

NEUROTOXICITY OF DEOXYCOFORMYCIN: EFFECT OF CONSTANT INFUSION ON ADENOSINE DEAMINASE, ADENOSINE, 2'-DEOXYADENOSINE AND MONOAMINES IN THE MOUSE BRAIN

S. HELLAND, O. J. BROCH and P. M. UELAND

Department of Pharmacology, University of Bergen, N-5016 Haukeland sykehus, Bergen, Norway

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Summary—The tight-binding adenosine deaminase inhibitor, 2'-deoxycoformycin (dCF), was continuously infused into mice by intraperitoneal implantation of microosmotic pumps delivering the compound at a rate of $0.16 \text{ mg hr}^{-1} \text{ kg}^{-1}$ for up to 6 days. The activity of cerebral adenosine deaminase was nearly totally inhibited. The amount of adenosine and 2'-deoxyadenosine was determined in the brain frozen in liquid nitrogen through the intact skull bone. The concentration of adenosine was about 1 nmol/g , and was essentially not altered following treatment with deoxycoformycin. Deoxycoformycin induced a progressive increase in cerebral content of 2'-deoxyadenosine, which after 1 day of treatment equalled the amount of adenosine. The concentrations of serotonin, dopamine and noradrenaline in the brain were not altered.

Key words: deoxycoformycin, adenosine deaminase, adenosine, 2'-deoxyadenosine, monoamines.

Deoxycoformycin (dCF), a tight-binding inhibitor of adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4), possesses lymphocytopenic and immunosuppressive properties and is currently undergoing clinical trials as an antileukemic agent (Glazer, 1980; Major, Agarwal and Kufe, 1981a; Poplack, Sallan, Rivera, Holcenberg, Murphy, Blatt, Lipton, Venner, Glaubiger, Ungerleider and Johns, 1981). The dose-limiting toxicity of the substance involves symptoms from the central nervous system (CNS), like lethargy and severe sedation (Major *et al.*, 1981a,b; Poplack *et al.*, 1981). In the light of the observation that adenosine depresses neuronal function both *in vivo* and *in vitro* (Fox and Kelley, 1978; Haulica, Abebei, Branisteanu and Topoliceanu, 1973; Phillis, Kostopoulos, Edstrom and Ellis, 1979), it has been suggested that the symptoms induced by deoxycoformycin in the CNS are mediated by adenosine (Major *et al.*, 1981b).

It was recently reported that deoxycoformycin inhibited adenosine deaminase in homogenates of rat brain (Skolnick, Nimitkitpaisan, Stalvey and Daly, 1978). No study has been devoted to the effect of deoxycoformycin on adenosine deaminase and adenosine levels of the brain *in vivo*. In the present paper the effect of infusion of deoxycoformycin on adenosine deaminase, adenosine, 2'-deoxyadenosine and monoamines in mouse brain are described.

METHODS

Chemicals and drugs

Sources of most chemicals have been given in a previous publication (Helland and Ueland, 1982).

2'-Deoxyadenosine was purchased from Sigma Chemical Co., St Louis, MO. 2'-Deoxycoformycin (dCF) was a gift from Parke-Davis Research Laboratories, Ann Arbor, MI.

Treatment of the animals and isolation of the brain

The deoxycoformycin was dissolved in 0.9% sodium chloride, and the solution was sterilized by filtration through Millex single use filters (Millipore, Bedford, MA). Alzet mini-osmotic pumps, model 2001 (Alza, Palo Alto, CA) were filled with the solution under sterile conditions as described by the manufacturer. The filled pumps were implanted intraperitoneally into mice. After 4 hr to 6 days, the animals were killed by cervical dislocation and the brain removed immediately and put into liquid nitrogen.

For measurement of the concentrations of adenosine and 2'-deoxyadenosine, the mouse was anesthetized with ether and the skull was exposed. The animal was then allowed to breathe in fresh air and submerged into liquid nitrogen just before it recovered from the anesthesia. The brain was removed while still frozen and stored at -80°C until use.

Assay for adenosine deaminase activity

One half of the brain was homogenized in 10 vol. of 0.1 M potassium phosphate buffer, pH 7.4. The activity of the enzyme was determined by a radiochemical method based on the conversion of [$8\text{-}^{14}\text{C}$]-adenosine to inosine. The incubation mixture contained $100 \mu\text{M}$ adenosine in 0.1 M potassium phosphate buffer, pH 7.4 and the temperature was 37°C . The radioactive metabolites were separated by

thin layer chromatography on PEI-cellulose plates (Fain and Shepherd, 1977).

Determination of adenosine and 2'-deoxyadenosine

The frozen brain was homogenized in ice-cold 0.8 M perchloric acid. The homogenate was centrifuged and neutralized with 1.44 M KOH/1.2 M KHCO₃. The nucleosides were determined by high-pressure liquid chromatography with a 3 μ M Hyper-sil ODS column (0.46 \times 10 cm) equipped with a 2 cm guard column packed with Pelliguard (Supelco). The mobile phase was 5% methanol in 15 mM acetate buffer, pH 4.5 and the flow rate was 2 ml/min. The detector was a Beckman fixed wavelength detector model 160. The detection limit was 2 pmol.

Determination of biogenic amines

Sodium bisulphite (1.25 mg/ml) was added to the perchloric acid extracts and the extracts were then neutralized as described above. Noradrenaline, dopamine and serotonin were determined by high-pressure liquid chromatography on a Partisil 10 SCX column (0.5 \times 25 cm) eluted at a flow rate of 1.5 ml/min with 50 mM ammonium formate buffer, pH 3.5, containing 1% propranolol and 0.1 mM EDTA. An electrochemical detector from Bio-analytical Systems (model LC-4A) was used. The detector potential was set at 0.7 V.

Table 1. Monoamine concentrations in the mouse brain after continuous intraperitoneal infusion of deoxycoformycin

Days	Noradrenaline	Dopamine	Serotonin
0	1.49 \pm 0.08	4.27 \pm 0.46	1.07 \pm 0.07
2	1.46 \pm 0.13	4.51 \pm 0.79	1.17 \pm 0.09
4	1.57 \pm 0.13	3.42 \pm 0.42	1.17 \pm 0.09
6	1.49 \pm 0.17	4.40 \pm 0.60	1.25 \pm 0.21

Values in nmol/g \pm SEM (means of 5–10 determinations).

RESULTS

The adenosine deaminase activity in extracts from brain was nearly totally inhibited following treatment with deoxycoformycin. The effect was apparent after 4 hr of treatment (Fig. 1A). Deoxycoformycin induced essentially no increase in the cerebral content of adenosine. In contrast, the concentration of 2'-deoxyadenosine increased from undetectable levels to amounts equalling the concentration of adenosine, i.e. about 1 nmol/g (Fig. 1B).

The treatment with deoxycoformycin had no effect on the amounts of noradrenaline, dopamine and serotonin in the mouse brain (Table 1).

DISCUSSION

The inhibition of adenosine deaminase in brain by deoxycoformycin *in vivo* (Fig. 1A) suggests that deoxycoformycin is distributed to the brain. This is in accordance with the finding that this agent passes into the CNS of both humans (Major *et al.*, 1981b) and monkeys (Blatt, Venner, Riccardi, Cohen, Gangji, Glazer and Poplack, 1982). The inhibition was not associated with a detectable increase in the amount of adenosine in the brain, indicating that adenosine deaminase does not play an essential role in the regulation of the adenosine content of the brain. A decrease in catabolism of adenosine may enhance the salvage of adenosine to adenosine monophosphate, catalyzed by adenosine kinase (Fox and Kelley, 1978). In addition, adenosine may be transported into the extracellular compartment. The present data do not provide information about the distribution of adenosine between the extra and intracellular compartment, nor on the regional distribution of adenosine. With this reservation, it is suggested that adenosine is not a mediator of the effects of deoxycoformycin on the CNS.

The determination of adenosine in the brain involved rapid freezing of the cerebral tissue through the intact skull. This procedure was used to avoid an artificial increase in the amount of adenosine, due to cerebral anoxia post mortem (Nordström *et al.*, 1977; Winn *et al.*, 1979; Wojcik and Neff, 1982). The present values for adenosine (about 1 nmol/g, Fig. 1B) equal those found by others taking similar precautions (Nordström, Rehnrona, Siesjö and Westerberg, 1977; Wojcik and Neff, 1982). When adenosine was measured in brains taken out immedi-

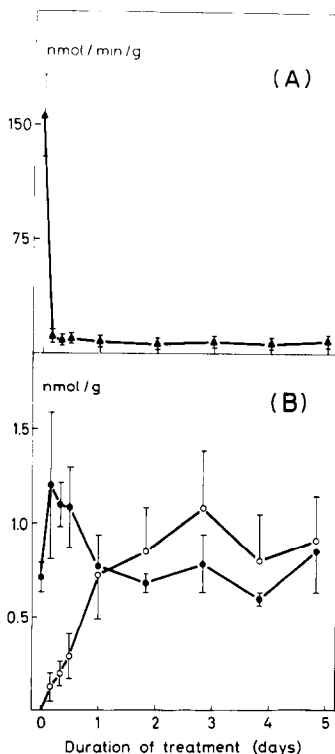


Fig. 1 (A and B) Adenosine deaminase activity (\blacktriangle — \blacktriangle), adenosine (\bullet — \bullet) and 2'-deoxyadenosine (\circ — \circ) in the mouse brain after continuous intraperitoneal infusion of deoxycoformycin. The values are means of four determinations \pm SEM.

ately after cervical dislocation, the values were in the range 50–300 nmol/g (unpublished results). These values reflect a post mortem increase, which is often overlooked (Volicer, Mirin and Gold, 1977; Gharib, Sarda, Chabannes, Cronenberger and Pacheco, 1982).

Although efforts were made to avoid any post mortem increase in the adenosine content of the brain, the possibility still exists that the low values obtained might be artificially elevated, and thereby obscure the effects of deoxycoformycin on the content of adenosine in brain. This should be considered in the light of the recent findings by Zetterström, Vernet, Ungerstedt, Tossman, Jonzon and Fredholm (1982) who reported elevated levels of adenosine in dialysates from intact rat brain following treatment of the animals with the adenosine deaminase inhibitor, erythro-9-(2-hydroxy-3-nonyl)adenine.

Deoxyadenosine, like adenosine, has a potent depressant action on neurones of the cerebral cortex (Fox and Kelley, 1978; Phillis *et al.*, 1979). Elevated concentration of 2'-deoxyadenosine in the brain of mice receiving deoxycoformycin by infusion points to a role for 2'-deoxyadenosine as mediator of the effects of deoxycoformycin in the CNS.

As no change was found in the amount of nor-adrenaline, dopamine and serotonin in the brain following treatment with deoxycoformycin there is no reason to suggest the involvement of these monoamines in the CNS depression.

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