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Short Communication

Histological insights in iminodipropionitrile-induced toxicity in rats

Khalid Elfakki Ibrahim[a], Haseeb Ahmad Khan[b,*], Fayza Ahmad Omer[c]

[a] Department of Zoology, College of Science, King Saud University, Riyadh, Saudi Arabia
[b] Department of Biochemistry, College of Science, King Saud University, Riyadh, Saudi Arabia
[c] Diagnosis Center, Veterinary Research Institute, Animal Resources Research Corporation, Khartoum, Sudan

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ABSTRACT

Iminodipropionitrile (IDPN) is a prototype nitrile compound that produces excitation, chorea and circling (ECC) syndrome in rodents. Previous studies have implicated vestibular hair cell degeneration in IDPN-induced behavioral abnormalities. Although the pathological changes in vestibular labyrinth of IDPN-treated rats are well documented, the effects of IDPN on other organ systems are not clearly understood. We therefore examined the histopathological alterations in inner ear, brain, liver and kidneys of rats exposed to IDPN. Adult male Wistar rats were divided into two groups of six animals each. Control rats received normal saline whereas the IDPN group was treated with IDPN (100 mg/kg, i.p.) daily for 7 days. All the animals were carefully observed for any behavioral abnormality and the dyskinetic movements including the vertical and horizontal head weaving, circling and backward walking were quantified. The animals were sacrificed on day 9 and the samples of cochlea, brain, liver and kidney were collected for histopathology. The results showed a direct correlation between the severity of behavioral deficits and the cellular damage in crista ampullaris in IDPN-treated rats. Histopathology of liver was severely influenced by IDPN treatment, leading to vacuolization of cytoplasm, distorted sinusoids, infiltration of mononuclear cells and necrotic zones. However, the severity of hepatic damage in IDPN-treated rats was independent of the magnitude of vestibular hair cell degeneration as well as the severity of behavioral deficits. Administration of IDPN in the vestibulotoxic doses did not produce any histological changes in the brain cortex and kidneys of rats.

1. Introduction

The dyskinetic syndrome in rodents exposed to iminodipropionitrile (IDPN) was initially reported by Delay and coworkers (1952). This irreversible behavioral syndrome in rodents is designated as ECC syndrome (excitation with choreiform and circling movements), and characterized by repetitive head movements, retropulsion, circling, hyperactivity, and swimming deficits (Delay et al., 1952). Llorens et al. (1993) suggested that administration of IDPN induces vestibular hair cell loss, which is mainly responsible for the gross changes in behavior associated with IDPN exposure. Subsequent studies noticed that other nitrile compounds of industrial importance including allylnitrile, crotonitrile and acrylonitrile also cause similar behavioral deficits (Tanii et al., 1991; Gagnaire et al., 1998). However, rats treated with IDPN, allylnitrile, and *cis*-crotononitrile developed the ECC syndrome, whereas those treated with *trans*-crotononitrile and hexadienenitrile exhibited a different syndrome, characterized by faltering movements (Boadas-Vaello et al., 2005). The findings indicate that different nitriles cause two distinct types of motor syndromes through either vestibular hair cell degeneration or neuronal degeneration (Boadas-Vaello et al., 2005; Khan et al., 2009).

The ease in quantification of IDPN-induced behavioral symptoms as well as the experimental reproducibility rendered this prototype nitrile as one of the most suitable compounds for validation of functional observational battery and motor deficits for screening of neurotoxic drugs (Delay et al., 1952). IDPN has also been recommended by the U.S. Environmental Protection Agency (1991) as a positive control for these testing guidelines (Crofton et al., 2008). IDPN-induced toxicity is not limited to vestibular and nervous systems but this compound also causes olfactory (Genter et al., 1992), ocular (Seoane et al., 1999) and reproductive (Takahashi et al., 2012) toxicities in rats. However, the toxicological effects of IDPN on other vital organs including liver and kidneys remained unknown and were evaluated in this study. We performed histopathological evaluation of crista ampullaris, brain, liver and kidneys of rats exposed to vestibulotoxic dose of IDPN as compared to normal tissues.

* Corresponding author at: Department of Biochemistry, College of Science, Bld 5, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia.
E-mail addresses: khan_haseeb@yahoo.com, haseeb@ksu.edu.sa (H.A. Khan).

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2. Materials and methods

2.1. Animals and treatment

Adult male Wistar rats weighing 200–250 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 rats received normal saline whereas the IDPN group was treated with IDPN (100 mg/kg, i.p.) daily for 7 days. The oral LD50 of IDPN in rats is 2700 mg/kg however oral route is rarely used for IDPN-induced movement disorder, probably due to inconsistency in the drug absorption from the gastrointestinal tract. There are two commonly used intraperitoneal dose regimens for producing ECC syndrome in rats: 100 mg/kg daily for 7–8 days (Al Kadasah et al., 2009; Tariq et al., 2007) or 400 mg/kg for 3 consecutive days (Khan et al., 2003; Boadas-Vaello et al., 2005). However, the former regimen is preferred for comparative studies as it results in gradual appearance of behavioral signs whereas the later regimen causes abrupt onset of severe behavioral deficits. The animals were sacrificed on day 9 and the samples of cochlea, brain, liver and kidney were collected for histopathology.

2.2. Behavioral analysis

All the animals were carefully observed for any behavioral abnormality before the daily administration of IDPN. Complete behavioral studies were started on day 7 (onset day of behavioral deficits) and repeated on day 8 and day 9, between 8 and 10 AM. 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 (back pedaling) for a period of 2 min, as described earlier (Khan, 2012; Tariq et al., 1995).

2.3. 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 postfixes in 10% neutral buffered formalin for 15 h. The bony labyrinth was decalcified by placing it in a decalcifying agent Cal-Ex (Fisher Scientific, USA) for 48 h. The decacified specimens were processed overnight for dehydration, clearing and impregnation using an automatic tissue processor (Sakura, Japan). The specimens were embedded in paraffin blocks using 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 4 μm and an autostainer (Leica 5020, Germany) was used for Hematoxylin & Eosin staining.

2.4. Statistics

The mean values of animals' bodyweights between the control and treated groups were compared by using independent samples *t*-test. The behavioral data in the form of severity scores were found to be non-normal hence a nonparametric statistical test, Mann–Whitney *U* test was applied for two-group comparisons.

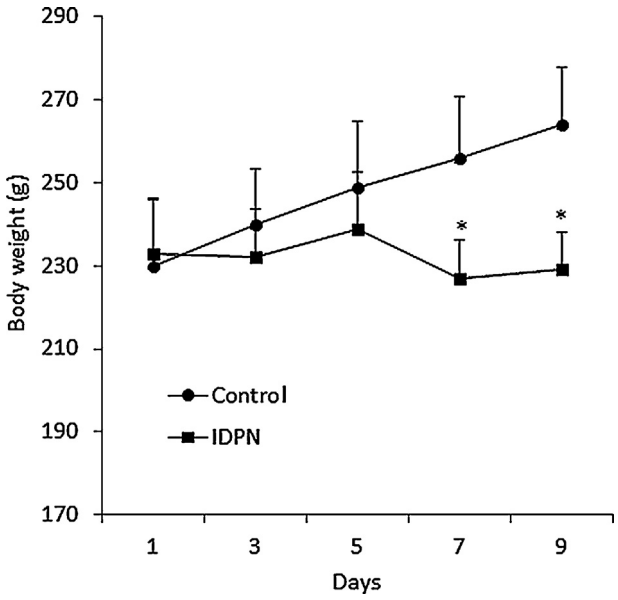

Fig. 1. Effect of IDPN on bodyweight of animals during the course of study. Intraperitoneal administration of IDPN reduced the bodyweight gain in rats. *$P<0.05$ as compared to control group using *t*-test.

Both the tests were performed using SPSS statistical package. *P* values <0.05 were considered as statistically significant.

3. Results and discussion

Administration of IDPN prevented the bodyweight gain in the animals (Fig. 1), which is in agreement with previous reports (Al Deeb et al., 2000; Tariq et al., 1998; Khan et al., 2009). Our behavioral studies showed that administration of IDPN produced abnormal symptoms associated with the ECC syndrome (Table 1). The severity of behavioral signs varied among the animals; animal no. 6 showed a high severity score whereas circling and back walking were totally absent in animal nos. 4 and 5 (Fig. 2). The onset and time-course progression of IDPN-induced behavioral syndrome was found to be consistent with our previous reports (Al Deeb et al., 2000; Khan et al., 2004; Tariq et al., 1998, 2004) suggesting the validity of IDPN as a reproducible positive control for neurovestibular toxicity studies in rats.

The results of histopathology of inner ear showed that the treatment of rats with IDPN for 7 days produced degeneration of vestibular sensory hair cells in crista ampullaris (Fig. 3). The crista ampullaris of control rats showed normal sensory epithelium with intact hair bundles. Our findings are in agreement with previous reports (Llorens et al., 1993; Khan, 2012; Al Deeb et al., 2000; Tariq et al., 1998) suggesting a close association between IDPN-induced neurobehavioral toxicity and degenerative changes in the

Table 1
Effect of IDPN on inducing behavioral abnormalities in rats.

Behavioral sign	Group	Day 7	Day 8	Day 9
Retrocollis	Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	IDPN	10.17 ± 4.54**	14.00 ± 6.68**	27.00 ± 9.46**
Laterocollis	Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	IDPN	1.00 ± 0.99	1.83 ± 1.27	4.66 ± 1.54*
Circling	Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	IDPN	2.50 ± 2.49	9.33 ± 8.55	12.66 ± 4.88*
Back walking	Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	IDPN	0.50 ± 0.49	0.50 ± 0.49	3.50 ± 1.45*

Values are means ± SEM. *$P<0.05$ and **$P<0.01$ versus control group using Mann–Whitney *U* test. The onset of behavioral abnormalities was on day 7.