N-3-pyridylmethyl-N'-p-nitrophenylurea ocular toxicity in man and rabbits

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SUMMARY Ingestion of the rat poison N-3-pyridylmethyl-N'-p-nitrophenylurea (PNU) produced ocular toxicity in three humans and in an animal model, the Dutch Belted rabbit. The electroretinogram b wave was especially susceptible to the effects of the rodenticide, and the target tissue appeared to be the retinal pigment epithelium. Injection of PNU itself did not produce ocular toxicity. The poison had to be administered orally. Gentamicin administered orally with PNU prevented the ocular toxicity. Presumably this antibiotic killed those gastrointestinal bacteria responsible for PNU's metabolism into an ocular toxin. L-tryptophan, a known antidote for the lethal effects of PNU, was an antidote for the ocular toxicity when administered orally but not when administered parenterally.

N-3-pyridylmethyl-N'-p-nitrophenylurea (Vacor, PNU) is a rat poison that attacks peripheral nerves and the β cells of the pancreas.^{1,2} It seems to have a low toxicity for pets and primates. While the LD₅₀ for rats was 12 mg/kg, those for dogs and rhesus monkeys were more than 500 mg/kg and 2000 mg/kg respectively. Unfortunately PNU was found to be much more toxic in humans than expected; the LD₅₀ for man has been estimated at 5 to 10 mg/kg. The minimum lethal total dose reported in an adult male was between 390 and 780 m.³ The nucleotide precursors, L-tryptophan and nicotinamide, were found to be effective antidotes in rats;⁴ nicotinamide became the recommended treatment of human poisoning.⁵

Diabetes mellitus was a common finding in human survivors. β Cell function tended to return to a variable degree two to three years after ingesting the poison.⁶ PNU also produced human peripheral neuropathy and encephalopathy.⁷ Orthostatic hypotension tended to be the most persistent and troublesome abnormality, though full recovery has been reported.⁸

In 1981 Mindel and coworkers⁹ reported on the ocular findings of a 20-year-old female who had ingested PNU in a suicide attempt 30 months previously. She ultimately developed many of the features of retinitis pigmentosa, namely, atrophic optic nerve heads, attenuated retinal vessels, extinguished electroretinograms (ERGs), and retinal pigment clumping. The role of PNU in producing this constellation of findings was not clear because the patient had received large doses of ergotamine tartrate, up to 6 mg a day for nearly one month, to correct her orthostatic hypotension. Ergotamine tartrate overdose has been associated with papillitis but not with retinopathy.¹⁰ The present study provides evidence that PNU is toxic to the human eye. In addition an animal model of PNU's ocular toxicity was developed.

Material and methods

HUMAN STUDIES

Three survivors of PNU (Vacor) ingestion were examined. One was female and two were male.

Flash ERGs were recorded under four stimulus conditions: (1) Photopic: cone responses to a 10 μ s strong white flash (Grass photostimulator S₈ white)

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against a 438 cd/m² background light (normal amplitude >94 µV). (2) Flicker: cone responses to a brief exposure of 30 per second strong flashes (normal b wave implicit time <33 ms). (3) Scotopic: an estimate of rod function was obtained using a weak white flash (S₁ white) after 20 minutes of dark adaptation (normal amplitude >194 µV and normal scotopic b wave implicit time <63 ms). (4) Combined rod and cone responses to a strong flash after 20 minutes of dark adaptation (normal amplitude >289 µV). Measurements were made of b wave amplitude (the a wave trough to the b wave peak) and of flicker b wave implicit times (stimulus onset to b wave peak).

RABBIT STUDIES

Adult Dutch Belted (pigmented) rabbits were used exclusively.

Drug administration. N-3-pyridylmethyl-N'-p-nitrophenylurea (PNU) was not soluble in water but could be dissolved 300 mg PNU per ml dimethylsulphoxide (DMSO). Multiple doses were each separated by a one-week interval.

An oral dose of PNU, 125 mg/kg body weight, was arbitrarily chosen and was administered by an oral-gastric tube after pentobarbital anaesthesia. Three oral doses were given to each of three rabbits.

PNU was administered by injection to six rabbits. One received intramuscular injections of 125 mg/kg, 250 mg/kg, and 875 mg/kg, that is, a total of 1250 mg/kg. A second rabbit received a single intramuscular injection of 675 mg/kg. The third rabbit received two retrobulbar injections, left eye, of 75 mg/kg, that is, a total of 150 mg/kg. Two additional rabbits received single intraperitoneal injections of PNU, 125 mg/kg. The sixth rabbit received an injection of PNU, 20 mg/kg, in the left carotid artery.

L-tryptophan and gentamicin were evaluated as prophylactic agents. L-tryptophan, 0.459 mM/kg, i.e., equimolar to PNU, 125 mg/kg, was administered intravenously 10 minutes before and 1 hour, 3 hours, and 6 hours after an oral dose of PNU, 125 mg/kg to four rabbits. L-tryptophan or gentamicin sulphate was administered orally three times to four rabbits as 1 g suspensions in 10 ml water. Administrations were 4 hours before, 1 hour before, and 4 hours after an oral dose of PNU, 125 mg/kg.

Electroretinograms were obtained prior to each PNU administration and at one week intervals after PNU administration. Rabbit pupils were dilated with tropicamide 1% and phenylephrine 2.5% eye drops. The animals were anaesthetised with pentobarbital or xylazine; ketamine was sometimes injected to supplement anaesthesia. Proparacaine hydrochloride 0.5% provided corneal anaesthesia for an 'ERG-JET' corneal contact lens electrode (Nicolet

Instrument Corp.). After photopic responses were recorded, the rabbit was dark adapted for 30 minutes and scotopic responses to white and blue light were obtained.

Histological examinations were performed one week after the last administration of PNU. Once the ERGs were recorded the animal was killed with intravenous pentobarbital. The eyes were fixed in glutaraldehyde. One eye of each rabbit was examined by means of light and electron photomicrographs of the retina and pigment epithelium.

Case histories and results

HUMAN STUDIES

Patient 1 was 18 years old when she attempted suicide by ingesting 780 mg of PNU. She acutely developed severe gastrointestinal haemorrhage and an infarct of the right occipital lobe. She survived but became an insulin-requiring diabetic with severe orthostatic hypotension. Eighteen months later she had her first ophthalmic examination. Her visual acuity was 6/6 in both eyes and her retinal vessels and retinal pigmentation were within normal limits. No electrophysiological studies were performed at that time. Subsequently she was put on ergotamine tartrate in increasing doses in an attempt to control her orthostatic hypotension. At a daily dose of 6 mg a day of ergotamine tartrate the patient's symptoms were controlled (blood pressure 130/70 mmHg supine and 120/75 mmHg standing). However, after three weeks of being maintained at this dosage she complained of severe head pain and mildly blurred vision. Ergotamine tartrate was discontinued. Three weeks later, and approximately eight months after her first ophthalmic examination, she was again examined.

Her visual acuity remained 6/6 in each eye, but the arterioles and venules of the retinas were now thread-like. Fluorescein angiography showed decreased vascularity of the optic disc and a tessellated pigmented epithelium. There was no abnormal retinal pigment clumping. Electroretinograms were extinguished bilaterally. Retinal thresholds after dark adaptation were markedly elevated in the peripheral retina but only moderately in the posterior pole. The findings on this point were reported in greater detail in 1981.⁹ At that time it was assumed that the cause of the retinal changes between the two eye examinations was the ergotamine tartrate. During the next three years she developed a progressive pigment retinopathy (Fig. 1). Mild opacifications and pigment accumulations were seen on the posterior lens capsules (Fig. 2). Her visual acuities were reduced to 6/7.5 bilaterally.

To help clarify the role of PNU in producing this progressive retinopathy, other survivors of Vacor

Fig. 1 Patient 1, fundus photograph 64 months after PNU ingestion. Pigmentary retinopathy is present.

ingestion were examined. Patient 2 was a 36-year-old white male who had ingested 3.7 g of PNU three years previously. He became an insulin-requiring diabetic. Severe orthostatic hypotension and peripheral neuropathy persisted for 10 months, but then these gradually cleared. He never received ergotamine tartrate. Patient 2 agreed to have electroretinography performed and to have his pupils

Fig. 2 Patient 1, slit-lamp photograph of left eye, posterior capsule lens opacification present 64 months after PNU ingestion. The visual acuity was 6/7.5.

pharmacologically dilated for this test. But he refused to co-operate for an ophthalmoscopic examination or to have a dark adaptation examination. The electroretinograms were abnormal in both eyes (Fig. 3). The scotopic response to the weak stimulus was subnormal in amplitude and delayed in implicit time (80 to 85 ms). The scotopic response to the strong stimulus consisted only of a decreased amplitude a wave with a longer than normal latency. Under photopic conditions, the amplitude of the b wave to the flicker stimulus was about half the size of the normal recording, with a slight delay in the implicit time (34 ms).

Patient 3 was a 32-year-old white male who had ingested 1.5 g of PNU six years previously. A more complete description of his case history has been reported.^{6,11} He was an insulin-requiring diabetic for the first two years after his acute poisoning, but sufficient islet cell function returned thereafter to permit control of the diabetes by diet alone. His toxic peripheral neuropathy, consisting of severe orthostatic hypotension and pain, also resolved after one year. He never received ergotamine tartrate. Patient 3 permitted an ophthalmoscopic examination. Although there was no optic atrophy, narrowing of vessels, or pigment clumping, there were large areas in the peripheral retina with apparent loss of normal pigmentation. The electroretinograms were bilaterally abnormal (Fig. 3). The scotopic b wave response to the weaker stimulus was subnormal in amplitude; the implicit time was difficult to measure. The scotopic b wave response to the brighter stimulus consisted solely of an a wave of normal amplitude. The photopic b waves evoked by a 30 Hz flickering light were within normal limits in amplitude and implicit times.

RABBIT STUDIES

Oral PNU, 125 mg/kg, resulted in loss of the rod portion of the b wave. Administration of two additional doses had little effect on the a wave or on the cone portion of the b wave (Fig. 4). Electron microscopic examination of eyes removed one week after the third oral dose of PNU revealed marked pathology of the retinal pigment epithelium (Figs. 5A–D). There was destructive vacuolisation with loss of phagosomes, nuclei, and cytoplasmic organelles and with a tendency to form large fracture areas. The visual retina was minimally involved. The ganglion cells and inner portions of Müller cells appeared normal. Photoreceptor inner segments had a mild increase in the number of vacuoles, and there was some dropout of photoreceptor outer segments. These photoreceptor abnormalities were considered to be secondary to the almost total destruction of the retinal pigment epithelium (RPE). Behaviourally the

Fig. 3 Electoretinograms of patient 2 and patient 3 compared with that of a normal 20-year-old female. The amplitudes of the scotopic responses of both patients were markedly reduced. The scotopic and photopic implicit times of patient 2 were abnormally prolonged. Strong = S_{g} white; weak = S_{1} white.

rabbits did not appear to suffer any visual impairment.

Although oral administration of PNU caused impaired ERG responses and histological evidence of destruction of the RPE, intramuscular, intracarotid artery, retrobulbar, and intraperitoneal injections were without effect. Neither oral nor parenteral PNU produced diabetes mellitus in rabbits.

Multiple intravenous doses of L-tryptophan given

within 6 hours of a single oral dose of PNU failed to prevent PNU toxicity. The ERG scotopic responses to blue and white light had been reduced by mean amounts of 64% and 36%, respectively, one week after a single administration of PNU without L-tryptophan. Administration of PNU with intravenous L-tryptophan was associated with corresponding reductions of 58% and 67%. Light and electron photomicrographs showed RPE degeneration. However, oral L-tryptophan and gentamicin

Fig. 4 Electoretinogram responses of a pigmented rabbit before PNU oral administration and one week after the first and third doses of 125 mg/kg/week. The first dose reduced the rod component. After the third dose essentially no rod responses remained. The animal was dark adapted for only 10 minutes because of difficulty maintaining prolonged anaesthesia.

Fig. 5A

Fig. 5B

Fig. 5 Photomicrographs of rabbit retinas comparing (5A, C) normal tissue with that (5B, D) obtained from the rabbit whose ERGs are shown in Fig. 4. The eyes of the latter were removed one week after the third oral dose of PNU, 125 mg/kg. The inner retinas of both appear normal (5A, B, $\times 330$). However, the pigment epithelium of the rabbit fed PNU is disrupted. Electron microscopy (5C, D, $\times 2600$) demonstrates that the pigment epithelium of the PNU-fed rabbit has absence of phagosomes, nuclei, and cytoplasmic organelles and large fracture areas. Bar is $2.9\ \mu\text{m}$.

sulphate prevented ERG and histological changes from oral PNU.

Discussion

The initial goal of these studies was to determine whether or not PNU was the probable cause of the progressive pigment retinopathy that developed in patient 1. Among the alternative possibilities were that (1) the large doses of ergotamine tartrate used to

Fig. 5C

Fig. 5D

treat the orthostatic hypotension had damaged the RPE; (2) PNU and ergotamine tartrate toxicities were additive; and (3) the patient had hereditary retinitis pigmentosa. Similar large doses of ergotamine had been used to treat orthostatic hypotension and had not been associated with ocular toxicity.^{12 13} Ergotamine is less toxic than ergot, and the two reports attributing eye damage to ergotamine ingestion described a papillitis,^{11 14} not pigment retinopathy. Nor has ergotamine tartrate overdose caused ERG abnormalities.¹⁵ On the other hand many cases of PNU poisoning have occurred without any mention being made of ocular abnormalities. Perhaps the eyes had not been adequately examined.

To investigate this possibility, two other PNU survivors were found who had not been treated with ergotamine. Finding subjects willing to participate was difficult. First, most adult PNU ingestions were suicide attempts, and the survivors were reluctant to be reminded of episodes that now embarrassed them. Secondly, survivors who wanted to commit suicide learned quickly that an overdose of the insulin needed to control their PNU-induced diabetes mellitus was an efficient mode of self destruction. The two survivors consenting to have ERG studies had extinguished scotopic b wave responses and mild changes in their a waves. This selectivity for the b wave was also demonstrated in pigmented rabbits fed small doses of PNU. There seemed to be reasonable evidence to conclude that PNU was an ocular toxin. It should be emphasised that it could not be stated that all, or even most, of patients 1's abnormal electrophysiological responses and anatomical defects were the direct result of PNU ingestion. Ischaemic hypoxia from her gastrointestinal bleeding, and a possible contributing effect from ergotamine, confounded any attempts to assign causality.

Patient 1 had more severe ocular damage, orthostatic hypotension, and diabetes mellitus than the other two patients despite her ingested dose of PNU having been less, that is, 780 mg (patient 1) versus 3.7 gm (patient 2) and 1.5 gm (patient 3). However, patient 1 did not have emesis or develop diarrhoea after ingesting the poison, while the other two did. She waited 48 hours before going to a hospital emergency room. Presumably her absorbed dose of toxin was larger than those of patients 2 and 3.

PNU appeared to alter the human electroretinogram by preferentially extinguishing the b wave, and in patients 1 and 3 seemed to damage the retinal pigment epithelium. An animal model was needed that would demonstrate similar effects. Rodents were not tested, because they were extremely sensitive to the lethal effects of PNU. Rabbits are lagomorphs and phylogenetically closely related to rodents. Dutch Belted rabbits were chosen because their

retinal pigment epithelia were pigmented as was man's. Rabbit eyes are relatively large, allowing the ERG electrodes and equipment designed for use on patients to be easily adapted. When Dutch Belted rabbits were fed PNU, they developed both a selective reduction in the ERG b wave and histological evidence of selective damage to the RPE. This damage to the RPE did not result in an ophthalmoscopically identifiable pigment retinopathy, presumably because the experiments were too short, that is, the rabbits were maintained for no more than three weeks after receiving PNU, while patients 1 and 3 developed pigment retinopathy more than four years after ingesting the poison. Behaviourally, the rabbits' vision did not appear impaired. At the dose of PNU used Dutch Belt rabbits also had a fortuitous absence of diabetes mellitus and peripheral neuropathy. Thus they could be easily maintained.

Oral administration of PNU to rabbits had the potential disadvantage of variable drug absorption from the gastrointestinal tract. More uniform blood and tissue levels should result from parenteral administration. However, intramuscular, retrobulbar, intracarotid artery, and intraperitoneal injections of PNU were without effect. As only oral PNU was toxic, it appeared that the drug had to be metabolised in the gastrointestinal tract to become active, presumably by the bacterial flora. Consistent with this theory was the prophylactic effect of oral gentamicin, a Gram-negative bactericidal antibiotic.

L-Tryptophan was also tested as an antidote. Tryptophan is the amino acid precursor of nicotinamide, a proved antidote of PNU's lethal toxicity in rats.⁴ Parenteral L-tryptophan did not prevent ocular toxicity from oral PNU. However, oral L-tryptophan did. This suggested that tryptophan was ineffective once the toxic metabolite was absorbed. Oral tryptophan may have competed for the bacterial enzymes that converted PNU or competed for an uptake system responsible for the absorption of the toxic metabolite.

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