

Nitric Oxide is Protective Against Mercury Induced Depression

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Article info:

Received: 6 June 2010

First Revision: 17 June 2010

Accepted: 25 June 2010

ABSTRACT

Introduction: Mercury is the second most metal pollutant in the world and has the potential to induce many pathologic conditions, especially in nervous system, such as depression. Here we tried to find out if nitric oxide has any possible role in the pathophysiology of depression induced by this metal. Although the role of nitric oxide has been shown in mood control, here we use specific doses of nitric oxide inducer and/or inhibitors which had no effect on normal rats.

Methods: 120 male wistar rats weighting 200-250 gram were divided into two main groups: control and methyl mercury(MM) treated. Each main group was divided into four different sub-groups: Saline, L-Arginine, L-Name or 7-nitroindazole (7-NI) respectively. The duration of taking MM or saline was daily for 15 days for both. After the 15th injection a forced swimming test was done. This test shows behavioral immobility (BI) or latency of attempt to escape (LAE), as a depression indicator.

Results: Our study showed that low dose L-arginine is protective against MM induced depression as it could turn behavioral immobility (BI) to normal levels in groups taking MM plus L-Arginine, while in group taking just MM, BI was much longer showing the intensity of depression. L-Name and 7-NI did aggravated depression in MM groups but not control ones, on the other hand just in the case of 7-NI the result was significant.

Discussion: Our results showed 1) MM could induce depression in rat 2) L-Arginine could improve depression to normal situation in MM group, while in control group has no effect 3) 7-NI, a selective nNOS inhibitor can aggravate mental depression in intoxicated rats. These results showed the important role of nNOS in protection against MM induced depression.

Key Words:

Methyl Mercury;
Mental Depression;
Inflammation;
Oxidative Stress;
Nitric Oxide

1. Introduction

Regard to DSM-IV depression has three subtypes, including: Dysthymia, chronic major depression, and major depression superimposed with dysthymia (American psychiatric association: Diagnostic and statistical manual of mental disorders, 2005). Diagnos-

tic criteria for dysthymic disorder are: low mood during the day or most times of the day for at least one year in adults or two years in children, with at least two of following items: decrease or increase of appetite, insomnia or hypersomnia, loss of energy, low self-confidence, concentration and decision making disorders, feel of sadness and hopelessness (Freidman, 1995).

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Depression is among the 10 most prevalent diseases in the world and the most prevalent mental disorder too. 25 percent of people will experience it at least once during their life. It has been cited as the first reason for employee absences around 2010, too. It is twofold more prevalent in women (American Psychiatric Association: Diagnostic and statistical manual of mental disorders, 2005). Depression can lead to concentration disability and therefore, problems in social and interpersonal relationships. In many cases it may cause family and job loss or even suicide (Freidman, 1995).

Genetic factors may prone people to evolve mental depression. Prevalence of depression is 57 percent in identical twins. Chromosome 6 genes are among the most suspicious ones. Polymorphisms of these genes predispose people to this condition and make them more prone to develop depression. It means environmental factors such as social and personal conflicts may switch on depression in genetically susceptible individuals (Swivan, 1981). Researchers in Rochester University have suggested that screening for depression-prone subjects may have a gene linkage possibly in chromosome 6 (Swivan, 1981). Although stress responses are physiologically regulated and occur normally in everyday life, if they become persistent and severe depression can result (Kessler et al., 1994). Stress hormones try to overcome the situation following stress, but in continuous, uncontrolled stress they have some deleterious effects on the brain, especially on the hippocampus (Kroenke, 2003; Schneider, 1958; Wu, Parkerson, & Doraiswamy, 2002). Environmental pollutants also may cause depression (Ventura et al., 2004). Mercury is the most prevalent “metal pollutant” in the world. Two different types of mercury are organic and inorganic ones. The main source of mercury is from inside the earth. Volcanoes, earthquakes, and deep wells (such as petroleum pumps), can cause mercury to seep to the surface of the earth, at any strata. Planktons in the water digest the mercury and change inorganic mercury to more toxic organic types. Fishes will then eat the plankton and the next ring of the chain will be humans. This is one of the most common ways to cause mercury intoxication; two other routes are through dental fillings with amalgam and vaccinations (Crinnion, 2000). However mercury intoxication in human makes different organ dysfunctions, of which the most prominent affected, is nervous system. Ataxia, dysarthria, and convulsions can be induced by mercury intoxication (Fukuda, Ushijima, Kitano, Sakamoto, & Futatsuka, 1999; Kauppinen, Komulainen, & H, 1989). However depression is also among the first signs and symptoms of this process (Ventura et al., 2004) (8). There is no clinical differentiating factor between other types of depression and mercury induced ones (Chuu, Liu, & Lin-Shiau, 2001; Kauppinen et al., 1989).

Previous studies have shown that nitric oxide (N.O.), as a multi-potent agent, has an important role in the pathophysiology of depression, which comes from its specific properties (Marek & Duman, 2002). However it should be considered that N.O. based on its concentration and location can act as both as an oxidant or as an antioxidant agent (Yamashita, 1997) .

2. Methods

120 male wistar rats, weighting 200-250 gram, with free access to water and standard food, living in 12 hour dark/cycle entered the study. Efforts were made to minimize animal suffering. Rats were divided to two main groups (Table 1), case (n=60) and control (n=60). Methyl mercury (M.M.) intoxication was induced in case groups orally (gavage). They were fed M.M. (5 mg/kg, daily) for 14 days with a feeding syringe. The control group took saline with the same plan. Each main group divided into four subgroups, taking one of the following agents for 14 days of treatment, including: 7 Nitro-indazole, a neuronal specific NOS inhibitor (5mg/kg, subcutaneous, twice a week), or L-Name, a nonspecific NOS inhibitor (10 mg/kg, IP, twice a week), or L-Arginine, a N.O. donor agent (50 mg/kg, IP, every other day), or saline (1ml/kg, IP, twice a week).

Table 1. shows groups of the study (n: 120)

Methyl mercury (n: 60)	Vehicle (n: 60)
Saline (n: 15)	Saline (n: 15)
L- Arginine (n: 15)	L- Arginine (n: 15)
L- Name (n: 15)	L- Name (n: 15)
7- Nitroindazole (n: 15)	7- Nitroindazole (n: 15)

NEUROSCIENCE

It has been shown that M.M. intoxication with this dose may affect normal gait after 17 days after intoxication (Yamashita, 1997). However the behavioral test (FST) was started in the 15th day after the first M.M. Administration. The rats were checked for any movement abnormality before each behavioral test session. Abnormal moving rats didn't enter the test phase.

Modified Forced Water Swim Test

Modified forced water swim test is a version of classic “forced water swim test” The forced water swim test was first introduced by Porsolt and his colleagues (Porsolt, Le Pichon, & Jalfre, 1977). This behavioral test helps to detect depression, or better to say its sever-

ity of course, and evaluation of antidepressant drugs in rodents (Mendes-da-Silva et al., 2002; Porsolt et al., 1977). The device used in this test is a container with 42 cm height, 105 cm in diameter, made of steel and black colored inside. The container was filled with water (with a temperature of 25 °C) up to 18 cm height (Mendes-da-Silva et al., 2002). After each trial the container was cleaned and washed. The test makes an inescapable situation for the experimental animals. As a result there is a spectrum in behavioral response from hyperactivity and try to escape risky situation (which is named “latency of attempt to escape” or “LAE”) to inactivity, no motivation for escape and just prevention of death by putting one’s nose up on the water surface (this reaction is named “behavioral immobility” or “BI”). The Longer time periods of BI shows the severity of depression based on length (Mendes-da-Silva et al., 2002; Porsolt, 2000). The time that it takes to escape from situation, swimming or climbing, is named “latency of the attempts to escape” or “LAE”. It shows that the animal still has the motivation to stay alive, has enough energy and tendency to get rid of lethal situations (Mendes-da-Silva et al., 2002). If the response while flooded in water was staying immobile just to ventilate with no attempt to escape, it is considered as a marker of despair or depression; it is named behavioral immobility or “BI.” All groups had a pretest trial session and a test the day after. In the pretest trial animals were put in the center of the container for fifteen minutes (Mendes-da-Silva et al., 2002). The day after, in the test session, each rat had the same experience but just for five minutes. After each trial rats were removed from water and dried gently with a cotton towel (Mendes-da-Silva et al., 2002).

3. Results

The mean behavioral immobility (BI) of eight different groups were compared using one-way ANOVA test and Tukey as post-hoc, for revealing the differences between groups. The results (Figure 1) showed that there was a significant difference in behavioral immobility score between studied groups ($p < 0.05$).

Effect of methyl mercury on behavioral immobility

Tukey post-hoc- analysis showed that BI is increased in the methyl mercury group (methyl mercury + saline) compared to vehicle treated group ($p < 0.05$).

Effect of nitric oxide modulators on behavioral immobility

The results indicated that none of the 7-nitroindazole, L-Name, or L-Arginine groups had significant effect on BI score in vehicle treated rats ($p > 0.05$). But in M.M. treated groups results were different.

Groups that have received 7-nitroindazole with M.M. showed more BI compared to M.M. plus saline ($p < 0.05$). L-Name also increased BI score but it was not significant ($p > 0.05$). On the other hand L-Arginine decreased BI score in M.M. treated rats ($p < 0.05$) to the level of control groups.

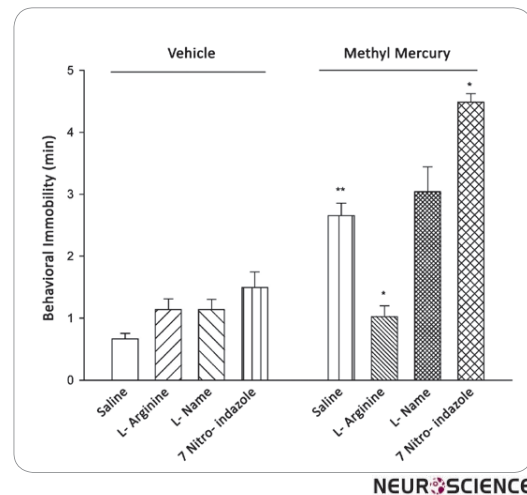


Figure 1. Results of one-way ANOVA test and tukey as post-hoc show the effects of L-Arginine (50 mg/kg, IP, every other day for 2 weeks), L-Name (10 mg/kg, IP, twice a week for 2 weeks), or 7 nitro-indazole (5mg/kg, s.c, twice a day for 2 weeks) on behavioral immobility (BI) in modified forced swimming test, induced by methyl mercury (5 mg/kg, by gavage, for 15 days) or vehicle (saline). Each group consists of 15 male wistar rats. * $p < 0.05$, difference is significant compare to Methyl Mercury+saline group. ** $p < 0.05$, difference is significant compare to Vehicle+saline group.

4. Discussion

In parallel or even exceeding to technical, industrial and medical developments, prevalence of mood and related behavioral disorders such as depression, anxiety, violence, alcoholism and aggression are also growing up (Siblerud, 1994). Recent studies have shown that environmental and food pollutions with heavy metals and certain medical procedures such as vaccination and dental filling with amalgam may have a role in this phenomenon. Mercury has attracted researcher attention in this filed and some epidemiologic studies have shown the relation of depression with mercury (Crinnion, 2000; Windham, 2010). Although the effects of mercury as a neurotoxic heavy metal well studied in cerebellar degeneration and related ataxia (Fukuda et al., 1999; Kauppinen et al., 1989; Yamashita, 1997) its possible role in the etiology of depression is less known. The etiology and involved neural systems in depression is not clearly identified. However, it has been

shown that inflammatory process may have a role in the pathogenesis of depression; especially in some animal models of depression there was a relationship between inflammatory process, hippocampus damage and depressive behavior (Holsboer, 2001; Leonard, 2007; Marek & Duman, 2002).

Results of the study show that chronic intoxication with methyl mercury increases behavioral immobility in modified forced swim test in rats. It means that chronic methyl mercury intoxication can induce depression. Another finding of the study is that nitric oxide may have a role in this process.

It has been shown that mercury intoxication can damage the nervous system with different mechanisms, such as release of free radicals, inflammation and interference with neurotransmitters release and uptake (Albrecht & Matyja, 1996; Bemis & Seegal, 2000; Brookes, 1992; Hobson & Rajanna, 1985; Hussain, 1997; Kim & Satoh, 2000; Mahboob, Shireen, Atkinson, & Khan, 2001).

On the other side 7-NI, L-Name or L-Arginine, with the dosage used in our study, had no considerable effect on BI in control groups. However, In M.M. treated groups that took NOS modulator agents, the results were different. 7-NI in M.M. group increased "BI" indicating severity of depression, and possibly more oxidative damage to brain. 7-NI is a nNOS (neuronal nitric oxide synthase) is a selective neural NOS inhibitor; it could be suggested that, after 7-NI administration, reduction of brain N.O. concentrations and its protection against oxidative stress, in result, yield an inflammatory reaction in brain. In such a condition clinical mental depression starts.

On the other hand L-Name in M.M. treated groups could increase but these changes would not be meaningful. Probably it means L-Name as a nonspecific NOS inhibitor cannot block nNOS enough to make considerable decrease in N.O. concentration; so there was an increased "BI," but again not significant. On the other hand it could be suggested that, in oxidative conditions in the brain, L- Name cannot stop antioxidant concentrations of N.O., so there will be enough active enzymes to inhibit the oxidative stress process.

And finally we could show that L-Arginine (a N.O. donor) could prevent mental depression in M.M. intoxicated rat (normal "BI" level). However, it should be considered that, this dose of L-Arginine had no obvious effects on the normal behavior of rat. It can be concluded that as M.M. makes oxidative stress, an acceptable modest increase in N.O. concentration can be helpful in protecting the brain from injury.

We can suggest that M.M. induced mental depression could be prevented by low doses of nitric oxide, and can worsen completely much more severely after nNOS inhibition, by 7-NI and to some extent L-Name, which is not a specific NOS inhibitor.

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