Pattern of neurobehavioral and organ-specific toxicities of β, β'-iminodipropionitrile in mice

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Abstract

Introduction: β, β'-iminodipropionitrile (IDPN) is a synthetic nitrile that produces a permanent movement disorder in rodents. Although IDPN-induced vestibular pathology is well documented, the mode of IDPN interaction with other organ systems is poorly understood. We examined the behavioral signs and histopathological changes in the vestibular labyrinth, brain, liver and kidneys of mice exposed to IDPN.

Material and methods: Adult male SWR/J mice were divided into 2 groups of 6 animals each. One group of mice received normal saline (control group) and the other group was treated with IDPN (400 mg/kg, *i.p.*) daily for 7 days. Dyskinetic movements including vertical and horizontal head weaving, circling and backward walking were quantified on days 7, 8 and 9.

Results: We observed a direct correlation between the severity of IDPN-induced behavioral deficits and the degeneration of vestibular hair cells in the crista ampullaris of mice. The brain cortex of both groups appeared similar, whereas the kidney histopathology revealed mild nephrotoxicity in some of the IDPN-treated mice. Administration of IDPN caused severe hepatotoxicity, but the intensity of hepatic damage was not correlated with the severity of behavioral deficits.

Conclusions: Degeneration of vestibular sensory hair cells plays an important role in the development of IDPN-induced behavioral deficits in mice. Exposure to IDPN also caused severe hepatotoxicity which was independent of the behavioral symptoms. These findings could be of potential relevance to human health, particularly after the observation that IDPN not only causes a movement disorder but also produces acute liver injury.

Key words: iminodipropionitrile, behavior, toxicity, crista ampullaris, brain, liver, kidney, mice.

Introduction

Occupational and environmental exposure to synthetic nitriles is of potential relevance to human health. β, β'-iminodipropionitrile (IDPN) is a synthetic nitrile that produces an irreversible behavioral syndrome in rodents, designated as ECC syndrome (excitation with choreiform and circling movements), and characterized by repetitive head movements, retropulsion, circling, hyperactivity, and swimming deficits [1, 2]. It has been observed that not only IDPN but also several other nitriles of industrial application such as crotononitrile, allylnitrile and acrylonitrile are able to produce motor deficits in experimental animals [3, 4]. However, within the nitrile compounds, IDPN, allylnitrile, and cis-crotononitrile produce the ECC

syndrome in rodents, whereas trans-crotononitrile and hexadienenitrile induce a different kind of syndrome, characterized by faltering movements [5]. Thus, nitriles cause two distinct types of motor syndromes through either vestibular hair cell degeneration or neuronal degeneration [5, 6]. Moreover, exposure to IDPN has also been shown to cause olfactory [7], ocular [8], developmental [9] and reproductive [10] toxicities in rats. However, the effects of IDPN on two important vital organs, liver and kidney, have not been evaluated.

In this study, we examined IDPN-induced behavioral deficits in mice and observed the histological changes in the crista ampullaris, brain, liver and kidneys of mice treated with IDPN as compared to normal tissues.

Material and methods

Animals and treatment

Adult male SWR/J mice weighing 30–35 g were used in this study. The animals were housed in polycarbonate cages with sawdust bedding, kept in a temperature-controlled room and maintained on 12-h light/dark cycles. Standard laboratory food and tap water were freely available to the animals throughout the study. The animals were divided into 2 groups of 6 animals each. Control mice received normal saline whereas the IDPN group was treated with IDPN (400 mg/kg, *i.p.*) daily for 7 days. The animals were sacrificed on day 9 and samples of the cochlea, brain, liver and kidney were collected for histopathology. The experimental protocol was approved by our Institutional Ethics Committee.

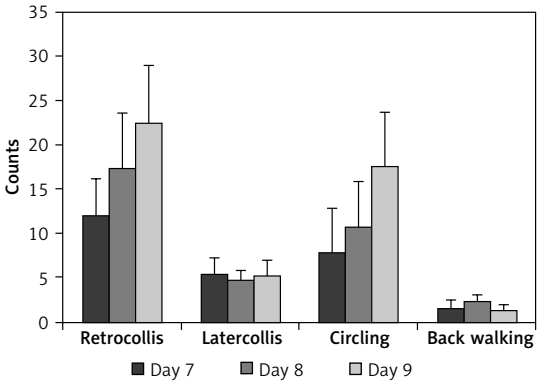

Figure 1. Time-course behavioral signs in IDPN-treated mice

Values are means of 6 animals ± SEM.

Behavioral analysis

All the animals were carefully observed for any behavioral abnormality before the daily administration of IDPN. The animals were placed individually in an observation chamber (50 cm × 50 cm) and were observed for dyskinetic movements including vertical (retrocollis) and horizontal (laterocollis) head weaving, circling and backward walking for a period of 2 min, as described earlier [11, 12].

Histopathology

The animals were subjected to cardiac perfusion with saline followed by 2.5% glutaraldehyde buffered with 0.2 M phosphate buffer solution (pH 7.4) under ethyl ether anesthesia. The temporal bones were quickly removed and postfixed in 10% neutral buffered formalin for 15 h. The bony labyrinth was decalcified by placing it in the decalcifying agent Cal-Ex (Fisher Scientific, USA) for 48 h. The decalcified specimens were processed overnight for dehydration, clearing and impregnation using an automatic tissue processor (Sakura,

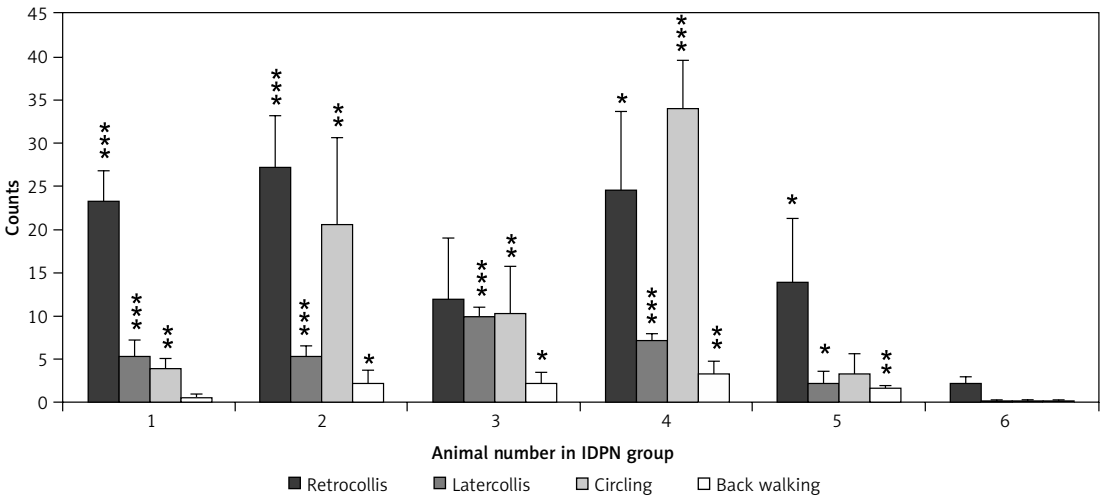

Figure 2. Intensities of behavioral deficits in individual animals of IDPN-treated group

*Values are means of 3 days ± SEM. *p < 0.05, **p < 0.01 and ***p < 0.001 versus animal no. 6 (animal with least symptoms).*

Japan). The specimens were embedded in paraffin blocks using an embedding station (Sakura, Japan) and serial sections of 5 μm thickness were cut using a microtome (Leica-RM2245, Germany) and stained with 1% toluidine blue for light microscopy observations. Other organs including brain, liver and kidney were fixed with 10% neutral buffered formalin for at least one day. Tissue processing and embedding were performed as above, whereas the thickness of specimens was set at

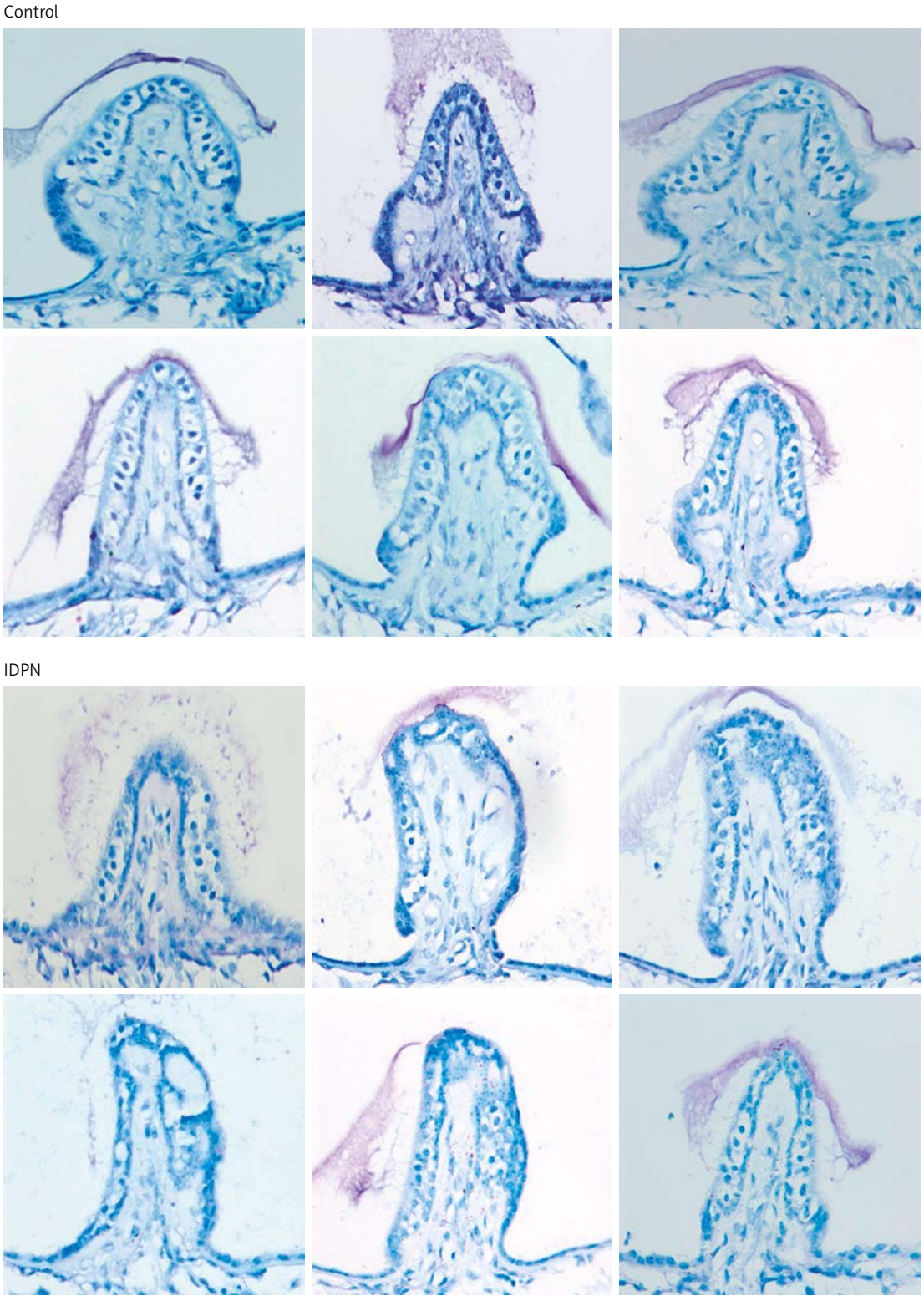

Figure 3. Light microscopic observation of crista ampullaris from control and IDPN-treated mice. Individual images correspond to individual animals in respective groups. Magnification 400×

4 µm and an autostainer (Leica 5020, Germany) was used for hematoxylin and eosin staining.

Statistical analysis

The mean values between control and treated groups were compared by using independent samples *t*-test with the help of the SPSS package. Values of $p < 0.05$ were considered as statistically significant.

Results and discussion

The onset of IDPN-induced behavioral deficits was on day 7 (Figure 1). The intensities of retrocollis and circling behaviors increased with time, whereas laterocollis and back walking did not show a time-course increasing trend in their intensities. Animal no. 4 showed the highest severity score followed by animals 2, 1, 3 and 5, whereas animal no. 6 had mild behavioral deficits in the form of retrocollis only (Figure 2). The results of histopathology of the vestibular organ showed that IDPN exposure caused degeneration of vestibular sensory hair cells in the crista ampullaris, whereas the crista of control mice showed normal sensory epithelium with intact hair bundles (Figure 3). Our findings are in agreement with previous reports [13–16] suggesting a close association between IDPN-induced neurobehavioral toxicity and degenerative changes in the crista ampullaris, including cytoplasmic vacuolation, detachment of hair cell-nerve terminal contacts, and loss of synaptic densification. Seoane *et al.* [17] compared the mode of hair cell degeneration in rats exposed

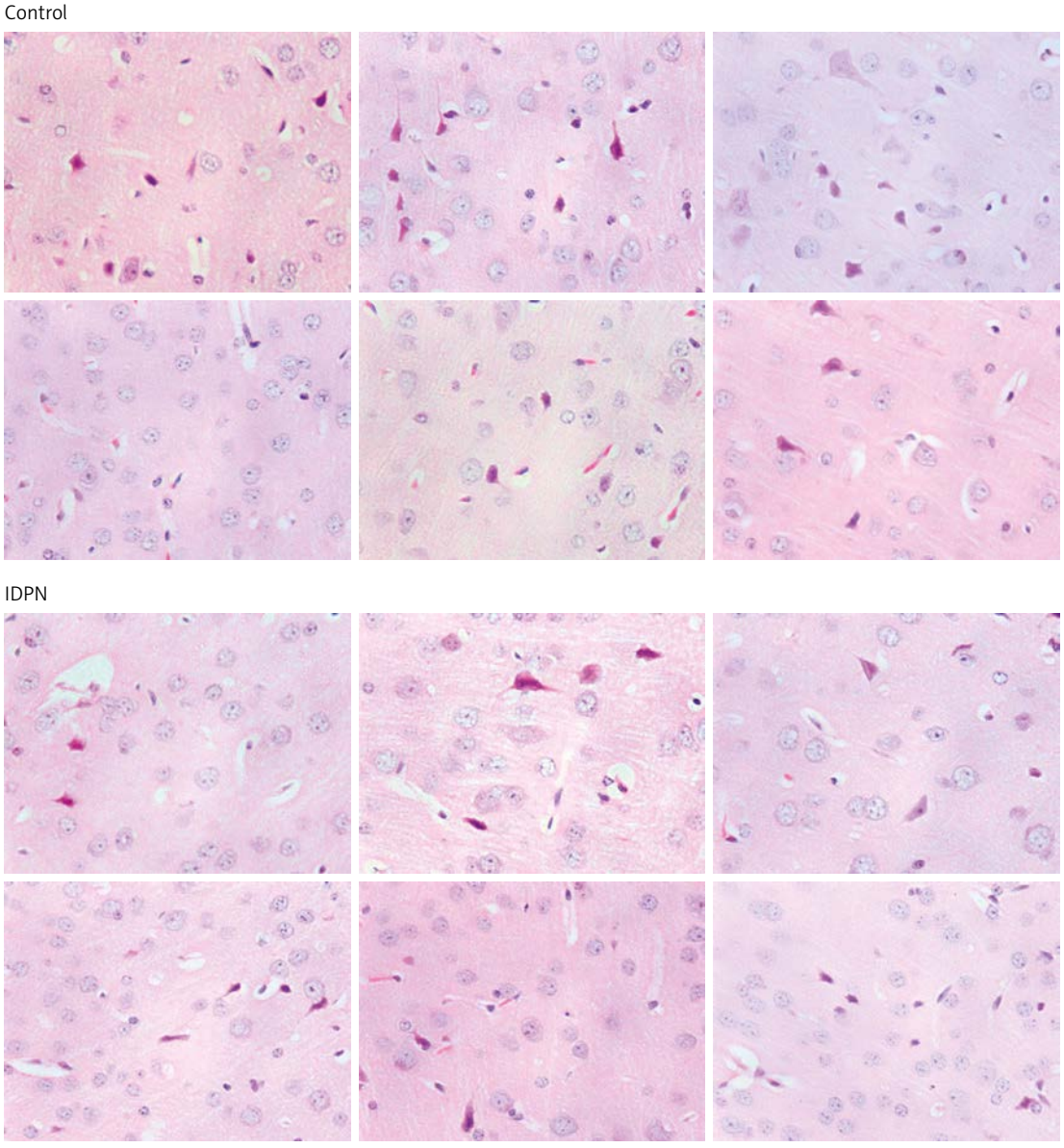

Figure 4. Light microscopic observation of brain cortex from control and IDPN-treated mice. Individual images correspond to individual animals in respective groups. Magnification 400×

to acute and sub-chronic dosages of IDPN and concluded that necrosis was most evident when the intensity was at its highest (acute exposure), whereas extrusion predominated when the intensity was at the lowest end of the scale (sub-chronic exposure).

Animal no. 4 with the highest severity score of behavioral deficits showed almost complete loss of hair cells in the sensory epithelium with no hair bundles seen (Figure 3). Animals 1, 2 and 3 (with moderate behavioral deficits) showed mild degeneration of hair cells and partial detachment of hair bundles. The sensory epithelia of animal no. 6 (with mild behavioral deficits) showed little degeneration of hair cells with intact hair bundles (Figure 3). These findings indicate a direct correlation between the severity of behavioral deficits and the cellular damage in the crista ampullaris of IDPN-treated mice. Khan *et al.* [18] also reported a direct association between the severity of IDPN-induced behavioral signs and the extent of vestibular hair cell degeneration, after administering graded doses of IDPN in rats of different age groups. Moreover, drugs that alleviated IDPN-induced behavioral deficits also reduced vestibular hair cell degeneration [19, 20], whereas the toxic interaction of drugs with IDPN synergistically aggravated both behavioral and vestibular toxicities [11, 15, 16, 21].

The results of brain histopathology did not reveal any prominent changes in the brain cortex of mice treated with IDPN as compared to controls (Figure 4). Several biochemical studies have shown that IDPN produces significant alterations

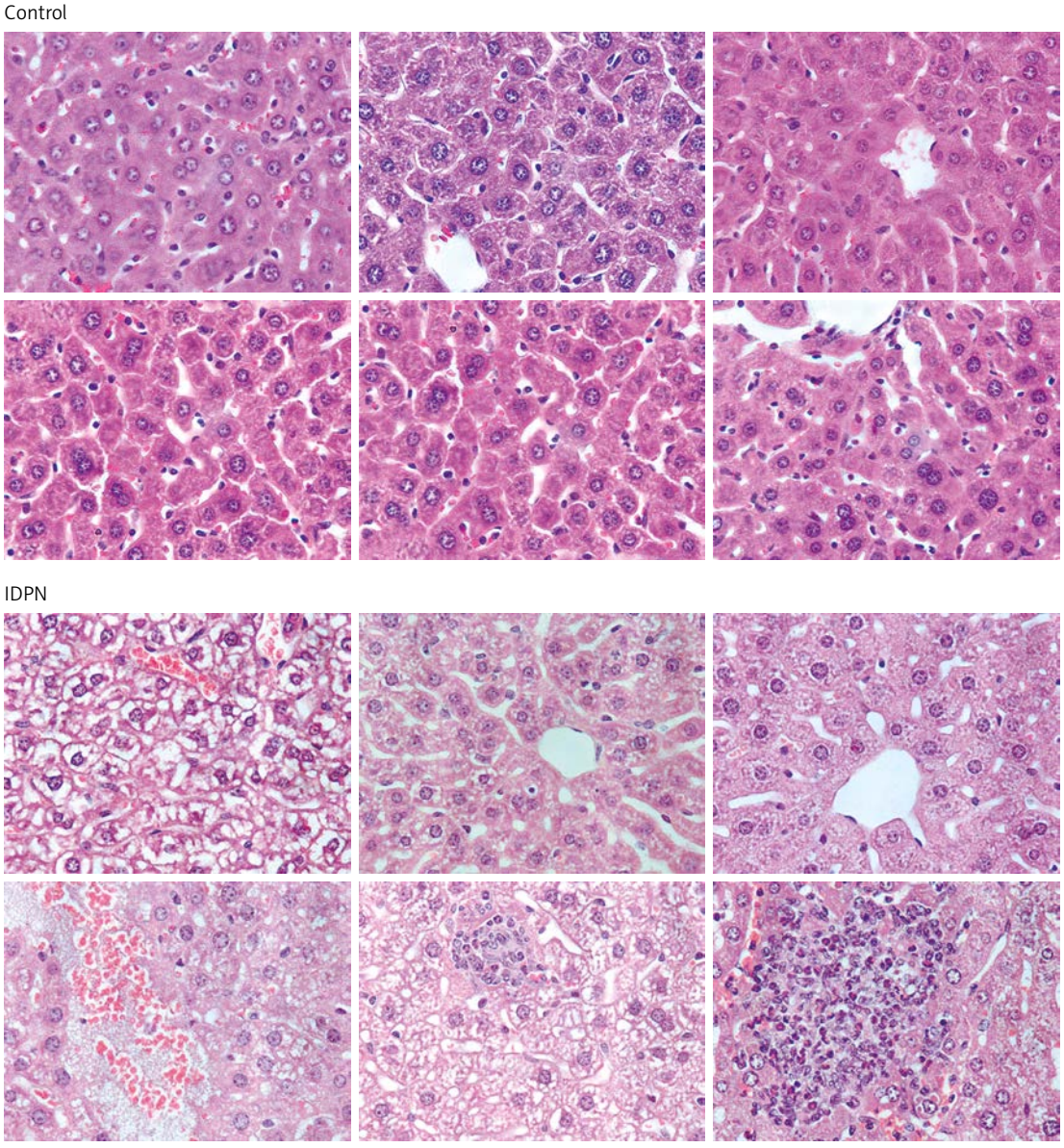

Figure 5. Light microscopic observation of liver sections from control and IDPN-treated mice. Individual images correspond to individual animals in respective groups. Magnification 400×

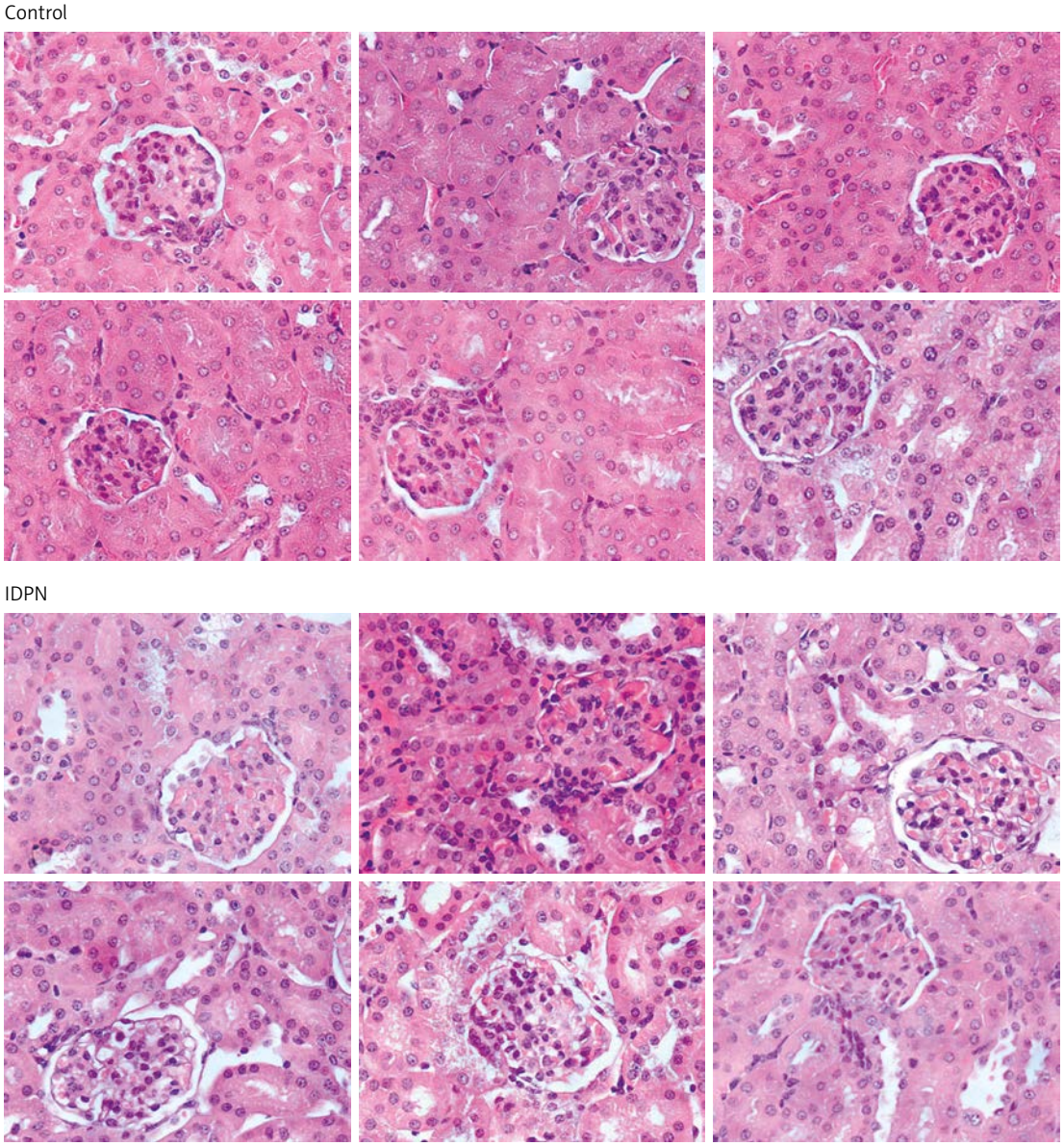

Figure 6. Light microscopic observation of kidney sections from control and IDPN-treated mice. Individual images correspond to individual animals in respective groups. Magnification 400×

in the brain neurotransmitters including dopamine [22], serotonin [23–25] and norepinephrine [26]. IDPN caused time- and dose-dependent increases in glial fibrillary acidic protein in the pons-medulla, midbrain, cerebral cortex and olfactory bulbs of rats; of these areas, the cortex and olfactory bulbs showed the highest effects [27]. Exposure to IDPN increased the expression of frontal cortical and thalamic vasoactive intestinal peptide, and striatal dynorphin, enkephalin and substance P [28]. Several studies have also reported significant alterations in the indices of oxidative stress and lipid peroxidation in brain of IDPN-treated rats [29–33]. The findings of the above studies indicate that IDPN produces significant biochemical and molecular alterations in the brain, but the neuronal morphology is not affected to such an extent as to be determined by light microscopy.

The results of liver histopathology showed severe hepatotoxicity in IDPN-treated mice (Figure 5). The prominent signs of hepatic damage were vacuolization of cytoplasm, distorted sinusoids, infiltration of mononuclear cells and necrosis. The severity of hepatic damage in IDPN-treated mice (Figure 5) was independent of the magnitude of vestibular hair cell degeneration in respective animals (Figure 3). It was reported previously that pretreatment with hepatotoxic dosages of carbon tetrachloride significantly increased the toxicity of IDPN, suggesting that hepatic transformation of IDPN to a toxic metabolite [34] may not be required for the manifestation of IDPN-induced neurotoxicity, but

instead may be involved in the detoxification of this compound [35]. Structural changes in rat liver have also been observed following subchronic exposure of IDPN [36]. Histopathological examination of kidney sections showed mild nephrotoxicity in animals 3, 4 and 5, in the form of mild tubular dilatation and vacuolation in glomeruli (Figure 6). A previous biochemical study in rats did not find any difference in blood urea nitrogen and serum creatinine levels between control and IDPN treated groups, suggesting that vestibulotoxic doses of IDPN do not impair the renal function of rats [15].

In conclusion, the severity of IDPN-induced behavioral deficits in mice is directly correlated with the degeneration of vestibular sensory hair cells in the crista ampullaris. Exposure to IDPN also caused severe hepatotoxicity in mice, but the extent of hepatic damage was not correlated either with the vestibular hair cell degeneration or with the intensity of behavioral deficits. Although this study was conducted in mice, the findings could be of potential relevance to human health, particularly after the observation that IDPN not only causes movement disorder but also produces acute liver injury. We recommend that environmental or occupational exposure to synthetic nitriles should be carefully monitored and that subjects at risk be evaluated not only for neurological symptoms but also with the liver function test.

Acknowledgments

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Conflict of interest

The authors declare no conflict of interest.

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