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Neurotoxic action of β-N-oxalyl-L-α,β-diaminopropionic acid

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β-N-OXALYL-L-α,β-DIAMINOPROPIONIC ACID (OX-Dapro) is a recently characterized neurotoxic amino acid, found in the seeds of *Lathyrus sativus*, prolonged consumption of which has been associated with human "neurolathyrism", a crippling disease.[1, 2] The structural requirements for the neurotoxic action of OX-Dapro in day-old chicks has also been recently reported.[3] Curiously enough OX-Dapro does not induce any neurotoxic effects in normal adult animals such as rats or mice.[1] We now report of our studies with this neurotoxin which could provide a new approach towards the study of this crippling disease, since preliminary studies reported here show that the innocuous nature of OX-Dapro to adult animals could be due to an effective blood brain barrier (BBB) system. It has been found that under certain experimental conditions OX-Dapro does induce neurotoxic effects in adult animals, showing that the BBB is altered under such conditions.

While day-old chicks (35–45 g) manifest typical neurological symptoms upon intraperitoneal administration of OX-Dapro* (10–20 mg/chick), adult birds do not respond to the neurotoxin at similar dosages but require higher dosages of the same. Thus a 25-day-old bird (60–70 g) requires a dosage of 1 mg/g to manifest clearcut neurotoxic effects. Adult rats and mice (1–3 months old) do not show any neurotoxic effects upon intraperitoneal administration of OX-Dapro at the level of 1 mg/g. Similar failures were also observed with the adult monkey.

* OX-Dapro used in these studies, isolated as described previously[1] had a melting point of 206°.

At this juncture, Dr. J. C. Watkins *et al.*[4] communicated to us that OX-Dapro is a very potent excitant of spinal interneurones and cortical Betz cells in the cat when administered microelectrophoretically. They have speculated that the lack of action in adult animals may be due to the failure of the neurotoxin to penetrate the BBB.[5a,b] Intraperitoneal administration of OX-Dapro (5–8 mg) to young rats (3–14 days) produced within 5 min, a generalized convulsive seizure sometimes resulting in death, but 17–21-day-old rats did not respond to even higher dosages (15–20 mg). Neurotoxic symptoms such as ataxic gait, sudden jerking movements, dragging of the legs or a hopping movement, rigidity of the neck were also induced by intraperitoneal administration of OX-Dapro in a 12-day-old dog weighing 550 g (dose 100 mg) and in an 8-day-old guinea pig weighing 70 g (dose 25 mg).

Clonic flexor convulsions followed by extensor spasms and death was also observed in adult rats and mice immediately after intracranial and intraventricular administration respectively, of OX-Dapro (dose: 100–250 μg for 45–50 g rats and 25–50 μg for 20–25 g mice). At lower dosages, a gross hyperactivity resulted and the animals recovered within 5–15 min. Neurotoxic effects which eventually resulted in permanent, total areflexic flaccid paraplegia has also been observed in the monkey upon intrathecal administration of OX-Dapro.[6]

ACIDOSIS AND SUSCEPTIBILITY TO OX-DAPRO

Treatment of adult animals with acid-forming salts like calcium chloride or ammonium chloride or drugs like Diamox (acetazoleamide), sulphanilamide or salicylic acid, all of which are known to cause an "acidotic" state[7–11] was found to make them susceptible to the neurotoxic action of OX-Dapro upon intraperitoneal administration.

Adult cockerels (650–750 g) treated with 2 g each of calcium chloride or ammonium chloride orally for 4 days, came down with typical paralytic symptoms upon intraperitoneal administration of 100 mg of OX-Dapro. Control birds treated similarly receiving 100 mg of aspartic acid did not show any effects. An hour after the administration of OX-Dapro the birds in the former case developed inability to stand, a weaving gait, stretching of the neck, drop of the wings, a characteristic wry neck, and an extensor paralysis of the legs. Although many birds recovered from such effects after 24 hr, a few birds occasionally died from such effects after 48–72 hr.

Adult rats (100–120 g) and mice (30–35 g) fed *ad libitum* for 3 days a diet containing 0·5% Diamox were administered OX-Dapro (0·5 mg/g) intraperitoneally. An hour after the administration, the animals developed characteristic neurotoxic symptoms similar to those observed upon intracranial or intraventricular administration of OX-Dapro. The animals recovered from such effects after 8–10 hours. Neurotoxic effects were also noticed upon intraperitoneal administration of OX-Dapro to rats treated with sulphanilamide (2% in the diet for 3 days) ammonium chloride (2·5% for 4–5 days) and salicylic acid (3% for 3 days).

A male monkey (1·5 kg) given orally, 2 g calcium chloride a day for 4 days was administered 200 mg of OX-Dapro along with 200 mg of calcium chloride intraperitoneally. After 30 min the monkey developed a gross ataxia, frequent stupor, rigidity of the neck, a generalized weakness with an attitude of universal flexion. The symptoms lasted for 6–8 hr. In all the experiments described above appropriate control animals were always employed and no neurotoxic symptoms were noticed.

It is thus evident that irrespective of the species, OX-Dapro could be neurotoxic to all the animals studied. The failure of the neurotoxin to effect neurotoxic action in adult animals while young animals are more easily susceptible to its action, is possibly due to an effective BBB system in the adult animal. Adult animals treated with compounds known to induce an "acidotic" state are evidently susceptible to the action of the neurotoxin. While no experimental data exists to show that the BBB is altered during "acidosis" it is evident from the studies reported here that OX-Dapro has in all probability overcome the BBB under such conditions. Attempts are being made to synthesize the labelled compound to investigate this aspect further. While the increased permeability under "acidotic" conditions may not be the result of a chief event it is likely that the activities of various enzymes could be altered resulting in alterations in the levels of certain metabolites which is reflected in an increased permeability or in the observed neurological effects.

It is likely that the incidence of "neurolathyrism" is not widespread even though a large population consume *L. sativus* seeds in some parts of India due to an effective BBB system under normal conditions towards OX-Dapro, the chief neurotoxic constituent, present in the seeds. However, it could be easily conceived that the BBB system could be altered in humans also owing to a metabolic acidosis resulting from endocrine disorders, disturbances in food intake, impaired liver function etc.[12] or due

to several other pathological conditions. Under such altered conditions OX-Dapro could exert its central effects, eventually resulting in the observed clinical condition of "neurolathyrism".

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Effects of salicylate congeners on glucose metabolism in the human red cell

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SALICYLATE and 2:6-dihydroxybenzoate have been shown to inhibit glycolytic reactions and the pentose phosphate pathway in mature erythrocytes.[1] The present report shows that these actions are shared to a varying degree by a number of related mono- and dihydroxybenzoates. An exception is 2:5-dihydroxybenzoate which caused stimulation of the pentose phosphate pathway. The incorporation of radioactivity from ^{14}C-labelled glucose into soluble metabolic intermediates and into $^{14}CO_2$ in the human red cell suspensions and the effects of the salicylate congeners were studied by the techniques described previously.[1]

The results in Table 1 show the amounts of ^{14}C from [^{14}C] glucose incorporated into the separated soluble intermediates in the presence or in the absence of 5 mM and 20 mM concentrations of the congeners. All the congeners resembled salicylate and 2:6-dihydroxybenzoate in causing diminished utilization of the labelled substrate and an increased formation of labelled pyruvate. However, at the 5 mM level, 3-hydroxy-, 4-hydroxy- and 3:4-dihydroxybenzoates were the only compounds to cause