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# Protective Effect of Hydrocortisone on Iminodipropionitrile-Induced Neurotoxicity in Rats

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*Abstract:* Occupational and environmental exposure of synthetic nitriles is of potential relevance to human health. Iminodipropionitrile (IDPN), a prototype nitrile toxin, has been shown to produce dyskinetic syndrome in rodents. This study reports the effect of concomitant exposure of rats to hydrocortisone and IDPN on behavioural abnormalities namely excitation, circling and chorea (ECC) syndrome. Four groups of female Wistar rats were given hydrocortisone (0, 10, 30 and 60 mg/kg, gavage, for 10 days) 30 min. before IDPN (100 mg/kg, intraperitoneally for 8 days). Two additional groups of rats were treated with either saline (control group) or 60 mg/kg of hydrocortisone (drug alone group). The animals were observed for neurobehavioural abnormalities including dyskinetic head movement, circling, tail hanging, air righting reflex and contact inhibition of righting reflex. After behavioural studies, the animals were killed, and the discrete brain regions and temporal bones were collected for biochemistry and inner ear histopathology, respectively. Hydrocortisone significantly and dose dependently attenuated the incidence and severity of IDPN-induced behavioural syndrome. Administration of hydrocortisone (60 mg/kg) alone significantly increased glutathione (GSH) levels in olfactory bulb and striatum, whereas IDPN alone significantly reduced GSH levels in olfactory bulb, striatum and hippocampus. Hydrocortisone (60 mg/kg) significantly compensated IDPN-induced depletions of GSH in different brain regions. Hydrocortisone also protected the animals against IDPN-induced vestibular hair cell degeneration. The protective effect of hydrocortisone may be attributed to its anti-inflammatory and antioxidant properties.

The neurotoxicity of iminodipropionitrile (IDPN) was first reported by Delay et al. [1] who observed an irreversible syndrome of behavioural abnormalities including repetitive head movements, circling, hyperactivity and swimming deficits in IDPN-treated rats. Selye [2] coined the term *ECC syndrome* (excitation with choreiform and circling movement syndrome) to designate IDPN-induced behavioural deficits. Occupational and environmental exposure of synthetic nitriles is of potential relevance to human health, especially after the discovery that not only IDPN but also several other nitriles of industrial application including crotonitrile, allylnitrile and acrylonitrile are able to produce motor deficits in experimental animals [3–6]. As a prototype nitrile compound, IDPN has been extensively used in experimental animals to induce ECC syndrome that can be quantitatively evaluated by an array of well-defined behavioural tests [7–10]. The mechanism of IDPN-induced neurotoxicity appears to be complex and multifactorial. The pioneer work of Llorens et al. [7,11] focused on the histopathology of inner ear revealed a direct correlation between vestibular hair cell degeneration and the severity of IDPN-induced behavioural deficits. On the other hand, neuropharmacological and biochemical studies implicated various neurotransmitters/neuromodulators [10,12–17] and oxygen-derived free radicals [9,18–22] in the development of IDPN-induced ECC syndrome.

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In the recent years, a pivotal role of immune system has been suggested in the neurodegenerative process [23–25]. Persistent activation of microglia, the resident immune cells of the central nervous system, generates potentially neurotoxic products including pro-inflammatory cytokines, glutamate and a flood of oxygen-derived free radicals [26,27]. Microglial activation following IDPN exposure has been observed in *pons medulla*, midbrain, cerebral cortex and olfactory bulb of the rat's brain [28]. However, blockade of microglial activation appears to be an effective neuroprotective strategy to disrupt the pro-inflammatory cascade and to protect animals against chemically induced neurotoxicity [29–32]. Consequently, the pro-inflammatory mediators have been assigned as the novel target sites for therapeutic approaches to prevent neurodegenerative diseases [33,34]. Corticosteroids are known to exert anti-inflammatory and immunosuppressive effects. In the present investigation, we have demonstrated the protective effects of a commonly used corticosteroid, hydrocortisone, on IDPN-induced ECC syndrome in rats.

## Materials and Methods

*Animals and drugs.* Adult female Wistar rats, weighing 220 ± 20 g were housed in a temperature-controlled room and maintained on 12-hr light:dark cycles, with free access to food and water. The development of IDPN-induced ECC syndrome in female rats is gradual and more reproducible as compared to male rats where the symptoms appear quite abruptly [8,35]. Therefore, female rats were

*Table 1.*

Effect of hydrocortisone (HC) on the incidence and severity of iminodipropionitrile (IDPN)-induced behavioural syndrome in rats.

| Treatment | Days | | |
|---|---|---|---|
| | 9 | 10 | 11 |
| Incidence of behavioural syndrome (%) | | | |
| Control | 0 | 0 | 0 |
| HC 60 mg/kg | 0 | 0 | 0 |
| IDPN | 75* | 100† | 100† |
| IDPN + HC 10 mg/kg | 25 | 75 | 100 |
| IDPN + HC 30 mg/kg | 0§ | 0§ | 57‡ |
| IDPN + HC 60 mg/kg | 0§ | 0§ | 0§ |
| Severity score (mean ± standard error) | | | |
| Control | 0 | 0 | 0 |
| HC 60 mg/kg | 0 | 0 | 0 |
| IDPN | 3.63 ± 0.98* | 10.00 ± 0.93† | 12.00 ± 1.04† |
| IDPN + HC 10 mg/kg | 1.63 ± 1.06 | 7.00 ± 1.78 | 9.25 ± 1.94 |
| IDPN + HC 30 mg/kg | 0‡ | 0§ | 3.87 ± 1.55‡ |
| IDPN + HC 60 mg/kg | 0‡ | 0§ | 0§ |

*$P < 0.05$ and †$P < 0.01$ versus control; ‡$P < 0.05$ and §$P < 0.01$ versus IDPN-alone group.

preferred for this study. The protocol of animal studies was approved by the Research and Ethical Committee of Armed Forces Hospital, Riyadh, Saudi Arabia. IDPN (Aldrich Chemical Company, Milwaukee, WI, USA) was dissolved in normal saline (McGaw Inc., Irvine, CA, USA) and injected intraperitoneally, while hydrocortisone (Sigma Chemical Company, St. Louis, MO, USA) was dissolved in distilled water (McGaw, Inc.) and administered orally.

*Dosing and testing.* The animals were divided into six groups of eight animals each. The rats in group 1 served as control and received vehicle only, while rats in groups 2, 3, 4 and 5 received hydrocortisone (0, 10, 30 and 60 mg/kg, respectively) 30 min. before IDPN (100 mg/kg). The animals in group 6 were treated with hydrocortisone (60 mg/kg) without IDPN and this group served as hydrocortisone-alone group. IDPN was administered daily for 8 days (onset of at least one symptom of ECC syndrome; appeared on day 9), while the treatment with hydrocortisone was continued until day 10 (until well-developed ECC syndrome in one of the groups). This subchronic dose regimen of IDPN is well tolerated by animals and found to be suitable for time-course evaluation of various drugs [9,10]. We avoided acute dose regimens [28,36] based on high doses of IDPN (up to 1000 mg/kg for three consecutive days) due to the sudden onset of a severe ECC syndrome that limits a dose–response quantitative evaluation of behavioural symptoms.

*ECC syndrome.* Each rat was examined for the presence or absence of the following signs: circling, dyskinetic head movements, tail hanging, air righting reflex and contact inhibition of the righting reflex using previously published behavioural testing battery [8] derived after the modification of an earlier protocol [7]. The animals were observed for a period of 2 min. to assess the severity of dyskinetic head movements and abnormal circling behaviour, while the tail hanging and the righting reflexes were tested at least three times for each animal for the grading of their severity, as described earlier in detail [8,10].

*Analysis of glutathione.* The measurement of glutathione (GSH) was done enzymatically according to the procedure reported by Owen [37]. The tissue was homogenized in ice-cold perchloric acid (0.2 M) containing 0.01% of ethylenediaminetetraacetic acid. The homogenate was centrifuged at 1700 g for 10 min. The enzymatic reaction was started by adding 100 µl of clear supernatant in a spectrophotometric cuvette containing 800 µl of 0.3 mM reduced nicotinamide adenine dinucleotide phosphate, 100 µl of 6 mM 5,5-dithiobis-2-nitrobenzoic acid and 10 µl of 50 units/ml GSH reductase (all these reagents were freshly prepared in a phosphate buffer at pH 7.5). The absorbance was measured over a period of 4 min. at 412 nm at 30°C. The GSH level was determined by comparing the rate of change of absorbance of the test solution with that of standard GSH.

*Inner ear histology.* The rats were anaesthetized with diethyl ether and perfused intracardially with 2.5% glutaraldehyde in 0.2 M phosphate buffer (pH 7.4). Temporal bones were removed and post-fixed in 10% neutral buffered formalin for 15 hr. The bony labyrinth was decalcified by placing it in a decalcifying agent, Cal-Ex (Fisher Scientific, Pittsburgh, PA, USA) for 48 hr. The specimens were then processed overnight for dehydration with increasing concentration of alcohol and clearing with acetone and chloroform using automatic processor (Shandon. Southern 2L Processor MkII, UK). The specimens were embedded in paraffin blocks and sections of 5 µm thickness were stained with 1% toluidine blue for light microscopy observations.

*Statistics.* The incidence of ECC syndrome was evaluated by Fisher's exact test using CalcFisher software (http://www.biometrica.tomsk.ru/programm_stat.htm). The results of severity scores of ECC syndrome and biochemical parameters were analysed by ANOVA using statistical software SPSS version 10 (Chicago, IL, USA). Dunnett's multiple comparison test was used to determine the significance level between the groups. A P-value of $< 0.05$ was considered as statistically significant.

## Results

*ECC syndrome.*

There were no behavioural abnormalities in the animals treated with vehicle (control) or hydrocortisone alone. The onset of ECC syndrome in IDPN-alone-treated rats occurred on day 9 and all the animals in this group became dyskinetic on day 10 (table 1). Co-treatment with hydrocortisone significantly and dose dependently delayed the onset and reduced the incidence and severity of IDPN-induced ECC syndrome (ANOVA $F = 6.38$ (day 9), $F = 29.98$ (day 10), $F = 23.05$ (day 11), $P < 0.001$).

*Oxidative stress.*

Administration of hydrocortisone (60 mg/kg) alone significantly increased GSH levels in olfactory bulb and striatum,

 

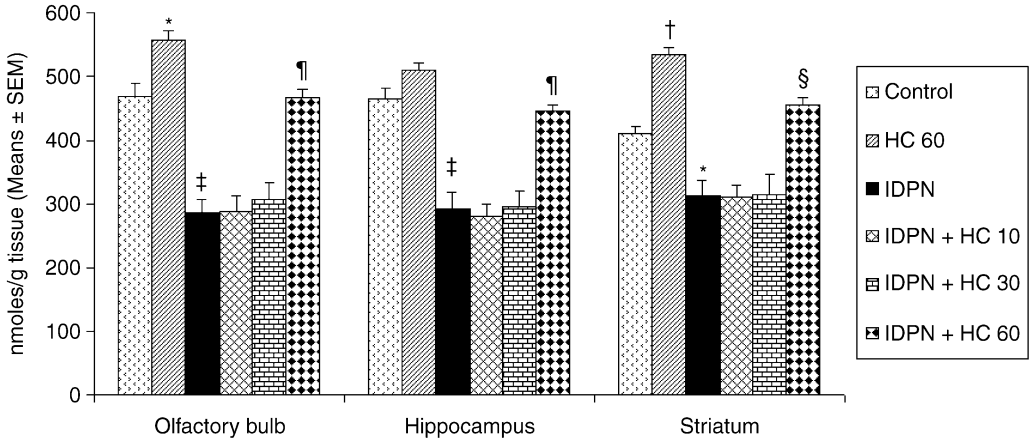


Fig. 1. Effect of hydrocortisone (HC) on iminodipropionitrile (IDPN)-induced changes in glutathione (GSH) levels in different regions of rat brain. *P < 0.05, †P < 0.01 and ‡P < 0.001 versus control group; §P < 0.01 and ¶P < 0.001 versus IDPN-alone group using Dunnett's multiple comparison test.

while the same dose of hydrocortisone insignificantly increased GSH in hippocampus of rats (fig. 1). IDPN alone significantly reduced GSH in these brain regions. Co-treatment with high doses of hydrocortisone (60 mg/kg) significantly reversed the effect of IDPN on GSH depletion in the olfactory bulb (ANOVA $F = 33.78$, $P < 0.001$), hippocampus (ANOVA $F = 23.35$, $P < 0.001$) and striatum (ANOVA $F = 13.32$, $P < 0.001$) (fig. 1). The low and medium doses of hydrocortisone were unable to compensate for IDPN-induced depletion of GSH in the discrete brain regions (fig. 1).

*Histological observation.*

The sensory epithelium in the crista of control as well as hydrocortisone-alone-treated rats showed no loss of hair cells. Administration of IDPN alone caused a severe degeneration of vestibular sensory hair cells in the crista ampullaris. Concomitant treatment with hydrocortisone protected the animals against the toxic effect of IDPN on sensory hair cells (fig. 2).

## Discussion

The results of behavioural studies clearly showed the protective effect of hydrocortisone against IDPN-induced ECC syndrome (table 1). Previous studies have reported the protective effects of hydrocortisone against experimental pancreatitis [38,39], cardiotoxicity [40], allergic encephalomyelitis [41] and neurotoxicity [42]. Our histopathological studies showed significant degenerative changes in sensory hair cells of crista ampullaris by IDPN that was markedly attenuated by hydrocortisone treatment (fig. 2). Earlier studies have demonstrated that IDPN-induced vestibular dysfunction is an important contributor to motor impairment in rats [7–9,11,43]. The mechanism of IDPN-induced vestibular toxicity is far from clear. The ototoxity of various drugs has been attributed to increased generation of oxygen-derived free radicals [44–46] and decreased cochlear blood flow [47,48]. However, the antioxidant effects of hydrocortisone have been associated with its ability to significantly improve cochlear vascular conductance [49].

Treatment of rats with hydrocortisone alone resulted in significant elevations in GSH levels in discrete brain regions, while IDPN alone significantly depleted GSH in olfactory bulb, hippocampus and striatum (fig. 1). Earlier studies have also demonstrated hydrocortisone-induced GSH increase, both *in vivo* and *in vitro* [50,51]. The first step of GSH synthesis is rate-limiting and catalysed by the enzyme γ-glutamylcysteine synthetase that is regulated by feedback competitive inhibition by GSH [52]. Hydrocortisone significantly increases the activity of γ-glutamylcysteine synthetase by increasing its synthesis suggesting an important role of glucocorticoids in the normal expression of this enzyme [50]. Moreover, administration of hydrocortisone has been shown to compensate for GSH depletion caused by bilateral adrenalectomy in rats [50]. Co-treatment with hydrocortisone (60 mg/kg) completely restored IDPN-induced GSH depletion suggesting that it may not directly block IDPN action on GSH, but compensates for IDPN-induced GSH depletion by increasing the synthesis of GSH (fig. 1). However, the failure of low (10 mg/kg) and medium (30 mg/kg) doses of hydrocortisone to recover GSH levels in IDPN-treated rats may be explained on the basis of behavioural toxicity seen in these animals on day 11 of the behavioural studies (table 1).

Administration of IDPN in rats produced a time- and dose-dependent increase in glial fibrillary acidic protein, a marker of microglial activation, in different regions of brain wherein maximum increases were observed in the olfactory bulb and cortex [28]. Seoane et al. [53] noticed peaked gliosis at 1 week in olfactory bulb and hippocampus following exposure to IDPN. The activated microglia are known to produce potentially neurotoxic species, including pro-inflammatory cytokines, arachidonic acid metabolites (eicosanoids and quinolinic acid) and oxygen-derived free radicals [26]. Pre-treatment with antioxidant drugs has been shown to protect animals against IDPN-induced ECC syndrome [9,18–20,22]. Hydrocortisone has been shown to profoundly

 

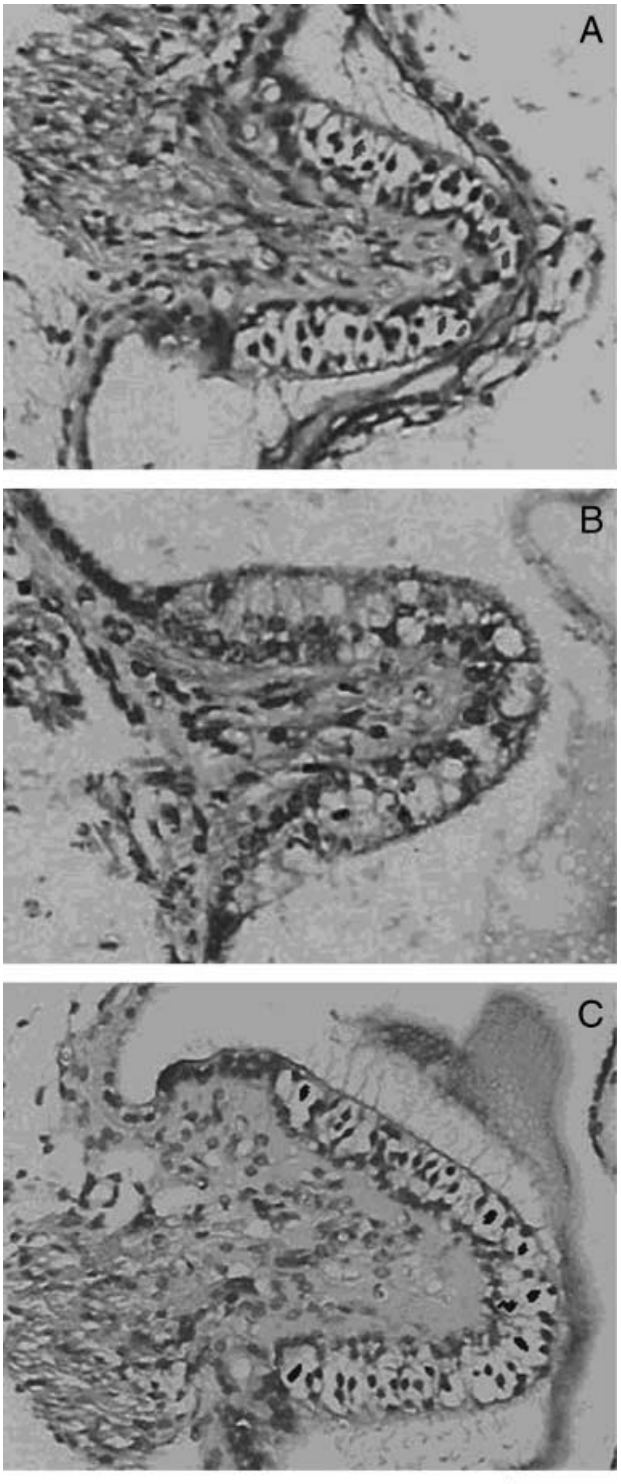


Fig. 2. Effect of hydrocortisone (HC) on iminodipropionitrile (IDPN)-induced hair cell degeneration in crista ampullaris. (A) control, (B) IDPN alone and (C) IDPN + HC, 60 mg/kg. Administration of HC attenuated the degeneration of hair cells in the sensory epithelium of IDPN-treated rats. IDPN alone produced severe loss of hair bundles which was prevented by HC treatment (scale bar, 20 μm).

suppress arachidonic acid break-down products [39], including quinolinic acid [54]. Moreover, it significantly inhibits the formation of pro-inflammatory cytokines [39] and offers protection against oxidative stress by inhibiting oxygen-derived free radicals [40,55,56]. The beneficial effects of hydrocortisone against IDPN toxicity may also be attributed to an early stage suppression of glial activation and/or inhibition of the pro-inflammatory cascade.

Finally, it is also known that IDPN is metabolized in the body to a toxic metabolite that in turn exerts its toxic effect [57–59]. Drugs that interfere with IDPN metabolism have been shown to modify IDPN-induced neurotoxicity [60–62]. High doses of hydrocortisone could affect the activity of drug-metabolizing enzymes [63], and the protective effect of hydrocortisone against tetraethylammonium bromide has been partially attributed to increased urinary excretion of the toxicant [64]. However, the effect of hydrocortisone on the bioavailability of IDPN warrants further study.

In conclusion, this study clearly demonstrated that hydrocortisone significantly and dose dependently attenuated IDPN-induced ECC syndrome. Its protective effect may be attributed to its anti-inflammatory and antioxidant properties.

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