LETTER TO THE EDITOR

On the Mechanism of Nitriles Toxicity

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This is apropos to some issues raised by Llorens et al. (2009) on our recent publication unraveling the mode of toxicities of acrylonitrile (ACN) and iminodipropionitrile (IDPN) in rats (Khan et al., 2009). In the following text, we have attempted to provide point-wise explanations to the queries of Llorens et al. in the light of relevant literature.

The transient behavioral effects appeared after ACN injections were evaluated by visual observation and the grading scores of − (absent), + (mild), ++ (moderate), and +++ (severe) were collectively assigned to each treatment group (Khan et al., 2009). In fact, our main concentration was to assess the behavioral abnormalities associated with the excitation, chorea, and circling (ECC) syndrome, for which IDPN was used as a positive control and a well-defined behavioral testing battery was applied (Khan et al., 2009). The use of subjective observation of behavioral and morphological signs is a common practice in pharmacological and toxicological studies. Llorens et al. themselves have used similar subjective grading of loss of vestibular function, rated from 0 (normal) to 4 (extreme alteration) using tail hang test (Seoane et al., 1999, 2003). Moreover, they have also evaluated the nitrile-induced corneal opacity by simple observation and subjective rating of the animals from 0 (no evidence of corneal opacity) to 4 (maximal corneal opacity) (Balbuena and Llorens, 2001, 2003; Seoane et al., 1999).

In their Letter to Editor, Llorens et al have objected the overlooking of certain references by us while discussing the behavioral effects of ACN (Khan et al., 2009). In fact, we preferred to cite the work of Gagnaire et al. (1998) being more recent and relevant to the dose regimen of ACN used by us. We have also discussed the findings of Rongzhu et al. (2007) on neurobehavioral alterations related to locomotor activities, motor coordination, learning, and memory in ACN-treated rats to portray a wider picture on this topic. Nevertheless, we feel that the selection of references be solely left on authors’ discretion. Of course the learned Reviewers of the manuscript are the most competent authorities to suggest any modification in the cited literature.

Llorens et al. (1993) have suggested an identity between the IDPN-induced ECC syndrome and the behavioral deficits resulting from bilateral labyrinthectomy; they justified their claim on the basis of only one rat subjected to bilateral labyrinthectomy. We agree that bilabyrinthectomy can produce behavioral deficits akin to those seen in IDPN exposed rats. However, the pathogenesis of both the conditions may not be identical unless evidences prove that bilabyrinthectomy can also cause axonal swelling similar to IDPN (Chou and Hartmann, 1964; Clark et al., 1980) as well as the drugs that modify IDPN-induced ECC syndrome also exert similar effects on the behavior of bilabyrinthectomized rats. Regarding the structure-function relationship, ACN (CH₂=CH-CN) is apparently more similar to allylnitrile (CH₂=CH–CN) than crotonitrile (CH₃–CH=CH–CN) as the later compound possesses the peculiar CH=CH group rendering it to conceive cis-trans isomerism.

Our findings on intense tyrosine hydroxylase (TH) immunostaining of dopaminergic neurons in the anterior striatum (but not the medial region) of IDPN-treated rats (Khan et al., 2009) are somewhat different from the observations of Llorens group who found no change in striatal dopamine (DA) concentrations following IDPN exposure in rats (data not shown in their paper) (Seoane et al., 1999). This discrepancy may be attributed to the differences in animal strain (Wistar vs. Long-Evans), dose of IDPN (8 × 100 vs. 3 × 400 mg/kg), analysis time post dosing (2 days vs. 4 weeks) used in our and their studies respectively. It is also important to note that DA turnover and not the DA content alone is a reliable predictor of dopaminergic status (Ogawa et al., 1990, 1991; Tariq et al., 1999). Moreover the role of dopaminergic neurotransmission in IDPN toxicity is supported by several studies demonstrating significant inhibition of IDPN-induced behavioral syndrome by DA antagonists (Cadet et al., 1987; Khan et al., 2004; Ogawa et al., 1991).

Another point raised by Llorens et al. is the use of a single rat per group for the histopathology of vestibular sensory
epithelia; the same animals were also used for TH immuno-
taining. We cannot simply justify this weakness on grounds of similar shortcomings in the studies of Llorens and coworkers in which they used only one animal for labyrinthotomy (Llorens et al., 1993) or histological assessment of vestibular receptors (Llorens et al., 1994). In fact, the dominant ethical position worldwide is that achievement of scientific and medical goals using animal testing is desirable provided that animal suffering and use is minimized (Amendment to Animal Welfare Act, 1985). According to European Centre for the Validation of Alternative Methods, the refinement of experimental protocol can significantly minimize the use of experimental animals. When the use of an animal is no longer required by an experimental procedure, in order to minimize the number of animals used in research, alternative uses of the animals should be considered (Guide for the Care and Use of Laboratory Animals, 1996). Thus, an experimental protocol with minimum number of animals and their optimum utilization would be more appropriate than using multiple sets of animals for various parameters. Although the histopathological findings reported in our study may not be statistically sound, they can genuinely serve as pilot for further studies. We also intentionally reduced the number of treatment groups (saving 14 animals) by studying the effect of concomitant exposure of IDPN with only the medium dose of ACN (15 mg/kg), whereas all the three doses of ACN were tested for their individual effects (Khan et al., 2009).

The method we used for vestibular histopathology was standardized by us (Tariq et al., 1998) and then applied in our various studies (Al Deeb et al., 2000; Khan et al., 2003, 2009; Tariq et al., 2002, 2006). This method is based on decalcification of vestibular labyrinth and is quite simple, reproducible and can be repeated in small laboratories. Being a light microscopic method it cannot attain the resolution of electron microscopy however it has proved its sensitivity to differentiate among various degrees of vestibular hair cell degeneration (Khan et al., 2003).

We used the technical grade of IDPN (90%) from Sigma-Aldrich in our study due to unavailability of pure compound. However, the probability that the 10% impurities in the technical grade of IDPN may have significant impact on brain glutathione (GSH) is less likely because the effects of IDPN on GSH levels are supported by earlier studied reporting the role of reactive oxygen species in the development of IDPN-induced neurotoxicity (Nomoto, 2004; Wakata et al., 2000). Moreover, the behavioral deficits caused by IDPN are significantly aggravated by GSH-depleting agent (Al Deeb et al., 1995) and attenuated by antioxidants (Lohr et al., 1988). These findings are in contrast to the claims of Llorens et al. (1993, 1994) about the irreversible nature of IDPN-induced behavioral deficits and warrant further studies on exploring therapeutic potential of antioxidants in nitrile toxicity.

Finally, we abstained to comment on the role of metabolic pathways in the toxicity profiles of ACN and IDPN (Khan et al., 2009) because we did not have enough pharmacokinetic data of these toxins. Moreover, this is a controversial issue as the inhibition of P450 activities with carbon tetrachloride has been reported to significantly decrease (Genter et al., 1994) or increase the toxicity of IDPN (Llorens and Crofton, 1991).

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REFERENCES


