 Parchure, M., Ambaye, R.Y., and Gokhale, S.V., Chemotherapy, in press (1984).
8 Cited from National Technical Information Service, US Dept. of Health and Human Services, NIOSH, Cincinnati, Ohio 1979.
9 Belies, R.P., Toxic. appl. Pharmac. *23* (1972) 537.
10 Windholz, M., Budavari, S., Stroumtsos, L.Y., and Fertig, M.N., in: The Merck Index, 9th edn, p. 543. Merck and Co. Inc. Rahway, New Jersey 1976.
11 Wade, A., in: Martindale, The extra pharmacopoeia incorporating Squire's companion, 27th edn, p.1683. The pharmaceutical press, London 1978.
12 Randelia, H.P., Hilgers, J., Hilkens, J., Krozen, V., Holmes, R., and Vandeberg, J., Lab. anim. *17* (1983) 280.
13 Miller, L.C., and Tainter, M.L., Proc. Soc. exp. Biol. Med. *57* (1944) 261.
14 Herbert, V., in: Modern nutrition in health and disease, 5th edn, p.221. Eds R.S. Goodhart and M.E. Shills, Lea and Febiger, Philadelphia 1974.
15 Goldin, A., Venditti, J.M., Macdonald, J.S., Muggia, F.M., Henney, J.E., and DeVita, V.T. Jr, Eur. J. Cancer *17* (1981) 129.
16 Olney, J.W., Fuller, T.A., and Gubareff, T., Nature *292* (1981) 165.
17 Roberts, P.J., Foster, G.A., and Thomas, E.M., Nature *293* (1981) 654.

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Circling behavior induced by phencyclidine in mice and its inhibition by naloxone

Y. Eshel and A.M. Korczyn*

Department of Physiology and Pharmacology, Sackler School of Medicine, Tel Aviv University, Ramat Aviv, 69978 Tel Aviv (Israel), 20 December 1983

Summary. Phencyclidine (PCP), when given to mice, induces general hyperactivity and rapid circling, similar to that caused by morphine. These effects are partially antagonized by naloxone.
Key words. Mice; circling behavior; phencyclidine; naloxone; hyperactivity.

Mice and rats manifest their activity by circling around the walls of a container in which they are placed. Animals have an innate preferred direction of circling, either clockwise or counter-clockwise. This tendency has been attributed to asymmetry between the two nigrostriatal systems[1].

Dopaminergic drugs increase the rate of circling and the degree of asymmetry[2]. Morphine, although not a direct dopaminergic agent, also increases, in mice, the rate of circling in the preferred direction[3,4].

Phencyclidine has previously been reported to induce rotatory behavior in rats with unilateral nigrostriatal lesions[5–7] as well as in intact rats[8]. This effect was attributed to interaction with the dopaminergic nigrostriatal system[5–8]. However, PCP shares some similarities with opiates[9] and in the present study we examined the possibility that the rotatory behavior induced by PCP is also mediated through opiate mechanisms.

Materials and methods. Male ICR albino mice weighing 20–35 g were studied. Rotation was measured as previously described[4]. Morphine sulfate (Assia), 40 mg/kg, phencyclidine hydrochloride (prepared by A. Kalir), 5 mg/kg and naxolone (Du Pont), 10 mg/kg, were injected i.p. Each dose was given to eight mice. Before drug administration each animal was harnessed to the rotometer for 1 h to verify its basic circling rate and preferred direction. Control groups were injected with saline prior to na-