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Original Article

Nevirapine mitigates monosodium glutamate induced neurotoxicity and oxidative stress changes in prepubertal mice

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Abstract

Aim: We investigated the effects of nevirapine on changes in body weight, lipid peroxidation/antioxidant status, apoptotic markers and neuromorphology following monosodium glutamate-induced neurotoxicity in prepubertal mice.

Material and Methods: Eighty male mice which were randomly divided into eight groups of ten mice each (n=10) were used. Mice in each group were administered oral vehicle, monosodium glutamate (MSG) at 2g/kg, or one of three doses of nevirapine (at 7.5, 15 and 30 mg/kg) alone or co-administered with MSG. Vehicle or nevirapine were administered daily for 28 days, while MSG was administered on days 1-7. On day 28, animals were euthanized and blood was collected for estimation of plasma malondialdehyde (MDA) and antioxidant levels; while sections of the cerebrum and hippocampus were either fixed and processed for general histology, or homogenized for the estimation of brain biochemical parameters.

Results: Results showed that administration of nevirapine alone (or when co-administered with MSG) was associated with a reduction in weight gain, increase in plasma/brain MDA levels, morphologic/morphometric evidence of dose-related hypercellularity; and varying degrees of neuroprotection, with co-administration. Nitric oxide levels and caspase-3 activity increased only with MSG, while superoxide dismutase activity decreased.

Conclusions: In conclusion, subchronic administration of nevirapine was associated with dose-related alterations of the measured parameters; indicating possible neuroprotection and mitigation of MSG neurotoxicity at some of the doses studied.

Keywords: HAART; Monosodium glutamate; Prepubertal; Oxidative stress; Apoptosis.

INTRODUCTION

Nevirapine (NVP) is a non-nucleoside reverse transcriptase inhibitor (NNRTI) that is used in the management of the human immunodeficiency virus infection (1). It is recommended for infants aged above 24 months; and those less than 24 months of age who have had no previous exposure to maternal/infant NVP or other NNRTIs used in the management of maternal retroviral infections, or the prevention of mother-to-child transmission (1,2). Nevirapine's use has been facilitated by its heat-stable liquid formulation, its pharmacokinetic properties (which include lesser occurrence of drug interactions than protease inhibitors), and a bioavailability not related to food intake (3); all these make it a drug of choice, particularly in sub-Saharan Africa. A dosing regimen that involved administering NVP at 150 mg/m2 twice daily was approved by the FDA (4); however, another dosing regimen which has found use in the resource-limited settings of sub-Saharan Africa is the administration of NVP based on weight bands, which requires no calculations (1)

The use of antiretroviral therapy (ART) has been associated with a significant decrease in the incidence of perinatal HIV-1 transmission (5) following the use of ARTs to control mother to child transmission. However, it has also resulted in an increase in the number of infants becoming exposed to ARTs in utero, with the possibility of development of ART-related toxicities; considering that little is known of the long-term effects of these exposures (6). While NVP's pharmacokinetics has been studied extensively in HIV-infected adults (7) and paediatric populations (8); recently, there are hints that exposure to ARTs may be linked to adverse effects in human

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subjects, irrespective of their HIV status (7,9); or even in rodents (10,11). Protease inhibitors and nucleoside analogs have been associated with mitochondrial toxicity and cardiovascular/metabolic complications (1). Also, NNRTIs like NVP have been linked to the development of toxic epidermal necrolysis, hepatotoxicity, and Stevens-Johnson syndrome in HIV infected individuals (12,13) and also in non HIV infected subjects (14). While the health status of mothers on highly active anti-retroviral therapy (HAART) regimens may be optimal, questions continue to be raised regarding the possible toxicities to the growing brain that may occur as a result of the exposure of the fetus or children to these potent drugs (1).

Extensive evaluations of the impact of ARTs on the liver, cardiovascular system, nutritional/ metabolic indices in adults (7,14-18), infants, children, and adolescents (8,19-22) in the context of HIV infection had been carried out. Some studies have also reported that administration of NVP to rodents was associated with the development of idiosyncratic reactions (10,11) and liver toxicity (23). While a number of studies have demonstrated ART-induced peripheral nervous system neurotoxicity (which have been linked to oxidative stress, alterations in protein/lipid metabolism and mitochondrial damage) (24), there are still gaps in our understanding of the possible neurochemical or neuromorphological effects of NVP, especially on the growing brain. In this study, we tested the hypothesis that repeated oral administration of NVP can alter oxidative stress, antioxidant status, apoptotic marker (caspase-3), and brain morphology/morphometry in healthy prepubertal mice or a mouse model of neurotoxic brain injury.

MATERIAL and METHODS

Drugs and chemicals

Monosodium glutamate (Ajinomoto®, 99% purity), Nevirapine (Oral suspension, Boehringer Ingelheim Roxane, Inc. Ohio, USA), Caspase-3 assay kit from Yeasen, China, Malondialdehyde (MDA), Superoxide-dismutase, and nitric oxide assay kits were sourced from Biovison, USA.

Animals

Male mice (Postnatal day 22) weighing between 8-12 g which were sourced from Empire Breeders, Osogbo, Osun state, Nigeria were used for these experiments. The mice were kept in a well-ventilated room and fed standard rodent chow (TOP® Feed LTD). They were allowed access to water ad libitum. Animals received care as outlined in the "Guide for the Care and Use of Laboratory Animals" as prepared by the National Academy of Sciences (2013). All procedures performed on the animals were in accordance with approved institutional protocols and as prescribed by the scientific procedures on living animals, European Council Directive (EU2010/63).

Experimental Methodology

Male mice (80) were used for this study. Animals were assigned into eight groups of 10 mice each. Mice in the groups received vehicle (distilled water at 10 ml/kg), MSG at 2g/kg (25) or one of three doses of nevirapine at 7.5, 15 and 30 mg/kg (26) administered alone or in combination with MSG. Vehicle or nevirapine (7.5, 15 and 30 mg/kg) was administered daily for 28 days (Postnatal day 50), while MSG was administered on days 1-7 (to induce MSG neurotoxicity) of the experimental period (25). Vehicle (distilled water), MSG or nevirapine were administered orally. Doses of nevirapine or MSG were reached by dissolving weighed quantities of the drug or salt (Ajinomoto®) in distilled water. Animals were weighed once every week. At the conclusion of the experiment, mice were sacrificed and blood collected through an intra-cardiac puncture for estimation of plasma antioxidant activity and malondialdehyde levels. The brains from mice in all groups were dissected out, observed grossly and weighed. Sections of the cerebrum and hippocampus were either fixed in formol-saline (n=6) or homogenized (n=6) and used to assess brain antioxidant status. Paraffin embedded sections of the cerebral cortex and hippocampus were cut and stained with haematoxylin and eosin for general histology.

Superoxide dismutase activity, caspase-3 activity, malondialdehyde and nitric oxide levels and were assayed from brain homogenates.

Homogenisation of brain tissue

Mice were first anaesthetized using diethyl-ether following which they were perfused transcardially with ice-cold saline. The cerebrum and hippocampus were dissected out and weighed. Homogenate (10%) of brain tissue was prepared with ice-cold phosphate buffered saline using a Teflon-glass homogenizer. The brain homogenate was then centrifuged at 5,000 rpm (4 °C) for 15 min. The pellet was discarded while the supernatant was used to assess antioxidant status and malondialdehyde levels. For the estimation of caspase-3, the brain homogenate was centrifuged at 10,000 rpm for 1 minute and the supernatant transferred to another tube, maintained on ice; and immediately used for analysis.

Assessment of plasma and brain antioxidant status

The activity of superoxide dismutase and levels of nitric oxide were assayed from plasma and brain homogenates as described in previous studies (27-29). Superoxide dismutase assay is based on enzyme's ability to inhibit phenazine methosulphate-mediated reduction of the nitro blue tetrazolium dye. The color change is measured at an absorbance of 560 nm over 5 minutes. Nitric oxide assay was measured spectrophotometrically using a dual process to measure total nitrate/nitrite concentration; the first steps involves the conversion of nitrate to nitrite through a process that is catalyzed by nitrate reductase. The second step involves the conversion of nitrite to a deep purple azo compound. The color change which is a reflection of nitric oxide (NOx) species in samples is measured at an absorbance of 540 nm.

Lipid peroxidation (malondialdehyde) levels

Malondialdehyde (MDA) kevel was measured from plasma or brain homogenate using the malondialdehyde assay kit, according to the manufacturer's instructions. Color change was measured at an absorbance of 532 nm (28).

Assessment of caspase-3 activity

The activity of Caspase-3 was assayed using the Caspase-3 assay kit (40313ES20) as previously described in a recent publication from our laboratory (28).

Histological preparation

The cerebral cortex and hippocampus were sectioned, processed for paraffin-embedding, cut at 5 µm and stained using haematoxylin and eosin (H&E) general histology stain.

Statistical Analysis

Data was analyzed with Chris Rorden's ezANOVA for windows version 0.98. Statistical analysis was by one way analysis of variance (ANOVA) with Tukey HSD, post-hoc test used for within and between group comparisons. Results were expressed as Mean ± S.E.M. and $p<0.05$ was considered an acceptable level of significant difference.

RESULTS

Effects of nevirapine on body weight

Figure 1 represents the percentage increase in body weight over the 28 day period. There was a significant ($F(7, 40) = 17.1$, $p<0.001$) decrease in body weight gain following administration of nevirapine alone at 15 and 30 mg/kg; an increase with monosodium glutamate (MSG) alone and in groups administered MSG with nevirapine at 7.5,15 and 30 mg/kg, compared to vehicle. Compared to mice that were administered MSG alone, bodyweight decreased in groups in which MSG was co-administered with nevirapine at 7.5, 15 and 30 mg/kg.

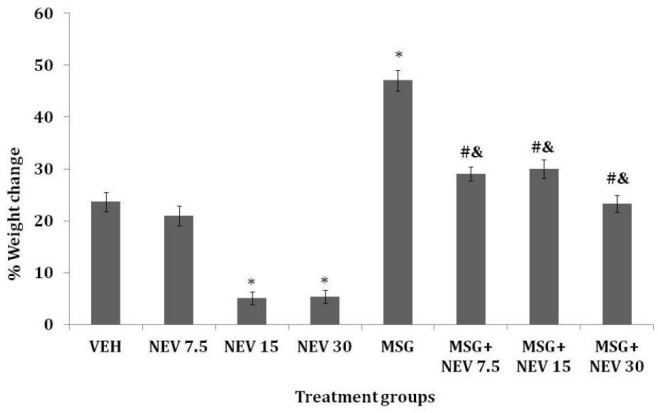

Figure 1. Effect of nevirapine on % change in body weight. Values are expressed as Mean ± S.E.M, *p<0.05 vs. VEH, #p<0.05 vs. MSG, #p<0.05 vs. MSG number of mice per treatment group =10; VEH: Vehicle, NEV Nevirapine, MSG: Monosodium glutamate

Effects of nevirapine on malondialdehyde levels and caspase-3 activity

Table 1 shows the effects of nevirapine on brain/plasma levels of malondialdehyde (MDA) and the activity of Caspase-3 activity in brain homogenates on day 28. There was a significant increase ($F(7, 40) = 45.1$, $p<0.001$) in plasma MDA levels in groups administered nevirapine alone (15 and 30 mg/kg), MSG alone, and MSG with nevirapine (MSG+NEV) at 7.5, 15 and 30 mg/kg compared to vehicle. Compared to MSG alone, MDA levels decreased significantly in groups administered MSG+ NEV 15.

Table 1. Effect of nevirapine on MDA levels and caspase-3 activity

Groups	MDA U/L	MDA U/g	Caspase-3 (ng/mg)
Vehicle	13.41±2.20	6.22±2.30	0.324±0.001
NEV7.5	17.07±2.21*	9.55±2.22*	0.332±0.001
NEV 15	16.77±2.18*	9.34±2.26*	0.335±0.001
NEV 30	16.27± 2.32*	10.12±2.45*	0.341±0.001
MSG	42.22±3.43*	22.12±2.44*	0.602±0.001*
MSG+NEV 7.5	47.33±3.32*	23.24±2.56*	0.595±0.001*
MSG+NEV 15	35.23±3.32*#	14.53±2.46*#	0.606±0.001*
MSG+NEV 30	45.26±3.34*	26.26±3.55*#	0.610±0.001*

Values are expressed as Mean ± S.E.M, *p<0.05 vs. VEH, #p<0.05 vs. MSG, number of mice per treatment group =6; VEH: Vehicle

Brain MDA levels increased significantly ($F(7, 40) = 19.55$, $p<0.011$) in groups administered MSG alone, and MSG with nevirapine (MSG+NEV) at 7.5, 15 and 30 mg/kg compared to vehicle. Compared to MSG alone, MDA levels decreased significantly in groups administered MSG+ NEV 15 and increased with MSG alone and nevirapine at 30 mg/kg.

Brain caspase-3 activity increased significantly ($F(7, 40) = 5.65$, $p<0.010$) in groups administered MSG alone, and MSG with nevirapine (MSG+NEV) at 7.5, 15 and 30 mg/kg compared to vehicle. Compared to MSG alone, caspase-3 activity did not differ significantly in any of the groups administered MSG+ NEV (7.5, 15, 30 mg/kg).

Effects of nevirapine on plasma and brain superoxide dismutase activity and nitric oxide levels

Table 2 shows the effects of nevirapine on plasma and brain superoxide dismutase activity and nitric oxide levels respectively. There was significant decrease in plasma ($F(7, 40) = 10.16$, $p<0.001$) superoxide dismutase activity in groups administered MSG alone, and MSG with nevirapine at 7.5, 15 and 30 mg/kg compared to vehicle; while compared to MSG alone, SOD activity increased significantly in mice that received MSG with nevirapine at 7,15 and 30 mg/kg. Brain SOD activity decreased significantly ($F(7, 40) = 24.22$, $p<0.001$) in groups administered MSG alone and MSG with nevirapine at 15 and 30 mg/kg compared to vehicle, while compared to MSG alone, SOD activity increased in mice that received MSG+ nevirapine at 7, 15 and 30 mg/kg.

Table 2. Effect of nevirapine on plasma/brain SOD activity and NO levels

Groups	Plasma SOD (U/L)	Plasma NO (U/L)	Brain SOD (U/g)	Brain NO (U/g)
VEH	4.34±0.12	24.62±2.11	23.67±0.53	14.53±3.42
NEV 7.5	4.17±0.22	24.07±2.16	20.97±2.76	14.41±3.00
NEV 15	4.42±0.32	26.16±2.24	22.76±1.44	14.66±3.23
NEV 30	4.24±0.33	23.14±2.66	22.70±1.56	14.84±3.16
MSG	1.65±1.23*	66.75±2.15*	10.45±8.65*	55.45±4.33*
MSG+NEV 7.5	2.46±1.75*	64.24±2.22*	12.99±7.34*	46.22±4.14*#
MSG+NEV 15	2.93±1.10*#	64.22±2.22*	19.42±12.22*	48.44±5.12*#
MSG+NEV 30	2.42±1.26*	65.33±2.22*	18.23±12.32*	51.34±3.23*#

Values are expressed as Mean ± S.E.M, *p<0.05 vs. VEH, #p<0.05 vs. MSG, number of mice per treatment group =6; VEH: Vehicle

Plasma nitric oxide levels increased significantly (F (7, 40) = 45.1, p<0.001) in groups that received MSG, and MSG with nevirapine at 7.5, 15 and 30 mg/kg compared to vehicle; and showed no significant differences when compared to the MSG alone group. Brain nitric oxide levels increased significantly in groups administered MSG alone and MSG + nevirapine at 7.5, 15 and 30 mg/kg compared to vehicle. However, compared to the MSG alone group, nitric oxide levels decreased in the groups administered MSG with nevirapine at 7.5, 15 and 30 mg/kg.

Effects of Nevirapine on morphology of the cerebral cortex

Examination of H&E stained sections of the cerebrum (figure 2a-h) revealed non-distinct layers of the cortex with pyramidal neurons, granule neurons and glia cell in groups of mice administered vehicle (figure 2a) or nevirapine alone at 7.5 (figure 2b), 15 (figure 2c) and 30 mg/kg (figure 2d); However, in the groups administered nevirapine alone, increased cellularity were also observed. Pyramidal neurons were multipolar with large, rounded, vesicular nucleus; the granular neurons had large open-faced nuclei, with prominent nucleoli and scanty cytoplasm. In groups of mice administered MSG (figure 2e), pale staining pyramidal and granule neurons with contracted pale-staining nuclei were observed; while in groups of mice administered MSG with nevirapine at 7.5 (figure 2f), 15 (figure 2g) and 30 mg/kg (figure 2h) respectively graded loss of cells and loss of cerebral architecture was observed while in the group administered MSG with nevirapine at 30 mg/kg (figure 2h) mild preservation of neuronal integrity was observed.

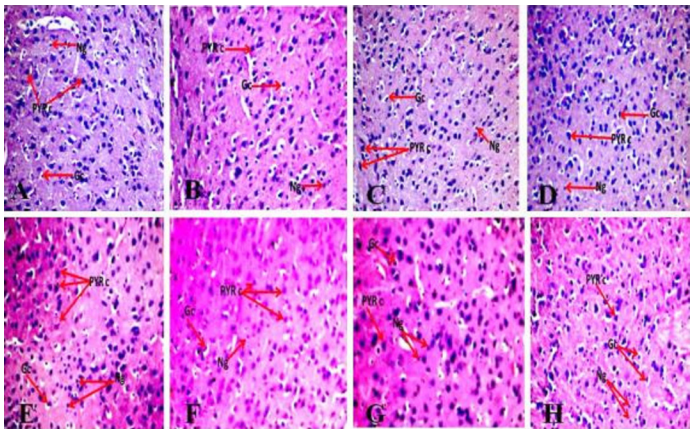

Figure 2 (A-H). Effect of nevirapine on mouse cerebral cortex morphology. A) Vehicle, B) Nevirapine (NEV) at 7.5 mg/kg, C) Nevirapine at 15 mg/kg, D) Nevirapine at 30 mg/kg, E) Monosodium glutamate (MSG) alone ,F) MSG+NEV at 7.5 mg/kg G) MSG+NEV at 15 mg/kg , H) MSG+NEV at 30 mg/kg. Representative slides showing pyramidal cells (PYRC), granule cells (Gc) and Neuroglia (Ng). H&E x100, scale bar 9.23 µm/pixel

Effects of Nevirapine on the morphology o the hippocampus

Figures 3(a-h) and 4(a-h) are representative slides of H&E-stained sections of the mouse hippocampus (cornus ammonis (CA1) and dentate gyrus regions). Examination of the cornus ammonis revealed large pyramidal cells of the stratum pyramidalis (figure 3a, 3b, 3c and 3d); while small granule neurons that are characteristic of the dentate gyrus (figure 4a, 4b, 4c and 4d)) were also observed in the group of animals administered vehicle and nevirapine alone at 7.5, 15 and 30 mg/kg respectively. Glia cells, dendrites and axons were also observed in the molecular layer (a region lying between the dentate gyrus and the compact zone of the cornus ammonis). In the group administered MSG alone, shrunken pale-staining nuclei were observed in the CA1 region (figure 3e) with sparsity of glia cells and neuronal processes in the molecular layer; while in the dentate gyrus (Figure 4e), degenerating granule cells with pale-staining nuclei were observed, with reduction in the cohesion between individual granule cells. In the groups of animals administered MSG with nevirapine at 7.5, 15 and 30 mg/kg, varying degrees of protection against neuronal injury are observed in the CA1 region (figures 3f, 3g and 3h) and dentate gyrus (figures 4f, 4g and 4h).

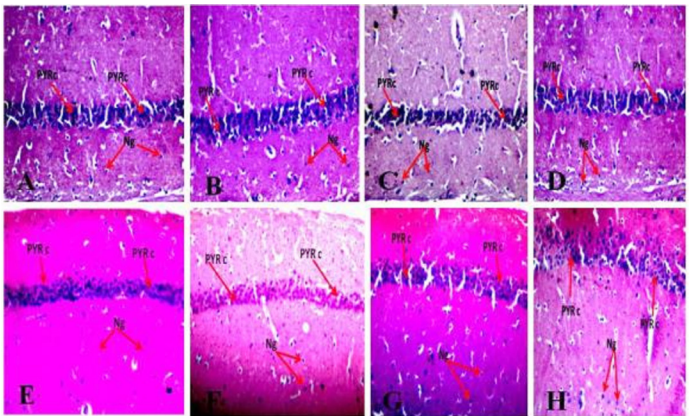

Figure 3 (A-H). Effect of nevirapine on the morphology of the Cornus Ammonis (CA) 1 region of the mouse hippocampus. A) Vehicle, B) Nevirapine (NEV) at 7.5 mg/kg, C) Nevirapine at 15 mg/kg, D) Nevirapine at 30 mg/kg, E) Monosodium glutamate (MSG) alone ,F) MSG+NEV at 7.5 mg/kg G) MSG+NEV at 15 mg/kg , H) MSG+NEV at 30 mg/kg. Representative slides showing pyramidal cells (PYRC) and Neuroglia (Ng) H&E x100, scale bar 9.23 µm/pixel

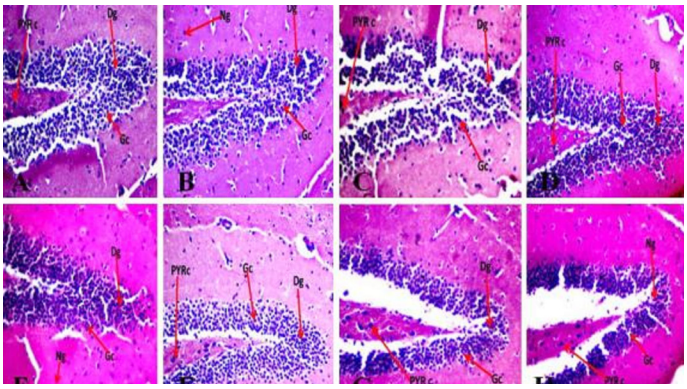

Figure 4 (A-H). Effect of nevirapine on the morphology of the dentate gyrus of the mouse hippocampus. A) Vehicle, B) Nevirapine (NEV) at 7.5 mg/kg, C) Nevirapine at 15 mg/kg, D) Nevirapine at 30 mg/kg, E) Monosodium glutamate (MSG) alone ,F) MSG+NEV at 7.5 mg/kg G) MSG+NEV at 15 mg/kg , H) MSG+NEV at 30 mg/kg. Representative slides showing the dentate gyrus (Dg), pyramidal cells (PYRC), granule cells (Gc) and Neuroglia (Ng) H&E x100, scale bar 9.23 µm/pixel

Effects of Nevirapine on the morphometry of the cerebral cortex

Morphometric analyses of H&E stained sections of the mouse cerebral cortex (Table 3) demonstrated significant ($F=12.34$, $p<0.035$) increase in total area covered by neuronal cells in the mice administered MSG and a significant decrease in groups administered MSG with nevirapine at 7.5, 15 and 30 mg/kg, compared to vehicle. In comparison to MSG, there was significant reduction in the total area covered by neuronal cells in groups administered MSG with increasing doses of nevirapine. Total cell count increased significantly with nevirapine alone at 15 and 30 mg/kg and decreased in groups of animals administered nevirapine alone at 7.5 mg/kg, MSG and MSG with nevirapine at 7.5, 15 and 30 mg/kg compared to vehicle. Compared to MSG, total cell count decreased significantly in groups of mice that received MSG with nevirapine at 7.5, 15 and 30 mg/kg respectively. Average cell size decreased significantly ($F=50.0$, $p<0.001$) in groups that received nevirapine at 30 mg/kg, MSG and MSG with nevirapine at 7.5, 15 and 30 mg/kg compared to vehicle. Compared to MSG, average cell size decreased in groups that were administered MSG with nevirapine at 7.5, 15 and 30 mg/kg respectively.

Table 3. Effect of nevirapine on morphometry of the cerebral cortex

Groups	Total Cell Count	Cell Size (um)	Total area covered by cells (m^2)
Vehicle	417.29 ± 8.65	0.38 ± 0.03	133.00± 1.11
NEV 7.5	338 25 ± 7.62*	0.38 ± 0.02*	148.34 ± 1.32
NEV 15	438.35 ± 8.24*	0.28 ± 0.02*	100.99 ± 0.11
NEV 30	101.26 ±10.55*	0.17±0.01*	165.66± 1.32
MSG	114.54 ±8.65*	0.30±0.02*	300.28±2.22*
MSG+7.5	22.25 ±3.43*#	0.05±0.001*#	0.50±0.11*#
MSG+15	42.25 ± 3.10*#	0.04 ± 0.001*#	0.61 ± 0.12*#
MSG+30	153.33± 4.55*#	0.014 ± 0.001*#	20.19 ± 1.01*#

Table represents Mean ± S.E.M, *p<0.05 vs. VEH, #p<0.05 vs. MSG, number of mice per treatment group = 6; VEH: Vehicle, NEV: nevirapine, MSG: monosodium glutamate

Effects of Nevirapine on the morphometry of the hippocampus

Table 4 shows effect of nevirapine on morphometry of H&E stained sections of the dentate gyrus and cornus ammonis (CA1) of the mouse hippocampus. A significant ($F=24.2$, $p< 0.001$) decrease in the width of the stratum pyramidalis layer of the CA1 region was observed in the groups of mice administered nevirapine alone (15 mg/kg), MSG alone and MSG with nevirapine at 7.5 and 15 mg/kg; while a significant increase was observed with MSG with nevirapine at 30 mg/kg, compared to vehicle. Compared to MSG, there was a significant increase in the width of the stratum pyramidalis of the CA1 region in the group administered MSG with nevirapine at 30 mg/kg Morphometric analysis of the dentate gyrus revealed a significant ($F=15.21$, $p< 0.015$) decrease in the width of the ascending and descending limbs in groups administered MSG, and MSG with nevirapine at 7.5, 15 and 30 mg/kg.

Table 4. Effect of nevirapine on the morphometry of the hippocampus

Dose	CA1(μm)	Dentate Gyrus (μm)	
		Ascending	Descending
VEH	9.31±0.11	8.67±1.83	16.01±3.33
NEV 7.5	11.40±0.95	7.82±1.13	14.87±1.62
NEV 15	9.07±0.21	7.42±1.16	14.96±2.06
NEV 30	7.65±0.21*	7.99±1.22	15.54±2.21
MSG	6.22±0.26*	6.32±1.11*	10.09±1.43*
MSG +7.5	6.51±0.22*	6.89±1.54*	10.55±1.21*
MSG +15	6.93±0.48*	7.23±1.12	12.11±1.90*
MSG +30	14.08±1.03*#	6.04±1.60*	10.88±0.93*

Table represents Mean ± S.E.M, *p<0.05 vs. VEH, #p<0.05 vs. MSG, number of mice per treatment group =6, VEH: vehicle, CA1: Cornus ammonis 1, NEV: nevirapine, MSG: monosodium glutamate

DISCUSSION

In this study, we examined the effects of increasing doses of nevirapine on body weight, lipid peroxidation, antioxidant status, caspase-3 activity and cerebral cortex/hippocampal morphometry and histomorphology in healthy prepubertal mice and in prepubertal mice exposed to a neurotoxic dose of MSG. Repeated administration of nevirapine co-administered with MSG resulted in a reduction of body weight, lipid peroxidation and nitric oxide levels, an increase in superoxide dismutase activity, no significant difference in caspase-3 activity; as well as varying degrees of protection against MSG-induced neuronal injury in the cerebral cortex and hippocampus.

In the study, administration of MSG to juvenile mice was associated with higher weight gain, when compared to vehicle-treated control. While the effects of MSG on body weight have continued to be contested, variations in results have been attributed to the differences in doses of MSG administered, route of administration and age at administration (30). For example, in a previous study in which MSG was administered at low doses (compared to doses used in this study) to adult mice, a decrease in body weight gain was observed (30). Studies have associated the administration of MSG to rodents in the neonatal period with the induction of obesity (31). This has been attributed to alteration in glucose and lipid metabolism (32). The administration of high dose MSG to mice during a period of life characterised by increasing growth may result in effects that are comparable to those occurring in the neonatal period. The observed increase in body weight was also accompanied by an increase in food consumption (data not included). Also, in this study, the administration of nevirapine to healthy mice was associated with a decrease in weight gain; while nevirapine in combination with MSG (2g/kg) appeared to mitigate MSG-induced weight gain. A number of studies have reported evidence of increased weight gain following nevirapine use in rodents (33,34) or its use as part of a treatment regimen (35). However, there have also been reports of no alteration in body weight following administration of nevirapine to adult rats (36). The increased weight gain observed in some of the studies

has been attributed to nevirapine's ability to increase food and water intake; however, this was not the case in the present study. Here, a decrease in food consumption was observed with nevirapine (with or without MSG) only when compared to MSG control, otherwise no significant difference from vehicle was observed.

A few studies have implicated increased oxidative stress and alteration of antioxidant status in the pathogenesis of MSG-induced toxicity. In this study, the administration of MSG at 2g/kg was associated with increased lipid peroxidation, a decrease in superoxide dismutase activity and an increase in nitric oxide levels in plasma and brain homogenates. These corroborate the results of studies that have also reported derangements in oxidant/antioxidant balance following high dose MSG (25). In the study, administration of nevirapine to healthy mice was associated with a slight increase in lipid peroxidation but no significant difference in SOD activity and No levels in the brain or plasma. However, in mice with MSG-induced neurotoxicity, an increase in Plasma and brain SOD and NO was observed; with nevirapine mitigating MSG-induced oxidative stress and antioxidant derangement when administered at 7, 15 and 30 mg/kg. A number of other studies have also reported increased lipid peroxidation and decreased antioxidant activity (superoxide dismutase and catalase) in the plasma or tissues of rats administered nevirapine alone with increasing doses (36,37).

Caspases are important during apoptotic cell death. Specifically, caspase-3 is important during neuronal development and it is also a marker of cell death under conditions of neuronal injury [38]. In this study, administration of nevirapine alone did not significantly alter brain caspase-3 activity. In groups of mice that were administered MSG with nevirapine, caspase-3 activity increased significantly compared to vehicle-treated groups; however, when compared to MSG group, there was no significant difference. Increased caspase-3 activity has been associated with apoptotic neuronal injury with reports that the administration of caspase-3 inhibitors could improve neurological deficits (38). Glutamate excitotoxicity (which is expected in MSG induced neurotoxicity) has been reported to occur via caspase-independent programmed cell death mechanisms that involve Calpain 1 (39).

Studies have demonstrated the ability of nevirapine to cross the blood-brain barrier in quantities that are significant enough to alter brain chemistry (40,41). There have been suggestions that neurotoxicity is a possible mechanism by which highly active antiretroviral therapy (HAART) could result in HIV-associated neurological disorders. Also, the administration of HAART to HIV patients has been associated with the development of progressive neuron loss (42). This effect has also been reported in animal models (24). Neurotoxicity has been linked to nucleoside reverse transcriptase inhibitors, protease inhibitors and non-nucleoside reverse transcriptase inhibitor. There have been reports that nevirapine significantly inhibited creatinine kinase activity (40) and cytochrome C oxidase (41) in the hippocampus, cerebrum and striatum of mice. In this study, administration of nevirapine alone was associated with increased cellularity (mainly small neurons which are likely to be glial cells) with increasing doses.

A number of studies have reported changes in brain morphology associated with the administration of MSG (25,30).In the present study, administration of MSG at 2g/kg resulted in morphological and morphometric changes in keeping with neurotoxicity. Administration of nevirapine (7.5 and 15 mg/kg) with MSG resulted in a worsening of MSG-induced toxicity. However, with nevirapine at 30 mg/kg, a mild preservation of cerebral cortex morphology and morphometry were observed. Also, in the cornus ammonis and dentate gyrus, similar effects were observed.

CONCLUSION

In this study, we observed that subchronic administration of nevirapine (either alone, or in a background of neurotoxic injury) was associated with variable dose-related changes in the parameters that were measured. The changes observed are generally indicative of protection against certain aspects of MSG-induced neurotoxicity, at some doses. However, the implication of these findings in relation to nevirapine use in the young, and a widening of the indications for its remains to be determined. Further studies would reveal the potential mechanisms for neuroprotection.

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