Histological study of the possible protective action of omega-3-fatty acids on the injurious effect induced by Bisphenol A on rat hippocampus

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ABSTRACT

Introduction: Bisphenol A (BPA), a well-known industrial chemical, has adverse effects on the brain even at relatively low exposure levels in rodents, primates and humans. Omega-3-fatty acids participate in a number of neuronal processes including neurogenesis, neuron differentiation, and neuro-protection.

Aim of the work: To investigate the possible protective action of omega-3-fatty acids on the injurious histological effect induced by Bisphenol A on rat hippocampus.

Materials and Methods: Eighteen adult male Wistar rats were divided into 3 equal groups; control, BPA (1.2mg/kg daily for 3 weeks, intraperitoneally) and the third group were given omega-3 (300mg/kg orally) in addition to BPA in the aforementioned dose and duration. Hippocampus sections were processed for hematoxylin and eosin staining, GFAB staining and electron microscopic examination.

Results: BBPA administration resulted into several histological alterations. Glial fibrillary acidic protein-positive cells were more abundant in the hippocampus of BPA-treated animals compared with the control animals. Ultra-structurally, the hippocampus of BPA-treated group showed nerve cells having nuclei with irregular outline and dilated perinuclear envelop, dilated RER and Golgi, swollen mitochondria with destroyed cristae. Some of the myelinated and unmyelinated nerve fibers showed degenerative changes. Concomitant administration of omega-3-fatty acids ameliorated these effects.

Conclusions: Omega-3-fatty acids partially minimized the severity of BPA-induced hippocampus injurious histological effects in Wistar rat.

Key Words: Bisphenol A, GFAB, Hippocampus, Histopathology, Omega-3-fatty acids

INTRODUCTION

Bisphenol-A (BPA); 2, 2, -bis (4-hydroxyphenyl) propane, is a necessary material for the production of plastics. BPA-containing material are used in the manufacture of many products, such as milk, water and infant bottles, food and beverage packaging, compact disks, eye glass lenses, dental sealants, artificial teeth, cans, drums, reinforced pipes, adhesives, nail polish and carbonless papers used in receipts. This extensive use of BPA products has led to great public concern since bad effects of BPA on human and animal reproduction are suspected.

This work aimed to study the histological changes induced by BPA in the hippocampus of Wistar rat and evaluate whether co-administration of omega-3 fatty acids could alter these effects.

PATIENTS AND METHODS

Animals

The present study was carried out on 18 adult male Wistar rats weighing (150–200 g). They were kept in clean...
properly ventilated cages under similar conditions and had free access to food and water throughout the experiment. They were accommodated to their new place one week before starting the experiment. All animals received humane care in accordance with the guidelines of research ethics committee, Sohag Faculty of Medicine, Egypt.

**Experimental design**

Rats were divided into 3 groups (6 animals each) as follows:

Group I (the control group): it was subdivided into 2 subgroups:

Subgroup IA: untreated group (3 animals).

Subgroup IB: animals received 1.5 ml corn oil (the solvent) intraperitoneally each day for 3 weeks.

Group II: animals received BPA, (Sigma, USA, purity 99%, CAT # 807-05-) dissolved in 1.5 ml corn oil (Sigma, USA, CAT # 80017-30-) and given in a dose of 1.2mg/kg intraperitoneally each day for 3 weeks[8].

Group III: Rats received omega-3 in a dose of 300 mg/kg orally via gastric tube[8] in addition to BPA in the same previous dose and duration. At the end of the experiment, all animals were anaesthetized with 4% halothane, perfused with paraformaldehyde 4% and sacrificed. Samples from the hippocampus were prepared for light microscopy, neutral-buffered formalin 10 % was used as fixative, followed by staining of the Sections (5µm thick) with hematoxylin and eosin stain[9].

**Immunohistochemistry for Glial fibrillary acidic protein (GFAP)**

Sections from hippocampus cut at 5 µm were fixed in 10% neutral buffered formalin for 2 days. Modified avidin -biotin peroxidase technique for glial fibrillary acidic protein GFAP (Thermo Scientific Co, Waltham, Massachusetts, USA) was applied to demonstrate the astrocytes as described previously[10]. Negative control sections were prepared by doing the same steps except that PBS was used in place of the primary antibody.

**Electron Microscopy**

Ten small pieces of tissue were fixed in 2.5% glutaraldehyde at pH 7.4 and 4°C. The specimens were then processed for electron microscopic examination. Semithin sections (0.5 um) were cut and stained with toluidine blue. Ultrathin sections (8090- nm) were cut using ultramicrotome. Uranyl acetate and lead citrate were used for staining[11]. The sections were examined by a JEOL electron microscope (Akishima, Tokyo, Japan) in the Faculty of Medicine, Tanta University.

**Quantitative evaluation for the immunostained sections**

The image analyzer system Leica Qwin 500 (Solms, Germany) in the National Institute of Research was used. GFAP-immunolabeled sections at 400 X magnification were examined and the positive data were calculated. The obtained data were analyzed using ANOVA and Post Hoc test. Significant P value was set at 0.05 (12).

**RESULTS**

**Light microscopy**

Group I

Normal histological structure of the hippocampus appeared after staining of the control group with H&E (Fig. 1A-F).

Group II (BPA-treated group)

Dentate gyrus: histological slides of the hippocampus samples of group II showed nerve cells of dentate gyrus that appeared degenerated and shrunken. The cells were surrounded by a large vacuolated pericellular space. The nuclei were darkly stained pyknotic (Fig. 2A).

Hippocampus proper: areas CA1, CA2 and CA3 of group II appeared disorganized with degenerated pyramidal cells. Loss of the normal arrangement of the pyramidal cells was evident. The cells were disorganized and irregular in shape. Shrunken cells with darkly stained nuclei that appeared surrounded by large vacuolated pericellular spaces were also found. Some cells appeared either with ghost-like shape or with degenerated fragmented nuclei, with loss of cellular characteristics (Figs. 2B-D).

Group III

Dentate gyrus: histological slides of samples of group III showed nerve cells of dentate gyrus with overall picture less severe compared to Bisphenol-treated group. Still there were some granule cells that were shrunken and having dark stained nuclei. Vacuolations were markedly decreased (Fig. 3A).

Hippocampus proper: areas CA1, CA2 and CA3 of group II appeared disorganized with degenerated pyramidal cells. Loss of the normal arrangement of the pyramidal cells was evident. The cells were disorganized and irregular in shape. Shrunken cells with darkly stained nuclei that appeared surrounded by large vacuolated pericellular spaces were also found. Some cells appeared either with ghost-like shape or with degenerated fragmented nuclei, with loss of cellular characteristics (Figs. 2B-D).

**Immunohistochemical results**

CA1 area was chosen to be studied in this research. The control group showed a few positive brownish star-
shaped GFAP immunoreactive cells dispersed in this area (Fig. 4A). Group II showed an apparent increase in the number and size of star-shaped GFAP immunoreactive cells (Fig. 4B). Group III showed less number and size of astrocytes of the CA1 area compared to Bisphenol-treated Rats (Fig. 4C).

**Morphometric results**

Statistical significant increase in the area percentage and the optical density of GFAP content in astrocytes was shown in group II (Bisphenol treated group) in comparison to group I (control group) and group III (Bisphenol+ Omeg-3-fatty acids). Group III showed significant increase compared to the group I and significant decrease compared to group II (Table1, Figs 4D&E).

**Electron microscopic results:**

**Group I**

Normal electron microscopic picture of the hippocampus was shown in samples of ultrathin sections of the hippocampus obtained from control rats (Fig. 5A-D).

**Group II**

Examination of ultrathin sections obtained from Bisphenol-treated animals showed prominent alterations. Some of the nerve cells showed nuclei with irregular outline and dilated perinuclear envelop. The cytoplasm showed vacuolation, dilated RER, dilated Golgi and swollen mitochondria with partial destruction of their cristae (Fig. 6A-C). Some of the myelinated nerve fibers showed irregular myelin sheath having thickening, partial disruption and the axoplasm revealed vacuolation and swollen mitochondria with destruction of their cristae (Fig. 6D and 7A). Some of the unmyelinated nerve fibers showed partial disruption and the axoplasm showed swollen mitochondria with destroyed cristae (Fig. 7B). As regards oligodendrocytes, some of them had shrunken eccentrically placed nucleus with dilated perinuclear envelop. Their cytoplasm revealed vacuolation and dilated RER (Fig. 7C).

**Group III**

Nerve cells showed normal nuclei and Golgi whereas it showed dilated RER and some mitochondria with destroyed cristae (Fig. 8A). Oligodendrocytes showed normal nuclei but its cytoplasm was still showing vacuolation and dilatation of RER (Fig. 8B). The myelinated nerve fibers showed almost normal axoplasm with normal mitochondria and microtubules. They preserved most of the compact lamellar structure with very minimal disruption (Fig. 8C). The unmyelinated nerve fibers looked almost normal with almost normal mitochondria and microtubules having only minimal disruption (Fig. 8D)
Fig. 1: A photomicrograph of a section of the hippocampus of Group I. (A) The hippocampus proper (CA1, CA2, CA3) and dentate gyrus (DG). (B) The dentate gyrus is formed of two limbs [suprapyramidal (S) and infrapyramidal (I)], crest (C), and hilus (H). (C) The three layers of the dentate gyrus: molecular (M), granular (G), and polymorphic (PL). The outer molecular layer contains neuroglial cells (curved arrow). The intermediate granular layer contains dense columns of granule cells (arrows). The inner polymorphic layer contains neuroglial cells (right angle arrow). (D) The CA1 area is formed of three layers: polymorphic (PL), pyramidal (P), and molecular (M). The pyramidal layer is formed of closely packed small pyramidal cells arranged in three or four rows with large vesicular nuclei, prominent nucleoli, and scanty triangular cytoplasm (curved arrow). Notice neuroglial cells (arrows). (E) The CA2 area is formed of polymorphic (PL), pyramidal (P), and molecular (M) layers. The pyramidal layer is formed of loosely packed small pyramidal cells (arrows). (F) The CA3 area is formed of polymorphic (PL), pyramidal (P), and molecular (M) layers. The pyramidal layer shows loosely packed triangular cells (arrows). H&E, A× 40, B× 100, C, D, E & F× 400.
Fig. 2: A photomicrograph of a section of the hippocampus of Group II. (A) The dentate gyrus with degenerated shrunken granule cells surrounded by a large pericellular space (arrows) and darkly stained nuclei (curved arrows). Note numerous vacuolations (V). (B) The degenerated, disorganized CA1 area. Some pyramidal cells appear shrunken with darkly stained nuclei and wide pericellular spaces (arrows), whereas others appear ghost-like (curved arrow) or with fragmented nucleus (right-angle arrow). (C) The CA2 area. Some pyramidal cells appear with darkly stained nuclei (arrows), whereas others appear ghost like (curved arrow). Note loss of normal pattern of pyramidal cell arrangement and vacuolations. (D) The CA3 area. Some of the pyramidal cells appear degenerated with darkly stained nuclei, shrunken and surrounded by a wide pericellular space (arrows) or ghost like (curved arrow).

H&E, × 400.
Fig. 3: A photomicrograph of a section of the hippocampus Group III rat. (A) The dentate gyrus with some shrunken granule cells surrounded by pericellular space (arrows) and darkly stained nuclei. Some vacuolations (V) are still seen. (B) The hippocampus at CA1 area of Group III shows less severe picture compared to Group II (less degeneration and less disorganization). Some pyramidal cells are still seen shrunken with darkly stained nuclei and wide pericellular spaces (arrows). (C) The CA2 area. The areas look more organized compared to the Bisphenol-treated group. Some pyramidal cells appear with darkly stained nucleus and pericellular spaces (arrows). (D) The CA3 area. Less number of pyramidal cells appears with darkly stained nucleus and pericellular spaces (arrows) compared to Group II. H&E, × 400.
Fig. 4: A photomicrograph of GFAP immunostained section of the hippocampus. (A) The control rat shows astrocytes in the CA1 area. The astrocytes appear as immunoreactive star-shaped cells having brown color (arrow). (B) The hippocampus of Group II rat shows astrocytes of the CA1 area. Note the apparent increase in the number and size of immunoreactive (brown) cells (arrows). (C) The hippocampus Group III showing less number and size of astrocytes of the CA1 area compared to Group II. GFAP immunostain, × 400. (D&E) are Graphs showing the mean percentage and mean field of optical density of GFAP positive astrocytes in the hippocampus of the tested groups. Data are represented as mean ± SEM. ***P < 0.001.
Fig. 5: An electron micrograph of a section in the hippocampus of Group I shows (A) Part of a normal nerve cell having euchromatic nucleus with less electron density (N), RER (arrow), polyribosomes (curved arrow), Golgi complex (G) and mitochondria(M), (B) Multiple nerve fibers which are either myelinated (curved arrow) or unmyelinated (double arrow), where numerous neurofilaments, microtubules and mitochondria (M) are seen. Notice the regular arrangement of myelin sheath (arrow), (C) An oligodendritic cell (arrow) with irregular nucleus (N) and nearby myelinated nerve fibers (curved arrows), (D) An astrocyte associated with a blood capillary (arrow and double arrow).
Fig. 6: An electron micrograph of a section in the hippocampus of Group II. (A) A nerve cell has a nucleus with irregular outline and dilated perinuclear envelop (arrow). The cytoplasm has dilated RER (curved arrow) and vacuoles (right angle arrow). (B) A nerve cell has a nucleus with irregular indented outline. Its cytoplasm has swollen mitochondria with partial destruction of their cristae (arrows). (C) A nerve cell has a nucleus with irregular outline and dilated perinuclear membrane (N). Its cytoplasm is vacuolated (arrows) and shows dilated Golgi (curved arrow). (D) Myelin sheath with thickening (arrow) and vacuolated axoplasm (V). Another one shows partial disruption (curved arrow) and swollen mitochondria with partial destruction of their cristae (right angle arrow).
Fig. 7: An electron micrograph of a section in the hippocampus of a rat of Group II. (A) Myelin sheath with thickening (arrow). Another one shows partial disruption (curved arrow). (B) Unmyelinated nerve fibers with partial disruption (arrows) and swollen mitochondria with destroyed cristae (curved arrow), (C) An oligodendrocyte with eccentric shrunken nucleus has dilated perinuclear membrane (arrow). Its cytoplasm shows vacuoles (right angle arrow) and dilated RER (curved arrow).
Fig. 8: An electron micrograph of a section in the hippocampus of a rat of group III. (A) A nerve cell has normal nucleus (N), normal Golgi (G), dilated RER (arrows) and swollen mitochondria with partial destruction of their cristae (curved arrows), (B) Oligodendrocyte with normal nucleus (N) cytoplasm is still showing dilated RER (arrow) and some vacuoles (curved arrow), (C) Normal myelinated nerve fiber having normal mitochondria and microtubules preserving most of its lamellar arrangement although still showing some thickening (arrow) and minimal disruption (curved arrow), (D) Normal unmyelinated nerve fiber with minimal disruption (arrow).
The effects of BPA on biological systems have been thoroughly researched both in vitro and in vivo, however the exact levels of exposure which can badly affect humans is under debate. Growing evidence suggests that BPA acts directly on neuronal functions as it is lipophilic and could accumulate in the brain. The present study revealed that low dose of Bisphenol A caused degenerative changes in the hippocampus of wistar rat. These results were in agreement with previous reports indicating that low exposure levels of Bisphenol A have adverse effects on the brain in both primates and rodents. One of these reports indicated that Bisphenol A exposure impaired the neurogenesis of hippocampus and affected memory and learning. Other reports indicated that the exposure in humans to BPA in early life affects neural development. Exposure to BPA during pregnancy is accompanied with manifestations of aggression and hyperactivity disorders in 2-year-old girls and with symptoms of anxiety and depression in young girls.

Our light microscopic results showed that the dentate gyrus in group II showed degenerative changes in the form of shrinkage and appearance of a large vacuolated pericellular space. The nuclei appeared darkly stained. It also showed that areas CA1, CA2 and CA3 of the Hippocampus proper in group II showed degenerated pyramidal cells that were disorganized. Disarrangement of the pyramidal cells might be explained by the adaptive response in the form of rearrangement of pyramidal cells. This might be a trial of pyramidal cells to regain their function. In line with these findings, other investigators proved marked disorganized pyramidal cell layer of the hippocampus.
in the brains of schizophrenic patients\textsuperscript{[20]}, Apoptosis might explain the appearance of dark neurons with markedly condensed cytoplasm and nucleoplasm\textsuperscript{[21]}. The appearance of vacuolated pericellular spaces was mostly due to shrinkage of nerve cells and withdrawal of their cytoplasmic processes secondary to disintegration of the cytoskeletal elements of these cells\textsuperscript{[22]}. It was proved that free radical attack on neural cells due to oxidative stress (OS) can provoke the degeneration of nerve cells. Bisphenol A exposure can lead to oxidative stress and DNA damage\textsuperscript{[23]}

GFAP is an intermediate filament protein that is found in numerous cell types of the CNS including astrocytes\textsuperscript{[24]} and ependymal cells\textsuperscript{[25]}. GFAP is known to be important in modulating astrocyte motility and shape by adding structural stability to astrocytic processes\textsuperscript{[26]}. GFAP is also shown as a specific marker for maturity of astrocytes of the CNS\textsuperscript{[27]}. It was found that injury of the CNS, whether because of trauma, disease, genetic disorders, or chemical factor lead to astrogliosis. In astrogliosis, rapid synthesis of GFAP takes place and is detected by immunostaining with GFAP antibody\textsuperscript{[28]}. Bisphenol A caused activation of astrocytes that was manifested in the present study in the form of increased expression of GFAP. This might be a compensatory mechanism for neuronal damage caused by Bisphenol A. These changes have been proved previously in other forms of neurotoxicity\textsuperscript{[29]}

Electron microscopic examination of Group II revealed some nerve cells with irregular nuclei and dilated perinuclear envelop. The cytoplasm showed vacuolation, dilated RER, dilated Golgi and swollen mitochondria with partial destruction of their cristae. Some of the oligodendrocytes had shrunk eccentrically placed nucleus with dilated perinuclear envelop. Their cytoplasm revealed vacuolation and dilated RER. The irregular nuclear outline or the shrinkage of the nucleus might be attributed to neuronal necrosis or DNA damage induced by Bisphenol A\textsuperscript{[30]}. The dilated perinuclear envelop, RER, Golgi and mitochondria can be accounted for by previous reports indicating that oxidative stress by Bisphenol A leads to increased intracellular ROS and oxidative cell damage\textsuperscript{[14 & 31]}. ROS is known to react with the polyunsaturated fatty acids of the biomembranes\textsuperscript{[32]}. Lipid peroxidation and fragmentation of protein, caused by Free radicals can lead to extensive cell damage\textsuperscript{[33]}. The cytoplasmic vacuolation was a result of lipid peroxidation theory indicated above, in addition to damage of the cell membrane as well as membranes of other cell organelles.

Ultrastructural examination of Group II also showed that some of the myelinated nerve fibers revealed irregular myelin sheath having thickening, partial disruption and the axoplasm revealed vacuolation and swollen mitochondria with destruction of their cristae. A possible explanation for this Bisphenol A-induced axonal degeneration indicated that reduced Na+/K+-ATPase activity could be an initiating event in ion disruption associated with swollen and, possibly, atrophied regions of distal axons\textsuperscript{[34]}. Other studies have reported that Ca2+ accumulation in the axon taking place as a reverse pathophysilogic process can start a destructive process of the axon\textsuperscript{[35]}. Axons discontinuity could be caused by the changes in basic myelin protein as a result of exposure to a toxic material. The myelin membrane is highly susceptible to damage from exposure to toxic materials, which can induce alterations in the myelin sheath\textsuperscript{[36]}. Some of the unmyelinated nerve fibers showed partial disruption and the axoplasm showed swollen mitochondria with destroyed cristae. This could be explained by the ‘dying back’ process of neuronal injury where injury of nerve cells made them incapable of maintaining their distal processes leading to axon degeneration\textsuperscript{[37]}

The present study results indicated that concomitant administration of Omega-3 fatty acids with Bisphenol A can help in reducing the neurodegenerative changes induced by Bisphenol A. DHA, a member of omega-3 fatty acids, is present in high concentration in the brain\textsuperscript{[38]}. DHA was found to participate in a number of neuronal processes including neurogenesis, neuron differentiation & survival and membrane integrity\textsuperscript{[39]}. DHA can protect the brain from reactive oxygen species and free radicals by increasing the activity of cerebral catalase and glutathione peroxidase\textsuperscript{[40]}. DHA may also be of great value for regenerative process of axons and dendrites after neuronal injury\textsuperscript{[41]}. Studies have reported that omega-3 PUFAs can give rise to significant neuroprotection after spinal cord injury. In an experimental study in rat hemisection and compression spinal cord injury, omega-3 PUFAs, addd early in the first hour after injury, can decrease neuronal and glial cell death, decline oxidative stress and the inflammatory cascade provoked by the primary injury, and lead to improved neurological function. Prominent studies in traumatic brain injury indicated a similar neuroprotective potential. Omega-3 PUFAs also possess neurotrophic properties\textsuperscript{[42]}

CONCLUSION

BPA exposure induced injurious histological effects in the hippocampus of Wistar rat. Concomitant administration of omega-3-fatty acids partially minimized the severity of these injurious effects. Further research is recommended in order to expand application of these results in clinical practice.

CONFLICT OF INTEREST

The authors have no conflict of interest.
EFFECT OF BISPHENOL A ON HIPPOCAMPUS

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EFFECT OF BISPHENOL A ON HIPPOCAMPUS

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Study of the possible protective effects of polyunsaturated fatty acids on the hippocampus of the rat: a histological study

Methodology:

Bisphenol A (BPA) is a chemically synthesized compound that has gained widespread use due to its potential for causing biological effects. However, the research on its potential for causing harm has increased, particularly due to reports of its neurotoxic effects. Bisphenol A has been shown to affect the hippocampus of rodents and primates at relatively low exposure levels, and it is known to disrupt the synthesis and differentiation of neural cells. In this study, the possible protective effects of polyunsaturated fatty acids (PUFAs) on the hippocampus of the rat were investigated.

Objectives:

The objective of this study was to investigate the possible protective effects of polyunsaturated fatty acids on the hippocampus of the rat exposed to Bisphenol A. The study was conducted on adult rats from the same strain. The animals were divided into three groups: the control group, the group exposed to Bisphenol A, and the group exposed to Bisphenol A and the protective effect of PUFAs. The study was carried out in three stages: histological, with the presence of histological changes and microscopic examination of the hippocampus; morphological, with the use of histochemical techniques; and behavioral, with the use of behavioral test batteries.

Results:

The results showed a significant histological change in the hippocampus of the rat exposed to Bisphenol A, including changes in the neuronal structure and the presence of reactive gliosis. The microscopic examination revealed the presence of neuronal atrophy and microglial activation. The histological and morphological changes were less pronounced in the group exposed to Bisphenol A and PUFAs. The behavioral tests showed a significant improvement in the animals exposed to PUFAs, indicating a protective effect of PUFAs on the hippocampus.

Conclusion:

It is concluded that polyunsaturated fatty acids have a protective effect on the hippocampus of the rat exposed to Bisphenol A. Future studies are needed to further investigate the protective effects of PUFAs on the hippocampus and to explore the mechanisms underlying these effects.

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The effects of Bisphenol A on the hippocampus of the rat: a histological study. The study was conducted on adult rats from the same strain. The animals were divided into three groups: the control group, the group exposed to Bisphenol A, and the group exposed to Bisphenol A and the protective effect of PUFAs. The study was carried out in three stages: histological, with the presence of histological changes and microscopic examination of the hippocampus; morphological, with the use of histochemical techniques; and behavioral, with the use of behavioral test batteries. The results showed a significant histological change in the hippocampus of the rat exposed to Bisphenol A, including changes in the neuronal structure and the presence of reactive gliosis. The microscopic examination revealed the presence of neuronal atrophy and microglial activation. The histological and morphological changes were less pronounced in the group exposed to Bisphenol A and PUFAs. The behavioral tests showed a significant improvement in the animals exposed to PUFAs, indicating a protective effect of PUFAs on the hippocampus. It is concluded that polyunsaturated fatty acids have a protective effect on the hippocampus of the rat exposed to Bisphenol A. Future studies are needed to further investigate the protective effects of PUFAs on the hippocampus and to explore the mechanisms underlying these effects.