American-Eurasian J. Agric. & Environ. Sci., 2 (1): 85-89, 2007
ISSN 1818-6769

β-Glucan Sex-Dependently Attenuates the Hyperalgesic Effects of Arsenite in Rats

[1]A.S. Oyadeyi, [2]V.A. Togun, [3]J.K. Oloke and [3]O.G. Oyeyiola

[1]Department of Physiology, Faculty of Basic Medical Sciences,
[2]Department of Animal Production and Health, Faculty of Agricultuiral Sciences,
[3]Biotechnology Center, Department of Pure and Applied Biology,
Ladoke Akintola University of Technology, P.M.B. 4000, Ogbomoso. Oyo State, Nigeria

Abstract: Against a backdrop of arsenite neurotoxicity, we investstigated the effects of arsenite on pain processing and whether the effects could be reversed by the administration of β-glucan, an antioxidant from Saccharomyces Cerevisae. Arsenic (5 mg kg^{-1}) significantly reduced tail flick latency 48 h after administration in animals. The hyperalgesia appeared sex-specific as female rats showed higher degree of hyperalgesia than male rats. Direct oral administration of β-glucan (0.5 mg kg^{-1}) significantly increased tail-flick latency while indirect administration through feeding of Saccharomyces Cerevisae-digested feed failed to produce any significant attenuation of the hyperalgesia. The analgesia produced by β-glucan also appeared to be sex specific. We conclude that arsenic produces hyperalgesia in rats probably through oxidative damage and that the hyperalgesic effect are reversible by antioxidants.

Key words: Arsenite · β-glucan · pain · hyperalgesia · nociception

INTRODUCTION

Humans and animals live in a complex environment and are subjected to various biological, Physical and environmental stimuli. Exposure to a variety of laboratory and environmental factors such as arsenite has been shown to induce a number of behavioural and physiological responses including alterations in nociceptive processing [1-3]. Arsenic is a nonessential trace element; it is a potent, toxic, mutagenic and xenobiotic metalloid. Arsenite has been growing rapidly during the last 5 years as a major pollutant of drinking water in several regions of the world [4, 5].

Arsenic, as trivalent arsenite (As^{3+}) or pentavalent arsenite (As^{5+}) is naturally occurring and ubiquitously present in the environment. Humans are exposed to arsenic mainly through either oral or inhalation routes. Oral exposure occurs via consumption of contaminated water, food and drugs [6]. Arsenic has been claimed to be of clinical utility in the treatment of syphilis, amoebiasis and certain other tropical diseases [7] and has also been used in Fowler solution in the treatment of arthritis [7]. Arsenic exposure results in endemic arsenic dermatosis along with hyperkeratosis, gangrene and skin cancer [8]. Arsenic intoxication in experimental animals has recently been associated with hepatic tumors [9], the inhibition of testicular steroidogenic function [10], incapacitation of Leydig cell function, negative effects on caudal epididymal milieu [11] and spermatogenesis [12]. It has also been implicated with severe metabolic disorders such as diabetes in humans [13]. Arsenic exposure has been reported to result in structural changes in the thymus of pregnant and newborn mice [14]. Long-term exposure of arsenic is associated with abortion, low birth weight and reduced lactation [15] as well as with embryonic cells toxicity *in vitro* [16]. Survey reports from the Ukraine, Taiwan and Bangladesh revealed that the intake of arsenic-contaminated drinking water caused reproductive disturbances in women [17], adverse pregnancy outcomes [18] and spontaneous abortion [19]. Acute arsenic exposure may promote immediate gastrointestinal tract infection [20], while chronic effects may exert degenerative, inflammatory and neoplastic changes of the respiratory, haematopoetic, cardivascular and nervous systems [21]. There is however a lack of literature data on the possible effects of arsenate on nociceptive processing, a possibility that is very strong in the light of arsenite-induced inflammatory perturbations on one hand [21] and its peripheral neuropathic effects on the other [6]. Peripheral neuropathy, among other

Correspondence Author: Dr. A.S. Oyadeyi, Department of Physiology, Ladoke Akintola University of Technology, P.M.B. 4000, Ogbomoso, Oyo State, Nigeria

impacts on the sensory system, will most definitely affect nociceptive processing because of the close association between the state of peripheral nerves and the sensory modality they transmit.

The deleterious effects of arsenite have been attributed to oxidative damage, thus an antioxidant should protect against or reverse its toxicity. The need for preventing arsenite-induced toxicity is underscored by the fact that there are no animal models to study the mechanisms of its toxicity while there is an increase in the population of those who are at risk of arsenite poisoning, especially in the developing world [22].

In an on-going effort to understand the mechanisms of arsenite poisoning as well as preventing it, we examined the effects of arsenite on nociception and the possibility of using β-glucan, a potent anti-oxidant, to prevent its toxic effects on nociception. As a secondary aim, we also attempted to determine whether the method of obtaining β-glucan had any effect on its potency.

MATERIALS AND METHODS

Animals: Adult male and female Sprague-Dawley rats were used. They were kept in a temperature (20-21°C) and light-controlled (12 h light:12 h dark cycle) room with free access to food and water.

Synthesis of β-glucan: β-glucan was synthesized as previously described by Hunter *et al.* [23]. Briefly, active dry yeast was added to 0.1 mol l^{-1} of NaOH and stirred for 30 min at 60°C. The material was then heated to 115°C at 8.5 psi for 45 min and then allowed to settle for 72 h. The sediment was re-suspended and washed in distilled water by centrifugation (350 g for 20 min). The alkali insoluble solids were with mixed 0.1 mol l^{-1} acetic acid and heated to 85°C for 1h, then allowed to settle at 38°C. The acid insoluble solids were drawn off and centrifuged as above. The compacted solid material was mixed with 3% H_2O_2 and refrigerated for 3 h with periodic mixing. The material was then centrifuged and the pellet washed twice with distilled water, followed by two washings in 100% acetone. The harvested solid material was dispersed on drying trays and dried under vacuum at 38°C for 2 h in the presence of Ca_2SO_4. It was then further dried overnight under vacuum at room temperature.

Synthesis of β-glucan indirectly from Saccharomyces cerevisae (SC)-digested feed: β-glucan was also synthesized indirectly by using SC to digest rice bran as previously described by Iyayi and Aderolu [24]. Briefly the rice bran was dried to constant weight at 60°C; 25 kg of the rice bran was autoclaved and oven-dried. The autoclaved material was then innoculated with SC under aseptic conditions after adjusting the moisture level to 25%. After 14 days, the biodegradation reaction was stopped and the material was then dried.

Nociceptive test: Nociceptive test was carried out by a modification of D'Amour and Smith's tail flick test [25]. Each animal was gently hand-held in a dry towel while the distal two-third of the tail was immersed in water maintained at 50±1°C. The time it took the animal to flick out its tail from the water was recorded as tail-flick latency.

Protocol: The animals were randomly divided into 4 groups and after basal nociceptive threshold was taken for all the rats, those in group I (n=8) were administered 0.2 ml of normal saline. Group II (n=7) were fed digested rice bran in addition to normal rat chow. Group III (n=8) served as control while Group IV rats (n=7) were administered 0.5 mg kg^{-1} (p.o) of the synthesized β-glucan.

Immediately after, all the groups received 5 mg kg^{-1} (i.p) arsenite. They were returned to their home cages and nociceptive testing was carried out 48 h later using the tail-flick test.

RESULTS

Effects of β-glucan on arsenite-induced hyperalgesia: Table 1 shows the baseline tail-flick latency in all animals.

Arsenic significantly produced hyperalgesia 48 h after administration in control animals (Table 2) of both sexes. This level of hyperalgesic effects appeared to be related as female animals showed higher degree of hyperalgesia than males.

Direct β-glucan significantly reduced the hyperalgesia while indirect administration via digested

Table 1: Baseline Tail flick latency in all groups of animals

	Tail flick latency (Secs)	
Groups	Male	Female
CNS	27.94±1.02	24.14±0.41
PRB	39.80±0.58	39.00±2.01
PBE	24.62±1.52	11.02±1.17
CSA	31.60±1.86	24.24±0.63

CNS - Arsenite + normal saline PRB - Arsenite + digested feed
PBE - Arsenite + β-glucan CSA - Arsenite alone

Table 2: Tail flick latency after 48 h

	Tail flick latency (Secs)	
Groups	Male	Female
CNS	28.10±1.01	24.20±0.52
PRB	17.44±0.25*	34.40±1.32*
PBE	32.78±0.33*	13.52±1.14*
CSA	22.16±0.69	12.58±1.20*

*Significant, compared with control, $p< 0.01$, student t-test

CNS-Arsenite + normal saline PRB-Arsenite + digested feed

PBE-Arsenite + β-glucan CSA-Arsenite alone

Table 3: Percentage effects on nociceptive processing in males and females

	Males	Females
CNS	0.50% Analgesia	0.24% Analgesia
PRB	56.18% Hyperalgesia	11.79% Hyperalgesia
PBE	33.14% Analgesia	22.69% Analgesia
CSA	29.88% Analgesia	75.66% Analgesia

feed failed to produce any significant alteration on hyperalgesia (Table 3). This analgesia effect was more prominent in the male animals than the females.

DISCUSSION

Arsenic produced a conspicuous hyperalgesia in rats. This hyperalgesia was evident in the significantly reduced tail-flick latency. Arsenic is however not the only substance that can cause facilitated nociceptive processing. Acute intra-peritoneal vitamin C [26] and acute restraint stress [27] have also been reported to induce hyperalgesia, although the underlying mechanisms may be different.

Hyperalgesia, generally occurs when the firing threshold of Aδ and C nociceptive afferent is lowered into the non-noxious range [28]. The mechanisms involve synthesis of arachidonic acid from membrane lipids via the steroid-sensitive enzyme Phospholipase A_2. Arachidonic acid is acted upon by the cyclooxygenase enzyme to produce prostaglandins, which act directly on the peripheral terminals of Aδ and C fibers and then lower their threshold [28]. Although we are not aware of any report that has examined the effect of arsenite on nociception, several links are possible. For instance, it has been severally documented that arsenite is a potent cytotoxic agent, whose cytotoxicity is not only rapid, as fast as 5 minutes after treatment [6] but also involves reactive oxygen species [22] which induce oxidative damage. Oxidative damage has been implicated in neurological disorders such as arthritis [29]. It is possible that arsenite induces hyperalgesia by damaging peripheral nerves.

Sex differences in nociception have been documented by several investigators with females more generally responsive to pain [30]. The result of higher arsenite-induced hyperalgesia in females compared to males, is consistent with the gamut of evidences that has shown females to respond more to noxious stimuli than males. Although we did not examine the mechanisms, gonadal hormones [31], menstrual cycle [32] and psychosocial factors [33] are some of the factors documented as been responsible for gender differences in pain processing. Our results also show greater analgesic effects of direct β-glucan in males than females. If we take β-glucan as a pharmacologic agent and it is, then this is consistent with studies that have also documented differential analgesic responses in males and females. For example, Icero *et al.* [34] have reported enhanced sensitivity to morphine in males compared to females, a fact that has been subsequently confirmed [35].

The lack of significant effect on account of the digested rice bran consumed by the rats is not surprising. In all reality, the time lapse of forty eight hours was too little for any significant deposition of β-glucan or any other fungal metabolite for that matter. One study that reported enhanced feeding value of rice bran after fermentation with Trichoderma viridae was carried out over several days [24]. It is known that the ability of fungi to degrade fiber lasts several days [36, 37]. Even if there had been adequate digestion of rice bran by Trichoderma viride, the short period of feeding would not have guaranteed sufficient intake of the substance contained in the feed by the animals.

In summary, we report the ability of β-glucan to sex dependently attenuate the hyperalgesia induced by arsenite in rats. This is in concord with reports that have shown that many fungal metabolites can be utilized to treat a wide variety of diseases like inflammation and arthritis [38].

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the assistance of the following students: Owojaiye Kehinde, Olarotimi Tolulope, Adurodolorun Olubukola, Eniolorunda Olayinka, Olawumi Oluwaseun, Aroyehun Adeola, Ajonijebu Chris.

REFERENCES

1. Amit, Z. and Z.H. Galina, 1986. Stress-induced analgesia: adaptive pain suppression. Physiol. Rev., 66: 1091-1120.
2. Bodnar, R.J., 1986. Neuropharmacological and neuroendocrine substrates of stress-induced analgesia Ann. N.Y. Acad. Sci., 467: 345-360.
3. Lewis, J.W., 1986. Multiple neurochemical and hormonal mechanisms of stress-induced analgesia. Ann. N.Y. Acad. Sci., 467: 194-204.
4. Chappell, W.R., B.D. Beck, K.G. Brown, R. Chaney, C.C. Richard, K.J. Irgolic and D.W. North, 1997. Inorganic arsenic: A need and an opportunity to improve risk assessment. Environ. Health Perspect., 105: 1060-1065.
5. Nickson, R., J. McArthur, W. Burges, K.M. Ahamed, P. Ravenserof and M. Rahman, 1998. Arsenic poisoning of Bangladesh ground water. Nature, 395: 338-338.
6. Liu, S.X., M. Athar, I. Lippai, C. Waldren and T.K. Hei, 2001. Induction of oxyradicals by arsenic: Implication for mechanism of genotoxicity. Proc. Natl. Acad. Sci., 98: 1643-1648.
7. Klaassen, C.D., 1990. Heavy metals and heavy metal antagonists. In The Pharmaceutical Basis of Therapeutics (G.A. Goodman, T.W. Rall, A.S. Nies and P. Taylor, Eds.). Pergamen Press, New York, pp: 1602-1605.
8. Chowdhury, T.R., B.K. Mandal, G. Samanta, G.K. Basv, P.P. Chowdhury, C.R. Chanda, N.K. Karan, D. Lodh, R.K. Dhar and D. Das, 1997. Arsenic in ground water in six districts of West Bengal, India - The biggest arsenic calamity in the world: The status report up to August, 1995. In Arsenic Exposure and Health Effects (C.O. Abernathy, R.L. Calderson and W.R. Chappell, Eds.). Chapman and Hall, New York, pp: 93-111.
9. Waalkes, M.P., J.M. Ward, J. Liu and B.A. Diwan, 2003. Transplacental carcinogenicity of inorganic arsenic in the drinking water: induction of hepatic, ovarian, pulmonary and adrenal tumors in mice. Toxicol. Appl. Pharmacol., 186: 7-17.
10. Sarkar, M., N.M. Biswas and D. Ghosh, 1991. Effect of sodium arsenite on testicular $^{5^-}$3ß, 17ß-HSD activities in albino rats: Dose and duration dependent responses. Med. Sci. Res., 19: 789-790.
11. Togun, V.A., A.S Oyadeyi, J.K Oloke, A.O. Oyewopo, 2005. Effect of sodium arsenite with or without vitamin E on epididymal sperm motility and concentration in Sprague-Dawley rat. Proceedings of the 39th Conference of the Agricultural Society of Nigeria, pp: 57-58.
12. Sukla, J. P. and K. Pandey, 1984. Impaired spermatogenesis in arsenic-treated fresh water fish *Colisa fasciatus* (Bl and Sch). Toxicol. Lett., 21: 191-195.
13. Tseng, C.H., C.P. Tseng, H.Y. Chiou, Y.M. Hsueh, C.K. Chong and C.J. Chen, 2002. Epidemiologic evidence of diabetogenic effect of arsenic. Toxicol. Lett., 133: 69-76.
14. Skal'naia, M.G., A.A. Zhavoronkov and A.V. Skal'nyi, 1995. Morphologic characteristics of the thymus in pregnant and new born mice. Arkhiv Patologii, 57: 52-58.
15. Donald, M.C., R.A. Edwards and J.F.D. Greenhalgh, 1995. Minerals. In Animal Nutrition (M.C. Donald, R.A. Edwards and J.F.D. Greenhalgh, Eds.). Longman ELBS, England, pp: 127-136.
16. Lee, T.C., M. Oshimura and J.C. Barrett, 1985. Comparison of arsenic-induced cell transformation, cytotoxicity, mutation and cytogenetic effects in Syrian hamster embryo cells in culture. Carcinogenesis, 6: 1421-1426.
17. Zadorozhnaja, T.D., R.E. Little, R.K. Miller, N.A. Mendel, R.J. Taylor, B.J. Presley and B.C. Gladen, 2000. Concentrations of arsenic, cadmium, copper, lead, mercury and zinc in human placentas from two cities in Ukraine. J. Toxicol. Environ. Health, A61: 255-263.
18. Yang, C.Y., C.C. Chang, S.S. Tsai, H.Y. Chuang, C.K. Ho and T.N. Wu, 2003. Arsenic in drinking water and adverse pregnancy outcome in an arseniasis-endemic area in north-eastern Taiwan. Environ. Res., 91: 29-34.
19. hmad, S.A., M.H. Sayed, S. Barua, M.H. Khan, M.H. Faruquee, A. Jalil, S.A. Hadi and H.K. Talukder, 2001. Arsenic in drinking water and pregnancy outcomes. Environ. Health Perspect., 109: 629-631.
20. Goebl, H.H., P.F. Schmidt, J. Bohl, B. Teltenborn, G. Kramer and L. Gutmann, 1990. Polyneuropathy due to arsenic intoxication: Biopsy studies. J. Neurol., 49: 137-149.
21. Neiger, R.D. and G.D. Osweiler, 1989. Effect of subacute low level dietary sodium arsenite on dogs. Fundam. Appl. Toxicol., 13: 439-451.

22. Hei, T.K., Liu Six and C. Waldren, 1998. Mutagenicity of arsenic in mammalian cells: role of reactive oxygen species. Proc. Natl. Acad. Sci., 95: 8103-8107.
23. Hunter, K.W., R.A. Gault and M.D. Berner, 2002. Preparation of micro particulate β-glucan from Saccharomyces cerevisae for use in immune potentiation. Lett. Appl. Microbiol., 35: 267-276.
24. Iyayi, E. and Z. Aderolu, 2004. Enhancement of the feeding value of some agro-industrial by-products for laying hens after their solid state fermentation with Trichoderma viride. Afr. J. Biotechnol., pp: 182-185.
25. D'Amour, F.E. and D.C. Smith, 1965. A method for determining loss of pain sensation. J. Pharmacol. Exp. Ther., 72: 74-79.
26. Yadeyi, A.S., F.O. Ajao, A.O. Afolabi, U.S. Udoh and G.F. Ibironke, 2005. Chronic vitamin C administration induces thermal hyperalgesia in male rats. Nig. J. Health Biomed. Sci., 4: 153-155.
27. Oyadeyi, A.S., F.O. Ajao, G.F. Ibironke and A.O. Afolabi, 2005. Acute restraint stress induces hyperalgesia via non-adrenergic mechanisms in rats. Afr. J. Biomed. Res., 8: 123-125.
28. Basbaum, A. and M.C. Bushnell, 2002. Pain: Basic mechanisms. In: Pain 2002-An updated review: Referesher course syllabus, M.A. Giamberardino (ed). IASP press, seattle, pp: 1-2.
29. Sen, C.K., 1995. Oxidants and antioxidants in exercise. J. Appl. Physiol., 79: 675-686.
30. Fillingim, R.B., 2000. Sex, gender and pain: A biopsychosocial framework. In: Sex, Gender and Pain, R.B. Fillingim (ed). IASP Pres, Seattle, pp: 1-5.
31. Aloisi, A.M., 2000. Sensory effects of gonadal hormones. In: Sex, Gender and Pain, R.B. Fillingim (ed). IASP Press, Seattle, pp: 7-24.
32. Fillingim, R.B. and T.J. Ness, 2000. The influence of menstrual cycle and sex hormones on pain responses in humans. In: Sex, Gender and Pain, R.B. Fillingim (ed). IASP press, Seattle, pp: 191-207.
33. Robinson, M.E., J.L. Riley III and C.D. Myers, 2000. Psychosocial Contributions to sex-related differences in pain responses. In: Sex, Gender and Pain, R.B. Fillingim (ed). IASP press, Seattle, pp: 41-68.
34. Icero, T.J., B. Nock and E.R. Meyer, 1996. Gender-related differences in the antinociceptive properties of morphine. J. Pharmacol. Exp. Ther., 279: 767-773.
35. Boyer, J.S., M.M. Morgan and R.M. Craft, 1998. Microinjection of morphine into the rostral ventromedial medulla produces greater antinociception in male compared to female rats. Brain Res., 796: 315-318.
36. Ofunya, C.O. and C.J. Nwanjiuba, 1990. Microbial degradation and utilization of cassava peel. World J. Microbiol. Biotechnol., 6: 144-146.
37. Iyayi, E.A. and D.M. Losel, 2001. Changes in the carbohydrate fractions of cassava peels following fungal solid state fermentation. J. Food Technol. Africa, 6: 101-103.
38. Lull, C., H.J. Wichers and H.F.B. Savel Koul, 2005. Anti-inflammatory and immunomodulating properties of fungal metabolites. Mediators Inflamm,. pp: 63-80.