Paradoxical Increase in 3-Nitropropionic Acid Neurotoxicity by α-Phenyl-tert-butyl-nitrone, a Spin-Trapping Agent

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Background: 3-Nitropropionic acid (3-NP), a mitochondrial toxin, impairs cellular energy generation by inhibiting succinate dehydrogenase. The basis of its neurotoxicity is oxidative stress in the wake of cellular energy failure. α-Phenyl-tert-butyl-nitrone (PBN), a spin-trapping agent with free radical-scavenging capability, has shown protective effects in various models of experimental brain insults. The effect of PBN on the 3-NP neurotoxicity paradigm was evaluated in this study.

Methods: Two groups of adult male mice receiving daily systemic 3-NP administration were pretreated with PBN or normal saline respectively for 5 days. After the treatment course, motor dysfunction and the volume of cerebral lesions were quantitatively evaluated. Cellular apoptosis and expressions of glial fibrillary acidic protein (GFAP) and cyclooxygenase-2 (COX-2) in the brain were compared between the 2 groups.

Results: All mice treated with normal saline and 3-NP survived but developed mild motor dysfunction. Apoptosis of striatal cells was noted in the absence of destructive cerebral lesions. In contrast, combined treatment with PBN and 3-NP resulted in more severe motor dysfunction and higher mortality in experimental animals. Destructive lesions with cellular necrosis, and enhanced expressions of GFAP and COX-2 were noted in the striatum.

Conclusions: 3-NP neurotoxicity was paradoxically accentuated by the combined treatment with PBN and 3-NP. Metabolic clearance of 3-NP is probably impaired by PBN and the increased oxidative stress caused by higher 3-NP levels may exceed the free radical-scavenging ability of PBN. The shift from apoptotic to necrotic changes with increased 3-NP toxicity is in accord with the theory that cellular energy reserves determine the pattern of cellular death.

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Key words: 3-nitroptopionic acid, spin-trapping agent, mitochondrion, apoptosis, necrosis.

Accumulated evidence has shown that mitochondrial dysfunction plays a pivotal role in the development of certain neurodegenerative disorders such as Parkinson's disease, Huntington's disease, and motor neuron disease.[1-3] Mitochondrial toxins with specific biochemical properties are thus often

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applied in elucidating pathogenic mechanisms of these clinical conditions. 3-Nitropropionic acid (3-NP), a mycotoxin which causes irreversible inhibition of succinate dehydrogenase,[4] may induce characteristic basal ganglion lesions in humans and animals treated with it. Due to the selective involvement of the striatal neurons, 3-NP-treated experimental animals may present pathological features mimicking those of Huntington's disease in humans.[5] Similar to other mitochondrial toxins, the neurotoxicity of 3-NP has been largely attributed to the production of reactive oxidative species (ROS) during cellular energy failure. Accordingly, specific management strategies for reducing oxidative stress have been shown to attenuate 3-NP-related neuronal damage and neurological dysfunction.[6-9]

Spin-trapping agents are a group of chemicals characterized by potent antioxidative effects. They are so-called "free radical scavengers" capable of capturing free radicals and converting them into stable abducts by transferring them to carbon-nitrogen bonds of related nitrones.[10] In various conditions of neuronal injury with increased ROS production including both global and focal ischemia,[11,12] traumatic brain injury,[13] and exposure to excitatory neurotoxins,[14] spin-trapping agents have been found to attenuate cerebral lesions and enhance neuronal tolerance in experimental animals or cultured cells. In this study, we examined the effects of a spin-trapping agent, α-phenyl-tert-butyl-nitrone (PBN), in 3-NP treated mice to assess its potential neuroprotective effect.

METHODS

Animals and materials

Male ICR mice weighing 30~35 g were used for the controls and experiments. Animals were maintained under a 12-h light-dark cycle with free access to food and drinking water. 3-NP (Sigma, St. Louis, MO, USA) was dissolved in normal saline (12.5 mg/ml) and adjusted to pH 7.4 with sodium hydroxide. PBN (Aldrich, Milwaukee, WI, USA) was dissolved in normal saline to a concentration of 12 mg/ml. Fresh solvents of both agents were made up every day.

Experimental design

For evaluating motor dysfunction, destructive brain lesions, and DNA fragmentation, mice were subjected to intraperitoneal injections of 3-NP in a dose of 50 mg/kg every 12 h for 5 consecutive days. One group of mice (the 3-NP/PBN group, n = 12) was also administered PBN intraperitoneally at a dose of 30 mg/kg, while the other group (3-NP/NS, n = 12) was given an equivalent amount of normal saline, 30 min before each 3-NP injection. For the control group (n = 6), normal saline in individually adjusted volumes was injected every 12 h. Twelve hours after the final injection, the mice were deeply anesthetized with ketamine (80 mg/kg) and xylazine (4 mg/kg) and decapitated. The brains were obtained and sectioned in 20-μm thicknesses on a freezing microtome (Leica Microsystems, Germany). Another 3 groups of mice received intraperitoneal injections of 3-NP and normal saline (the 3-NP/NS group, n = 6), 3-NP and PBN (the 3-NP/PBN group, n = 6), or normal saline only (the control group, n = 3) for 3 days, respectively, and were used to examine the expressions of glial fibrillary acidic protein (GFAP) and cyclooxygenase-2 (COX-2). Animals were anesthetized with ketamine and xylazine and subsequently perfused transcardially with ice-cold heparin (10 U/ml) and 3.7% formaldehyde. The brains were removed, placed in 3.7% formaldehyde overnight, and sectioned in 50-μm thicknesses on a vibratome (Pelco International, CA, USA).

Evaluation of motor function

Motor function was evaluated before the first injection and prior to sacrifice by a grading scale adapted from that of Ludolph et al.[15] The 6 grades of the scale are as follows: grade 0, normal activity; grade 1, general slowness in movement due to hindlimb impairment; grade 2, prominent gait abnormality with poor coordination; grade 3, nearly complete hindlimb paralysis; grade 4, inability to move due to impairment of all 4 limbs; and grade 5, recumbency and lying on its side.

Evaluation of brain lesions and cellular morphology

Cryosections of brain tissue were stained with cresyl violet. Lesion volumes were calculated by summing the unstained areas of each section and multiplying the distance between sections (0.5 mm). Measurements were made with the help of an image analysis system (Image-Pro Plus, Media Cybernetics,

Silver Spring, MD, USA. Morphological changes in cells were observed under light microscopy.

Terminal deoxytransferase-mediated dUTP-biotin nick-end labeling (TUNEL)

To detect DNA fragmentation, TUNEL was performed according to the method described by Gavrieli *et al*. with modification.[16] Frozen brain sections were air-dried and fixed with 3.7% formaldehyde in 0.1 mM phosphate-buffered saline (PBS) for 45 min. Endogenous peroxidase activity was blocked with 60 mM hydrogen peroxide for 30 min. Slides were placed in 1 terminal deoxynucleotidyl transferase (TdT) buffer (Boehringer Mannheim, Indianapolis, IN, USA) at room temperature for 15 min. They were then treated with TdT and biotinylated 16-uridine-5'-triphosphate (Boehringer Mannheim) at 37° for 60 min. The reaction was stopped by washing with 6 mM sodium citrate and 60 mM sodium chloride twice for 15 min. The tissue was incubated with 2% bovine serum albumin in PBS for 30 min. After 3 washes with PBS, they were incubated in a moisture chamber with the avidin-biotin-peroxidase complex (Vector, Burlingame, CA, USA) for 30 min at room temperature. Slides were washed with 1 PBS for 10 min, followed by 0.175 M sodium acetate twice for 15 min. Finally the staining was visualized with 0.025% 3,3' diaminobenzidine tetrahydrochloride (DAB) and 0.25% hydrogen peroxide with 10 mg/ml nickel sulfate and counterstained with 0.5% methyl green.

GFAP and COX-2 immunohistochemistry

GFAP and COX-2 expressions of formaldehyde-fixed brain tissue were examined by an immunohistochemical method using a free-floating technique. Endogenous peroxidase activity was eliminated by 0.66% hydrogen peroxide in PBS with 0.3% triton-X (T-PBS). The tissue was washed and then incubated with 10% goat or rabbit sera in T-PBS for 90 min to reduce any non-specific binding. After being washed in T-PBS, the tissue was reacted overnight at 4°C with polyclonal rabbit antiserum against GFAP (Dako A/S, Glostrup, Denmark) at a dilution of 1: 500 or with polyclonal goat antiserum against COX-2 (Santa Cruz, CA, USA) at 1: 200. Sections were washed with T-PBS 3 times and incubated with 1: 300-diluted biotinylated anti-rabbit or anti-goat immunoglobulin G (Vector, Burlingame, CA, USA), followed by 1% avidin-biotin-peridoxidase complex in the Vector ABC kit. After the PBS wash, the immunoactivity was developed with 0.02% diaminobenzidine and 0.2% hydrogen peroxide, and sections were counterstained with 0.5% methyl green. In the negative control, samples were treated using the same procedure with the omission of the primary antibody.

GFAP immunoreactivities were observed under a light microscope and were assessed according to the sizes, staining intensities, and cytoplasmic processes of the positively stained astroglial cells. COX-2-positive cells were observed and counted in 12 separate fields (at 600x magnification) of the striatum and averaged for comparison.

Statistical analysis

Motor dysfunction in the 3-NP/NS and 3-NP/PBN groups was compared using the Mann-Whitney U-test. Numbers of COX-2-positive cells were expressed as the mean ± standard deviation (SD), and multiple comparisons between groups were made by 1-way ANOVA with the *post hoc* Bonferroni test. A p value of less than 0.05 was considered statistically significant.

RESULTS

In general, development of neurological impairment followed a similar pattern in mice which received 3-NP. The first manifestation of motor dysfunction was a distinctive "stretching-of-the-body" posture on initiation of walking due to hindlimb weakness, which developed on the third day of 3-NP administration. Later on, the mice walked with the abdomen dragging along the ground, and their movements on the vertical axis ("standing on hindlimbs") decreased. No mouse in the 3-NP/NS group subsequently developed motor dysfunction greater than grade 3. In the 3-NP/PBN group, the initial presentations were similar to those of the 3-NP/NS group, while the final outcomes were variable. Of particular note, more-severe movement abnormalities developed, including tremors, dystonic posture, complete paralysis of the hindlimbs, no ambulation due to involvement of all 4 limbs, and complete recumbency. All mice in the 3-NP/NS group survived, while 4 mice in the 3-NP/PBN group had died by the time the treatment was complete. The median grades

(ranges) of motor dysfunction were 0 (0 to 1), 2 (1 to 3), and 2 (1 to 4) in the control, 3-PN/NS, and 3-NP/PBN groups, respectively. The difference between the 3-NP/NS and 3-NP/PBN groups was not statistically significant ($p = 0.068$) (Fig. 1).

Histologically, no mice in the 3-NP/NS group demonstrated any evident cerebral lesions. Of special note, cells with shrunken cytoplasm, punctuate chromatin condensation, and fragmented nuclei were found in the striatum where TUNEL staining showed a large number of positively stained cells, especially in the dorsolateral portion (Fig. 2B, D). In the 3-NP/PBN group, destructive lesions in animals with grade 3 and 4 motor dysfunctions were limited to the striatum, and lesion volumes were 9.3 and 21.3 mm3, respectively (Fig. 2A). In the striatal lesions, swollen cells with pale cytoplasm were evident and were considered to be undergoing necrotic change. Only a few TUNEL-positive cells were found at the periphery of the lesions (Fig. 2C, E).

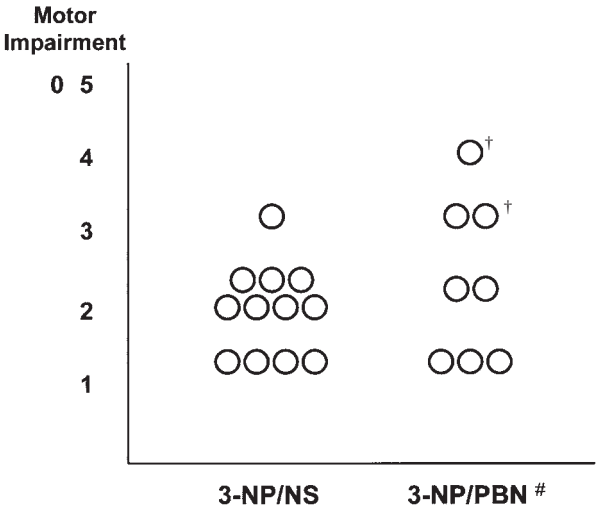
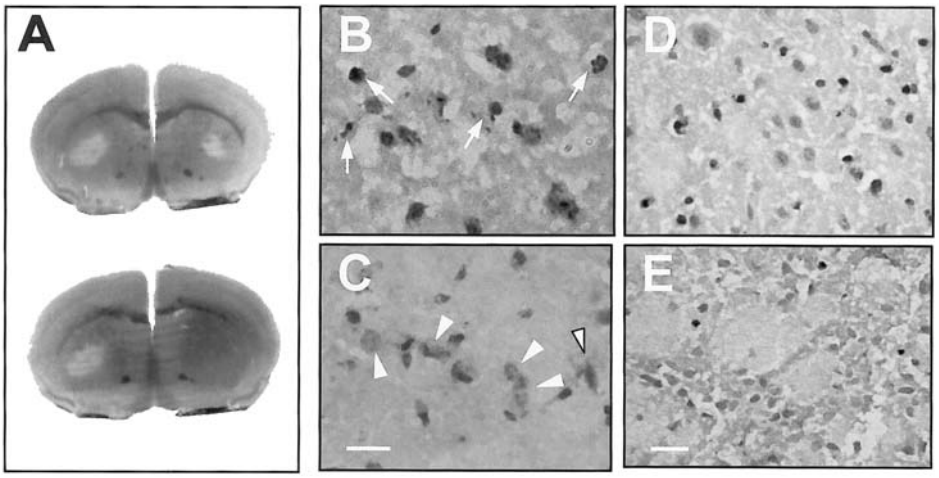


Fig. 1 Comparison of motor dysfunction of mice between the 3-NP/NS group (n = 12) and the 3-NP/PBN group (n = 12) ($p = 0.068$, Mann-Whitney U-test; #: not including 4 animals which died; †: with destructive striatal lesions).

Expression of GFAP in the striatum had obviously increased in 3-NP treated mice. Furthermore, its expression was more prominent in the 3NP/PBN group compared to the 3NP/NS group. Similarly, expression of COX-2 in the striatum was accentuated in both the 3-NP/NS and 3-NP/PBS groups, and the number of COX-2-positive cells in this region in the 3-NP/PBN group (47.9 ± 11.9) was significantly higher than those in the 3-NP/NS (39.2 ± 9.6) and control groups (25.2 ± 6.5, $p < 0.01$) (Figs. 3-4).

DISCUSSION

Results of this study indicate that PBN tended to



Figs. 2 Cresyl violet staining in the 3-NP neurotoxicity model on day 5 of treatment showing (A) destructive striatal lesions in a 3-NP/PBN-treated mouse, (B) typical apoptotic cells with condensed and fragmented nuclei (arrows) in the lateral striatum of a 3-NP/NS-treated mouse, and (C) cells with necrotic appearance (arrowheads) in the striatum of a 3-NP/PBN-treated mouse. TUNEL staining revealing (D) numerous reactive cells at the same region in B, but (E) only a few at the periphery of the lesion in C (bar: 20 μm in B and C, and 25 μm in D and E).

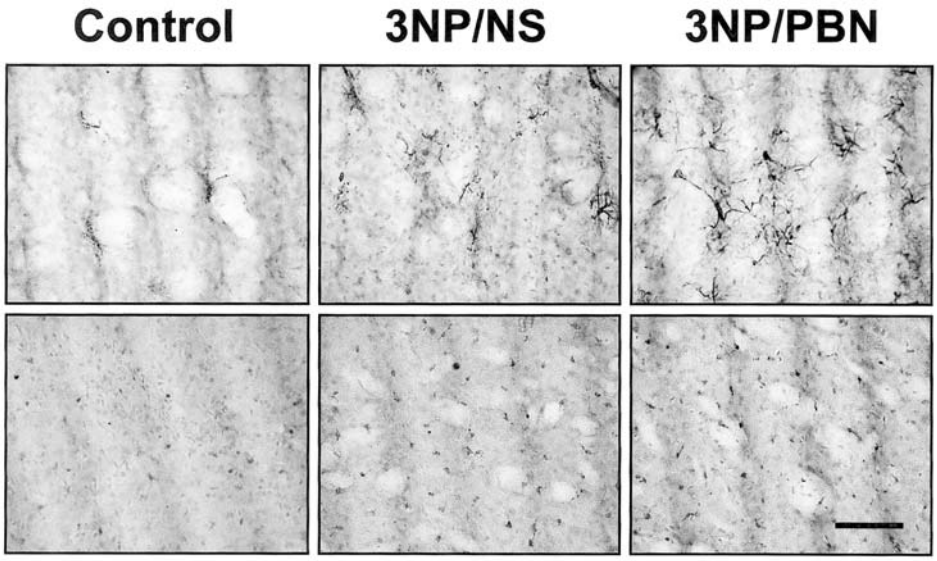


Fig. 3 Immunohistochemical reactions of GFAP (upper row) and COX-2 in the striatum (lower row) in the control, 3-NP/NS, and 3-NP/PBN groups following 3 days of treatment (bar: 100 μm).

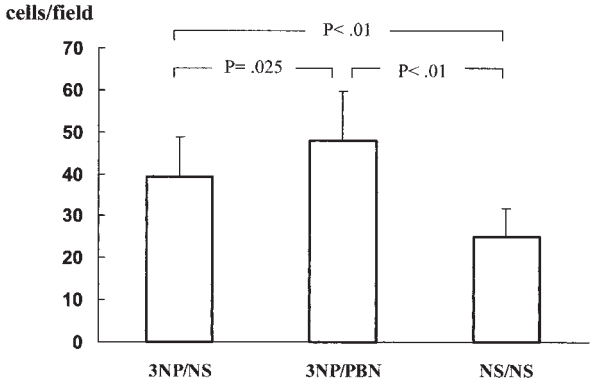


Fig. 4 Numbers of striatal cells with COX-2 immunoactivity in the 3-NP/NS (n = 6), 3-NP/PBN (n = 6), and control (n = 3) groups following 3 days of treatment ($p < 0.01$ by one-way ANOVA).

accentuate 3-NP neurotoxicity with evidence of increased mortality and motor dysfunction, and development of destructive cerebral lesions. These findings were accompanied by enhanced expressions of COX-2 and GFAP in the striatum. 3-NP has been shown to increase striatal GFAP expression, the extent of which parallels 3-NP toxicity.[17] Although COX-2 expression in the brain has not been studied in a 3-NP paradigm, COX-2 was found to be upregulated in response to conditions producing impaired cerebral energy metabolism, such as with cerebral ischemia and cyanide intoxication.[18,19] Under such conditions, inflammatory reactions and increased ROS production, i.e., the same reactions evoked by 3-NP toxicity, contribute to brain damage.[20] Thus, the accentuated expressions of GFAP and COX-2 in the current study suggest that increased 3-NP toxicity occurred with PBN treatment.

Although PBN has been shown to exert neuroprotective effects in several models of brain insult,[11-14] controversies still remain. In an infant rat model of group B streptococcal meningitis, PBN effectively reduced oxidative injury and attenuated neuronal damage in the cortex and hippocampus.[21] In contrast, PBN augmented neuronal apoptosis in the hippocampus and learning deficits in rats infected with *Streptococcus pneumoniae*.[22] This discrepancy could have been due to the pathogen-specific ability to modulate the environmental oxidative-reductive state and pathogen-dependent activation of antiapoptotic pathways. In a traumatic brain injury model, PBN increased neuronal apoptosis in the early stage, but this was accompanied by reduced cortical lesions and favorable behavioral outcomes.[23] Reports concerning the effects of PBN in a 3-NP neurotoxicity model are also conflicting. In an experiment using 3-NP treatment of cultured neurons, PBN was added to

reduce oxidative stress.[24] Under the conditions in that study, neuronal death caused by 3-NP was ameliorated by PBN. In another study using an intrastriatal injection of 3-NP in rats, neither motor deficits nor brain lesions were altered by the systemic administration of PBN.[25] Although the inadequate elimination of free radicals by PBN was postulated, the possibility of involvement of other mechanisms could not be excluded. Furthermore, Schulz *et al*. evaluated the effects of PBN and 2 other spin-trapping agents, *n*-tert-butyl- α-(2-sulfophenyl)-nitrone (S-PBN) and 5,5-dimethyl-1-pyrroline-*n*-oxide (DMPO), in reducing the toxicity evoked by the systemic administration of 3-NP in rats.[8] They found that only DMPO had a protective effect, while PBN and S-PBN were both detrimental. It was further shown that the plasma content of 3-NP was higher in rats pretreated with S-PBN, a phenomenon not noted with DMPO treatment. They inferred that either S-PBN or PBN may impede metabolic clearance of 3-NP via inhibition of hepatic cytochrome p450 monooxygenase, and the accentuated oxidative stress might eventually have exceeded the free radical-scavenging capability. In our study, a wide range of susceptibilities among the mice receiving 3-NP plus PBN treatment could be explained by interindividual variations in the activities of catalytic enzymes.[26] Therefore, when applying an agent with protective effects in vitro to in vivo conditions, its modulation of host metabolic or other physiological functions, which may unexpectedly lead to adverse reactions, must also be taken into consideration.

In our study, 3-NP-induced apoptosis of striatal neurons shifted to necrotic changes with the combined PBN and 3-NP treatment. Considering that inhibition of the mitochondrial function of energy generation underlies 3-NP toxicity, the above finding could be regarded as the presentation of 2 distinct features of cellular death in response to a gradual deficiency in the energy supply.[27] With mild to moderate energy depletion in neuronal cells, dysfunction of the N-methyl-D-asparate (NMDA) receptors allows excessive entry of calcium ions.[5] Subsequently, overt uptake of intracellular calcium ions by mitochondria may lead to release of apoptotic activators,[28] thus triggering the apoptotic process. On the other hand, with severe depletion of cellular energy, NMDA receptors are fully activated, and the intracellular overload of calcium ions disrupts the electron transport chain. This further aggravates the energy deficiency and, in association with the massive release of free radicals, causes necrosis.

Finally, the current study also showed that 3-NP can cause selective striatal damage without gross tissue destruction. Of particular interest, experimental animals developed motor deficits and neuronal apoptosis with a quite fixed pattern. These findings warrant further characterization to establish a novel animal model of apoptosis in the striatum, in the hope of opening more avenues for the testing of antiapoptotic strategies.

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REFERENCES

1. Mann VM, Cooper JM, Krige D, Daniel SE, Schapira AH, Marsden CD. Brain, skeletal muscle and platelet homogenate mitochondrial function in Parkinson's disease. Brain 1992;115:333-42.
2. Gu M, Gash MT, Mann VM, Javoy-Agid F, Cooper JM, Schapira AH. Mitochondrial defect in Huntington's disease caudate nucleus. Ann Neurol 1996;39:385-9.
3. Swerdlow RH, Parks JK, Cassarino DS, Trimmer PA, Miller SW, Maguire DJ, Sheehan JP, Maguire RS, Pattee G, Juel VC, Phillips LH, Tuttle JB, Bennett JP Jr, Davis RE, Parker WD Jr. Mitochondria in sporadic amyotrophic lateral sclerosis. Exp Neurol 1998;153:135-42.
4. Coles CJ, Edmonson DE, Singer TP. Inactivation of succinate dehydrogenase by 3-nitropropionic acid. J Biol Chem 1979;254:5161-7.
5. Brouillet E, Condé F, Beal MF, Hantraye P. Replicating Huntington's disease phenotype in experimental animals. Prog Neurobiol 1999;59:427-68.
6. Fu YT, He FS, Zhang SL, Zhang JS. Lipid peroxidation in rats intoxicated with 3-nitropropionic acid. Toxicon 1995;33:327-31.
7. Beal MF, Ferrante RJ, Henshaw R, Matthews RT, Chan PH, Kowall NW, Epstein CJ, Schulz JB. 3-Nitropropionic acid neurotoxicity is attenuated in copper/zinc superoxide dismutase transgenic mice. J Neurochem 1995;65:919-22.
8. Shulz JB, Henshaw DR, MacGarvey U, Beal MF. Involvement of oxidative stress in 3-nitropropionic acid neurotoxicity. Neurochem Int 1996;29:167-71.
9. Kim GW, Copin JC, Kawase M, Chen SF, Sato S, Gobbel

GT, Chan PH. Excitotoxicity is required for induction of oxidative stress and apoptosis in mouse striatum by the mitochondrial toxin, 3-nitropropionic acid. J Cereb Blood Flow Metab 2000;20:119-129.
10. Kotake Y. Pharmacologic properties of N-tert-butylnitrone. Antioxid Redox Signal 1999;1:481-99.
11. Sakamoto A, Ohnishi ST, Ohnishi T, Ogawa R. Protective effect of a new anti-oxidant on the rat brain exposed to ischemia-reperfusion injury: inhibition of free radical formation and lipid peroxidation. Free Radic Biol Med 1991,11:385-91.
12. Cao X, Phillis JW. α-Phenyl-tert-butyl-nitrone reduces cortical infarct and edema in rats subjected to focal ischemia. Brain Res 644:267-72,1994.
13. Awasthi D, Church DF, Torbati D, Carey ME. Pryor WA. Oxidative stress following traumatic brain injury in rats. Surg Neurol 1997;47:575-82.
14. Ferger B, van Amsterdam C, Seyfried C, Kuschinsky K. Effects of α-Phenyl-tert- butylnitrone and selegiline on hydroxyl free radicals in rat striatum produced by local application of glutamate. J Neurochem 1998;70:276-80.
15. Ludolph AC, He F, Spencer PS, Hammerstad J, Sabri M. 3-Nitropropionic acid – exogenous animal neurotoxin and possible human striatal toxin. Can J Neurol Sci 1991;18:492-8.
16. Gavrieli Y, Sherman Y, Ben-Sasson SA. Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. J Cell Biol 1992;119:493-501.
17. Wullner U, Young AB, Penney JB, Beal MF. 3-Nitropropionic acid toxicity in the striatum. J Neurochem 1994;63:1772-81.
18. Iadecola C, Foster C, Nogawa S, Clark HB, Ross ME. Cyclooxygenase-2 immunoreactivity in the human brain following cerebral ischemia. Acta Neuropathol 1999;98:9-14.
19. Li L, Prabhakaran K, Shou Y, Borowitz JL, Isom GE. Oxidative stress and cyclooxygenase-2 induction mediate cyanide-induced apoptosis of cortical cells. Toxicol Appl Pharmacol 2002;185:55-63.
20. O'Banion MK: Cyclooxygenase-2: molecular biology, pharmacology, and neurobiology. Crit Rev Neurobiol 1999;13:45-82.
21. Leib SL, Kim YS, Chow LL, Sheldon RA, Tauber MG. Reactive oxygen intermediates contribute to necrotic and apoptotic neuronal injury in an infant rat model of bacterial meningitis due to group B streptococci. J Clin Invest 1996;98:2632-9.
22. Loeffler JM, Ringer R, Hablutzel M, Tauber MG, Leib SL. The free radical scavenger alpha-phenyl-tert-butyl nitrone aggravates hippocampal apoptosis and learning deficits in experimental pneumococcal meningitis. J Infect Dis 2001;183:247-52.
23. Lewen A, Skoglosa Y, Clausen F, Marklund N, Chan PH, Lindholm D, Hillered L. Paradoxical increase in neuronal DNA fragmentation after neuroprotective free radical scavenger treatment in experimental traumatic brain injury. J Cerebr Blood Flow Metab 2001;21:344-50.
24. Olsen C, Rustad A, Fonnum F, Paulsen RE. Hassel B. 3-Nitropropionic acid: an astrocyte-sparing neurotoxin in vitro. Brain Res 1999;850:144-9.
25. Nakao N, Brundin P. Effects of alpha-phenyl-tert-butyl nitrone on neuronal survival and motor function following intrastriatal injections of quinolinate or 3- nitropropionic acid. Neuroscience 1997;76:749-61.
26. Transon C, Lecoeur S, Leemann T, Beaune P, Dayer P. Interindividual variability in catalytic activity and immunoreactivity of three major human liver cytochrome P450 isoenzymes. Eur J Clin Pharmacol 1996;51:79-85.
27. Beal MF. Energetics in the pathogenesis of neurodegenerative disease. Trends Neurosci 2000;23:298-304.
28. Green DR, Reed JC. Mitochondria and apoptosis. Science 1998;281:1309-12.

旋電捕捉劑α-苯基-三-丁基-硝基化合物反常地加重三-硝基丙酸之神經毒性

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背 景：粒腺體毒物三-硝基丙酸（3-nitropropionic acid）可抑制琥珀酸脫氫酶（succinate dehydrogenase）而降低細胞能量的生成。其具有的神經毒性之作用機轉，係經由細胞能量不足時，所產生的氧化壓力上升所致。α-苯基-三-丁基-硝基化合物（α-phenyl-tert-butyl-nitrone）是一種具有清除自由基功能的旋電捕捉劑。在某些腦損傷的實驗模式中，它被證實具有神經保護之作用。本研究係探討α-苯基-三-丁基-硝基化合物對於三-硝基丙酸所產生神經毒性之影響。

方 法：兩組成年雄性小鼠在每日注射三-硝基丙酸前先行給予α-苯基-三-丁基-硝基化合物或生理食鹽水，在完成5日之上述處置後，分別比較兩組動物之運動功能異常程度、腦中病灶體積、紋狀體中細胞凋亡、神經膠質纖維酸性蛋白（glial fibrillary acid protein）和第二型環化氧酶（cyclooxygenase-2）表現之情況。

結 果：接受生理食鹽水及三-硝基丙酸之小鼠在療程結束時雖全部存活，但皆產生輕微的運動異常。其腦部在紋狀體處出現大量凋亡細胞，但並未有壞死之病灶。同時接受α-苯基-三-丁基-硝基化合物與三-硝基丙酸處置之小鼠則在療程中死亡或結束時產生較嚴重之運動異常，其在紋狀體處出現破壞性病灶與細胞壞死現象外，此處神經膠質纖維酸性蛋白與第二型環化氧酶之表現程度亦較前組小鼠更爲顯著。

結 論：在本實驗中α-苯基-三-丁基-硝基化合物反而加重三-硝基丙酸之神經毒性，其原因可能是α-苯基-三-丁基-硝基化合物抑制三-硝基丙酸在身體中的代謝，使三-硝基丙酸累積造成的氧化壓力增加之情況超過α-苯基-三-丁基-硝基化合物的自由基清除功能。此外，由三-硝基丙酸毒性上升，致使紋狀體細胞由凋亡轉變成壞死變化的現象，則符合細胞能量存餘決定細胞死亡形成之理論。

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關鍵字：三-硝基丙酸，旋電捕捉劑，粒腺體，凋亡，壞死。

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