

Assessment of the effects of oseltamivir and indomethacin on dopamine, 5-HIAA, and some oxidative stress markers in stomach and brain of *Salmonella typhimurium*-infected rats

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Abstract

OBJECTIVES: The purpose of this study was to measure the effect of oseltamivir and indomethacin on dopamine and 5-HIAA levels and some oxidative biomarkers in brain and stomach of young rats in conditions of infection.

METHODS: Female Sprague Dawley rats in absence or presence of a live culture of *Salmonella typhimurium* (*S. Typh*), were treated as follows: PBS, group 1 (control); oseltamivir (100 mg/kg), group 2; indomethacin (67 µg/kg) group 3; oseltamivir (100 mg/kg) + indomethacin (67 µg/kg), group 4. The drugs were administered intraperitoneally every 24 hr for 5 days while *S. Typh* was give orally in the first and third day. C-reactive proteins was measured in blood on sacrifice, and from brain extract, dopamine and 5-HIAA levels as well as GSH, calcium, and H₂O₂ and total ATPase activity were measured by validated methods.

RESULTS: Dopamine increased significantly in cortex and cerebellum/medulla oblongata of groups that received indomethacin and oseltamivir. 5-HIAA increased significantly in all groups that received *S. Typh*. H₂O₂ decreased significantly in cortex regions of animals that received oseltamivir and indomethacin in presence of *S. Typh*. Total ATPase increased significantly in cortex and hemispheres of groups that received oseltamivir as well as in cerebellum/medulla oblongata and stomach of animals that received oseltamivir and indomethacin combined with *S. Typh*. GSH increased and calcium decreased significantly in stomach of animals that received oseltamivir or indomethacin alone or combined with *S. Typh*.

CONCLUSION: These results demonstrate the association between inflammatory response, oxidative stress, dopaminergic, and serotonergic metabolism in an experimental inflammatory animal model.

INTRODUCTION

Oseltamivir is used for the treatment of influenza virus infections and generally it is well tolerated by adults with the most common adverse effects being nausea and vomiting. However, neuropsychiatric behaviors including jumping and falling from balconies by young patients being treated with oseltamivir have been reported (Yoshino *et al.* 2008). Some authors suggest that the increase in dopamine during oseltamivir treatment may have caused these abnormal behaviors. It is likely that the administration of oseltamivir phosphate in the presence of inflammation leads to an increase in the brain concentration of both parent drug and active metabolite and this may explain the central nervous system side-effects observed with this drug (Oshima *et al.* 2009). The pharmacological mechanism of the neuropsychiatric effects of oseltamivir in adults and in very young pediatric population remains unclear. Oseltamivir (Tamiflu) is now being stockpiled by Mexican governments as a first line treatment for an anticipated outbreak of swine influenza caused by AH1N1, which came into effect in late March 2009, due to an outbreak of a respiratory illness that was later proved to be caused by H1N1 (S-OIV) virus, a novel swine-origin influenza A.

The pharmacologic process of oseltamivir's neuropsychiatric effect could be traced to the action of lipopolysaccharides (LPS) or endotoxins that activate hypothalamic-pituitary-adrenal axis and cerebral catecholamine systems. In mice, it increases the brain concentrations of dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and 5-hydroxytryptamine catabolites as well as 5-hydroxyindoleacetic acid (5-HIAA) (Dunn 1992) as a consequence of LPS-induced inflammation. Likewise, LPS inhibits gastric antral contractions in conscious rats by its regulatory action on the expression of corticotropin-releasing factor type 2 receptor (CRF2) in the rat's stomach. Nozu et al in their study suggest that indomethacin, as a member of non-steroidal anti-inflammatory drug class, plays a special role in the treatment of headaches, and that it does not alter motor index but blocks the inhibitory action of LPS (Nozu et al, 2014). Although, some studies have proved that indomethacin has a therapy potential in protecting cerebral noradrenergic and dopaminergic systems against lipopolysaccharide-induced acute phase reactions (Adham *et al.* 2012), but the mechanisms are still unclear. Employing antioxidant enzymes as biomarkers of oxidative stress, Mehta et al., (1999) used enterotoxin of *Salmonella.typhimurium* (*S.Typh.*) to probe the generation of reactive oxygen species (ROS), potent mediators of inflammatory disorders, on cellular or animal models and found a loss of cell viability.

GSH, a tripeptide known as reduced glutathione, is also a ubiquitous reducing agent whose absence induces severe oxidative stress (Driver *et al.* 2000). It has been suggested that GSH interacts with nitric oxide (NO)

by giving S-nitrosoglutathione (GNSO), in the presence of endogenously produced oxygen (Benuck *et al.* 1995). GSH is the main redox equilibrium regulator and it plays an important role in the protection of the tissues suffering from damage by oxidative agents. Recent studies indicated that the use of zinc induces defensive mechanisms to the brain by diminishing free radical-induced lipid peroxidation (Bediz *et al.* 2006). Free radicals are reactive oxygen or nitrogen species with impaired electrons, which may induce oxidative damage to biologically important molecules, though membrane lipids are the main target (Beckman *et al.* 1990), and the central nervous system (CNS) is particularly susceptible to this type of damage.

Membrane lipids are known to strongly interact with the lipid bilayer structural proteins (Swapna *et al.* 2005), such as Na⁺-K⁺ ATPase, which is responsible for ion interchange across the membrane (Neault *et al.* 2001). The inhibition of Na⁺-K⁺ ATPase promotes the excitatory amino acid release in CNS (Hernández 1982). It is necessary to determine the effects of oseltamivir and indomethacin under an infection condition in order to establish methods for its safe administration as contemplated in this study and taken into consideration that swine influenza by AH1N1 produces inflammation. For this, the aim of this study was to determine the effect of these substances in an infected animal model on dopamine and 5-HIAA levels and some biomarkers of oxidative stress in juvenile rat brain.

MATERIAL AND METHODS

Female Sprague Dawley rats each with a weight of 80±5 g (4 weeks old) were recruited and divided into four experimental groups (n=6 each) in the presence and absence of a live culture of *S.Typh.* (1×10⁶CFU/ml) and treated as follows: Group 1, control (PBS); group 2, oseltamivir (100 mg/kg); group 3, indomethacin 67 µg; group 4, oseltamivir (100 mg/kg) + indomethacin (67 µg). All treatments were given intraperitoneally every 24 hours for 5 days. Oral administration of 1×10⁶CFU/ml of *S.Typh.* was done only in the 1st and 3rd days of treatment. The rats were sacrificed by decapitation 60 minutes after receiving the last dose of oseltamivir and indomethacin and the brains and stomachs were extracted and stored in NaCl 0.9% at 4 °C. Serum was obtained from the blood of the animals on sacrifice and used to measure C-reactive proteins. Brain dissection was carried out by sagittal cutting. The left cut was homogenized in 5 volumes of TRIS-HCl 0.05 M, pH 7.4 and used to assess H₂O₂ and Na⁺, K⁺ATPase. The right cut was homogenized in 5 volumes of perchloric acid (HClO₄) 0.1M and used to evaluate the levels of calcium, Glutathione (GSH), 5-HIAA, and dopamine. The rats were procured from bioterium of Centro Médico Nacional Siglo XXI, IMSS, Mexico City, and housed in groups of 4 or 5 in clean plastic cages and allowed to acclimatize in the room

environment for 1 day. Animals were maintained in a mass air displacement room with a 12-h light: 12-h dark cycle at $22\pm 2^\circ\text{C}$ with a relative humidity of $50\pm 10\%$. Balanced food (Rodent diet 5001) and drinking water were given to the animals ad libitum. Animal experiments were carried out under strict compliance with the Guidelines for Ethical Control and Supervision in the Care and Use of Animals and all experimental procedures were done following national and international rules.

Innoculation of rats

Rats were inoculated with *S. Typh* strain from strain bank (ceparium) of Experimental Bacteriology laboratory of National Institute of Pediatrics, Mexico City. The strain was re-identified and an aliquot of maintenance medium was inoculated in SS agar (Salmonella Shigella culture medium). The cultures were incubated for 18–24 h at 37°C . The isolated colonies with morphologies suggestive of *S. Typh* were selected and confirmed by conventional biochemical tests.

Inoculation preparation was carried out by sowing the strain in TSA (Trypticasein Soya Agar) and incubated at 37°C for 18 h. The bacterial biomass was collected with hyssop, re-suspended in buffer PBS, pH=6.8 and adjusted to an $AS_{450\text{nm}}=0.175$ (equivalent to 3×10^8 UFC/ml) using DU 640 spectrophotometer, (BECKMAN). It was later diluted to obtain a concentration of 1×10^6 UFC/ml (Thygesen *et al.* 1994). The inoculation was carried out by oral administration of non-lethal volumes of 1 ml per animal using orogastric tube.

Measurement of C Reactive Protein (PCR-Látex)

The procedure to measure PCR was performed from blood serum of all animal groups using direct agglutination side slide test for the determination of C Reactive Protein (PCR-Látex kit) (Wiener Lab Rosario, Argentina). A stereoscopic microscope (Carl Zeiss, West Germany) for assay agglutination was used to read results and these were reported as positives or negatives.

Technique for the measurement of dopamine (DA)

DA levels were measured in the supernatant of tissue homogenized in HClO_4 after centrifugation at 9,000 rpm for 10 min in a microcentrifuge (HettichZentrifugen, model Mikro 12-42, Germany), with a version of the technique reported by Calderon *et al.* (2008). An aliquot of the HClO_4 supernatant, and 1.9 ml of buffer (0.003 M octyl-sulphate, 0.035 M KH_2PO_4 , 0.03 M citric acid, 0.001 M ascorbic acid), were placed in a test tube. The mixture was incubated for 5 min at room temperature in total darkness, and subsequently, the samples were read in a spectrofluorometer (Perkin Elmer LS 55, England) with 282 nm excitation and 315 nm emission lengths. The FL Win Lab version 4.00.02 software was used. Values were inferred in a previously standardized curve and reported as nMoles/g of wet tissue.

Measurement of 5-hydroxyindole acetic acid (5-HIAA)

5-HIAA levels were measured in the supernatant of tissue homogenized in HClO_4 after centrifugation at 9,000 rpm for 10 min in a microcentrifuge (HettichZentrifugen, model Mikro 12-42, Germany), with a modified version of the technique reported by Beck *et al.* (1977). An aliquot of the HClO_4 supernatant, and 1.9 ml of acetate buffer 0.01 M pH 5.5 were placed in a test tube. The mixture was incubated for 5 min at room temperature in total darkness, and subsequently, the samples were read in a spectrofluorometer (Perkin Elmer LS 55, England) with 296 nm excitation and 333 nm emission lengths. The FL Win Lab version 4.00.02 software was used. Values were inferred in a previously standardized curve and reported as nMoles/g of wet tissue.

Technique for the measurement of Glutathione (GSH)

The levels of GSH were measured from a sample of the floating tissue homogenized in HClO_4 which was got after being centrifuged at 9,000 rpm for 5 min. (in a microcentrifuge Mikro 12-42, Germany), according to the technique reported by Hissin and Hilf (1976). 1.8 ml of Phosphate Buffer at pH 8.0 with EDTA at 0.2%, an aliquot of 20 μl of the floating tissue in HClO_4 , and 100 μl of ortho-phthaldialdehyde (OPT) in concentration of 1 mg/ml in methanol, were put in an assay tube and incubated for 15 minutes at ambient temperature in total darkness. At the end of incubation, the samples were read in a PERLIN ELMER LS 55 spectrofluorometer with excitation longitude of 350 nm and emission of 420 nm. FL Win Lab version 4.00.02 software was used. The values were inferred in a previously standardized standard curve and were reported in nM/g of wet tissue.

Technique for the measurement of ATPase dependent on calcium and magnesium

The technique was carried out by using approximately 1 mg of the brain homogenate in 0.05 M tris-HCl at pH 7.4. This was incubated for 15 min, in a medium which contained 3 mM MgCl_2 , 7 mM KCl, 100 mM NaCl, with 4 mM of tris-ATP that was added to the homogenate after the 15 min. of incubation and were again incubated for 30 min. at 37°C with agitation in Dubnoff Labconco bath. The reaction was stopped by using 100 μl of trichloroacetic acid at 10%. The samples were centrifuged at 3,500 rpm for 5 minutes at 4°C (Calderón-Guzmán *et al.* 2005), and an aliquot of the floating tissue was used to measure inorganic phosphate (P_i) using the method proposed by Fiske and Subarrow (1925). The absorbance of the floating was measured at 660 nm using Helios-a of UNICAM spectrophotometer. ATPase dependent on calcium and magnesium was expressed in $\mu\text{M Pi/g}$ of wet tissue/min.

Measurement of calcium

The procedure to measure calcium was performed using supernatant liquid from the brain homogenate of

all animal groups with Ca-Color Arsenazo III AA direct colorimetric method kit (Wiener Lab Rosario, Argentina). The concentration was obtained utilizing an internal standard, and was reported in mg/g wet tissue.

Measurement of H₂O₂

The determination of H₂O₂ was made using the modified technique of Sinha (1972). Each brain region (cortex, hemispheres, cerebellum/medulla oblongata), and stomach were homogenized in 3 ml of tris-HCl 0.05 M pH 7.4 buffer. From the diluted homogenates, 100 µl was taken, and added to 1 ml of potassium dichromate solution (K₂Cr₂O₇) and anhydride acetic acid. The mixture was heat to boiling point for 10 min. (Thermomix 1420). The sample was later placed in an ice bath for 5 min. and centrifuged at 3,000 g for 5 min. (Sorvall RC-5B Dupont). The absorbances of the floating were read by triplicate at 570 nm in a spectrophotometer (Helios-a of UNICAM). The concentration of H₂O₂ was expressed in µMoles.

Analysis of results

Kruskal-Wallis statistical test and analysis of variance (ANOVA) with their respective contrasts after being subjected to variances homogeneity test were used. The values of $p < 0.05$ were considered statistically significant (Castilla-Serna 2011). To carry out the tests, JMP Statistical Discovery Software version 8.0.0 from SAS was used.

RESULTS

The levels of dopamine in cortex of *S. Typh.*-infected rats (*S. Typh.*-i) treated with oseltamivir and indomethacin (Table 1), increased significantly ($p < 0.02$) in the groups that were treated with indomethacin with respect to the control group. H₂O₂ concentration in cortex of *S. Typh.*-i

rats treated with oseltamivir (Osel) and indomethacin (Indo) (Table 1) decreased significantly ($p < 0.04$) in the groups that were treated with Osel and Indo in presence of *S. Typh.*-I when compared with the control group.

Calcium levels in cortex of *S. Typh.*-i rats treated with Osel and Indo (Table 1) decreased in the groups that received Osel plus Indo in comparison with all the groups with *S. Typh.*-i treatment. ATPase enzyme dependent on calcium and magnesium in cortex of *S. Typh.*-i rats treated with Osel and Indo (Table 1) increased significantly ($p < 0.02$) in the group that received Osel versus control group.

Dopamine levels in hemispheres of *S. Typh.*-i rats treated with Osel and Indo (Table 2) decreased significantly ($p < 0.04$) in the group that were treated with *S. Typh.*-I with respect to the control group. Concentration of 5-HIAA in hemispheres of *S. Typh.*-i rats treated with Osel and Indo (Table 2) increased significantly ($p < 0.01$) in all groups that received *S. Typh.*-I when compared with the control group. Ca²⁺, Mg²⁺ ATPase in hemispheres of *S. Typh.*-i rats treated with Osel and indo (Table 2) increased significantly ($p < 0.02$) in the group treated with oseltamivir. The calcium levels in hemispheres of *S. Typh.*-i rats treated with Osel and Indo (Table 2) decreased in all groups that received Osel alone or combined with Indo in comparison with the group that received Osel plus *S. Typh.*-i.

GSH levels in hemispheres of *S. Typh.*-i rats treated with Osel and Indo (Table 2) decreased significantly ($p < 0.01$) in the group that received Osel plus Indo plus *S. Typh.*-I versus control group.

Dopamine levels in cerebellum/medulla oblongata of *S. Typh.*-i rats treated with Osel and Indo (Table 3) increased significantly ($p < 0.02$) in the group that received Osel, and decreased in the group that received Osel plus *S. Typh.*-i. H₂O₂ levels in cerebellum/medulla

Tab. 1. Dopamine and 5-HIAA levels and some oxidative biomarkers in cortex of *Salmonella typhimurium*-infected (*S. Typh.*) rats treated with oseltamivir (Osel) and indomethacin (Indo).

Groups	Dopamine (µM/g tissue)	5-HIAA (nM/g tissue)	H ₂ O ₂ (µM/g tissue)	Calcium (mg/dL)	Ca ²⁺ , Mg ²⁺ ATPase (µM Pi/g tissue/min)	GSH (nM/g tissue)
Control (PBS)	5.35±1.84	334.66±33	0.149±0.02	5.16±0.02	272.04±52	198.84±48
Osel (100 mg/kg)	5.28±0.76	353.97±54	0.151±0.01	5.25±0.24	356.89±48*	220.30±42
Indo (67 µg/kg)	6.37±0.95*	337.87±63	0.149±0.02	5.25±0.17	330.77±42	174.23±26
Osel (100 mg/kg)+indo (67 µg/kg)	4.52±1.04	300.85±86	0.143±0.02	4.98±0.13*	292.29±75	192.52±33
<i>S. Typh.</i> + Control (PBS)	4.55±0.75	302.24±57	0.142±0.01	5.37±0.14	258.86±13	186.36±53
<i>S. Typh.</i> + Osel (100 mg/kg)	4.32±0.87	336.48±107	0.121±0.02*	5.37±0.26	328.92±27	197.34±23
<i>S. Typh.</i> + Indo (67 µg/kg)	4.48±0.60	299.18±57	0.127±0.01*	5.32±0.16	343.13±54	210.07±37
<i>S. Typh.</i> +Osel (100 mg/kg)+indo (67 µg/kg)	4.87±0.91	317.47±66	0.133±0.03	5.46±0.18	311.82±15	212.58±32

Mean values ± SD. Kruskal-Wallis test. Steel-Dwass comparisons. * $p < 0.05$

Dopamine: Indo vs *S. Typh.* control, *S. Typh.* + Osel + Indo, *S. Typh.* + Indo, Osel + Indo * $p < 0.02$

H₂O₂: *S. Typh.* + Osel vs control, osel * $p < 0.04$ and *S. Typh.* + Indo vs control, Indo * $p < 0.006$

Ca²⁺, Mg²⁺ATPase: Osel vs Control * $p < 0.02$

Calcium: Osel + Indo vs *S. Typh.* + Osel + Indo, *S. Typh.* + Osel, Indo, *S. Typh.* control, *S. Typh.* + Indo * $p < 0.02$

Tab. 2. Dopamine and 5-HIAA levels and some oxidative biomarkers in hemispheres of *Salmonella typhimurium*-infected (*S.Typh.*) rats treated with oseltamivir (Osel) and indomethacin (Indo).

Groups	Dopamine ($\mu\text{M/g tissue}$)	5-HIAA (nM/g tissue)	H_2O_2 ($\mu\text{M/g tissue}$)	Calcium (mg/dL)	$\text{Ca}^{+2}, \text{Mg}^{+2}$ ATPase ($\mu\text{M Pi/g tissue/min}$)	GSH (nM/g tissue)
Control (PBS)	9.09 \pm 2.6	348.02 \pm 65	0.171 \pm 0.02	5.40 \pm 0.06	419.17 \pm 86	124.78 \pm 10
Osel (100 mg/kg)	6.51 \pm 0.8	278.72 \pm 74*	0.162 \pm 0.04	5.39 \pm 0.13	459.21 \pm 44	123.90 \pm 24
Indo (67 $\mu\text{g/kg}$)	6.35 \pm 1.3	304.54 \pm 115	0.178 \pm 0.02	5.49 \pm 0.18	367.91 \pm 77	132.85 \pm 27
Osel (100 mg/kg)+indo (67 $\mu\text{g/kg}$)	7.25 \pm 1.0	403.01 \pm 41	0.179 \pm 0.02	5.41 \pm 0.09	389.86 \pm 119	135.06 \pm 9.1
<i>S.Typh.</i> + Control (PBS)	8.49 \pm 1.5*	392.80 \pm 62	0.181 \pm 0.01	5.68 \pm 0.62	449.60 \pm 61*	117.87 \pm 17
<i>S.Typh.</i> + Osel (100 mg/kg)	6.58 \pm 1.0	412.19 \pm 65	0.186 \pm 0.02	7.08 \pm 2.15*	420.57 \pm 72	118.77 \pm 33
<i>S.Typh.</i> + Indo (67 $\mu\text{g/kg}$)	6.63 \pm 1.0	431.61 \pm 69*	0.164 \pm 0.03	5.35 \pm 0.15	348.23 \pm 69	154.23 \pm 45
<i>S.Typh.</i> +Osel (100 mg/kg)+indo (67 $\mu\text{g/kg}$)	6.80 \pm 1.3	415.75 \pm 68	0.174 \pm 0.01	5.39 \pm 0.13	415.08 \pm 45	146.27 \pm 18*

Mean values \pm SD. Kruskal-Wallis test. Steel-Dwass comparisons. * p <0.05

Dopamine: *S.Typh.* control vs Osel, *S.Typh.* + Indo, *S.Typh.* + Osel * p <0.04

5-HIAA: Osel vs *S.Typh.* control, *S.Typh.* + Indo, *S.Typh.* + Osel + Indo, *S.Typh.* + Osel, Osel + Indo * p <0.01

S.Typh. + Indo vs Indo * p <0.02

$\text{Ca}^{+2}, \text{Mg}^{+2}$ ATPase: *S.Typh.* control vs Osel * p <0.02

Calcium: *S.Typh.* + Osel vs Osel, control, Osel + Indo, *S.Typh.* + Indo, *S.Typh.* + Osel + Indo * p <0.01

GSH: *S.Typh.* + Osel + Indo vs control, *S.Typh.* control * p <0.01

Tab. 3. Dopamine and 5-HIAA levels and some oxidative biomarkers in cerebellum/Medulla oblongata of *Salmonella typhimurium*-infected (*S.Typh.*) rats treated with oseltamivir (Osel) and indomethacin (Indo).

Groups	Dopamine ($\mu\text{M/g tissue}$)	5-HIAA (nM/g tissue)	H_2O_2 ($\mu\text{M/g tissue}$)	Calcium (mg/dL)	$\text{Ca}^{+2}, \text{Mg}^{+2}$ ATPase ($\mu\text{M Pi/g tissue/min}$)	GSH (nM/g tissue)
Control (PBS)	7.50 \pm 1.0	328.43 \pm 83	0.169 \pm 0.01	5.22 \pm 0.13	300.89 \pm 37*	97.36 \pm 16
Osel (100 mg/kg)	7.68 \pm 0.6	379.13 \pm 64	0.171 \pm 0.03	4.95 \pm 0.10*	294.72 \pm 100	90.99 \pm 18
Indo (67 $\mu\text{g/kg}$)	7.15 \pm 1.4	354.85 \pm 33	0.179 \pm 0.01	6.61 \pm 1.86	229.67 \pm 56	90.55 \pm 16
Osel (100 mg/kg)+indo (67 $\mu\text{g/kg}$)	6.41 \pm 0.7	369.11 \pm 54	0.167 \pm 0.02	4.89 \pm 0.13*	182.72 \pm 28*	85.26 \pm 8
<i>S.Typh.</i> + Control (PBS)	7.56 \pm 1.2	43706 \pm 92	0.193 \pm 0.02*	5.37 \pm 0.81	254.58 \pm 51	93.32 \pm 12
<i>S.Typh.</i> + Osel (100 mg/kg)	6.47 \pm 1.8*	345.07 \pm 48	0.182 \pm 0.03	5.44 \pm 0.47	347.34 \pm 12*	95.72 \pm 26
<i>S.Typh.</i> + Indo (67 $\mu\text{g/kg}$)	6.71 \pm 1.3	412.10 \pm 69	0.168 \pm 0.02	5.26 \pm 0.45	248.73 \pm 59	101.54 \pm 18
<i>S.Typh.</i> +Osel (100 mg/kg)+indo (67 $\mu\text{g/kg}$)	5.83 \pm 0.9*	362.48 \pm 58	0.166 \pm 0.03	5.20 \pm 0.50	157.33 \pm 51*	85.65 \pm 10

Mean values \pm SD. Kruskal-Wallis test. Steel-Dwass comparisons. * p <0.05

Dopamine: *S.Typh.* + Osel + Indo vs *S.Typh.* control, Osel, control * p <0.005 and *S.Typh.* + Osel vs Osel * p <0.02

H_2O_2 : *S.Typh.* control vs control * p <0.02

Calcium: Osel + Indo vs *S.Typh.* + Osel, *S.Typh.* + Indo, Control, Indo * p <0.02 and Osel vs *S.Typh.* + Osel, control, Indo * p <0.02

$\text{Ca}^{+2}, \text{Mg}^{+2}$ ATPase: *S.Typh.* + Osel + Indo vs *S.Typh.* + Osel, *S.Typh.* control, *S.Typh.* + Indo, Osel * p <0.02

Osel + Indo vs *S.Typh.* + Osel, *S.Typh.* + control, *S.Typh.* + Indo, Osel, control * p <0.03

and Control vs Indo, *S.Typh.* + Osel * p <0.04

oblongata of *S.Typh.*-i rats treated with Osel and Indo (Table 3) increased significantly (p <0.02) in the group that received *S.Typh.*-I with respect to the control group. Calcium concentration of cerebellum/medulla oblongata of *S.Typh.*-i rats treated with Osel and Indo (Table 3) increased significantly (p <0.02) in Osel or Indo with *S.Typh.*-i treatment.

$\text{Ca}^{+2}, \text{Mg}^{+2}$ ATPase enzymes in cerebellum/medulla oblongata of *S.Typh.*-i rats treated with Osel and Indo (Table 3) increased significantly (p <0.02) in all the groups that received Osel or Indo alone or combined with *S.Typh.*-i.

H_2O_2 levels in stomach of *S.Typh.*-i rats treated with Osel and Indo (Table 4) increased significantly (p <0.02) in the group that received Osel alone or combined with Indo plus *S.Typh.*-i. Calcium concentration in stomach of *S.Typh.*-i rats treated with Osel and Indo (Table 4) decreased significantly (p <0.02) in the group that received Osel plus Indo.

$\text{Ca}^{+2}, \text{Mg}^{+2}$ ATPase enzymes in stomach of *S.Typh.*-i rats treated with Osel and Indo (Table 4) increased significantly (p <0.01) in all the groups that received Osel or Indo alone or combined with *S.Typh.*-i. GSH levels in stomach of *S.Typh.*-i rats treated with Osel and Indo

(Table 4) increased significantly ($p < 0.003$) in all the groups that received Osel or indo alone or combined with *S.Typh*-i. C reactive protein in serum of *S.Typh*-i rats treated with Osel and Indo (Table 4) showed positive agglutination in all the groups that received oral *S.Typh*-i.

DISCUSSION AND CONCLUSION

Inflammatory diseases associated with pain are often difficult to treat in the clinic due to insufficient understanding of the nociceptive pathways involved. Systemic inflammatory response is associated with the production of ROS, nitric oxide (NO), which in turn deplete the endogenous GSH (Corsi 2010). This result is in accordance with the reports of the present study for the decrease of GSH in the group that received oseltamivir plus indomethacin in the presence of inflammation-inducing *Salmonella Typhimurium*.

Dopamine levels increased in the cortex regions of animals that received indomethacin, and decreased in the hemisphere regions of animals that received *Salmonella Typhimurium*. These results are in accordance with the reports of Noworyta *et al.* (2013) who suggest that a single ip LPS (10 mg/kg) administration increases hydroxyl radical production but does not affect extracellular dopamine although, repeated ip LPS (5×5 mg/kg) treatments decrease extracellular level of dopamine for the damage of dopamine neurons that was (is observed 72 h after local LPS administration.

With respect to 5-HIAA, an increase in hemispheres of animals that received *Salmonella Typhimurium*, as consequence inflammation response, was observed. This demonstrates that a causative relationship between inflammation and depression is gradually gaining consistency as suggest by Martín-de-Saavedra *et al.* (2013) who indicate that chronic inflammation due to a deletion of Nrf2 can lead to a depressive-like phenotype.

Several drugs used to reduce inflammation may affect cerebral functions and thus provoke health complications. The use of non steroidal anti-inflammatory drugs (NSAIDs) as indomethacin is associated with a broad spectrum of untoward side-effects such as gastrointestinal ulceration (Corsi 2010). Indomethacin induces mitochondrial dysfunction and generation of reactive oxygen species. This redox imbalance was reflected by decreased mucosal glutathione (GSH), nitric oxide, and glutathione peroxidase contents or enzymatic activity along with elevated lipid peroxides (El-Abhar 2010). This result is in accordance with the reports of the present study for the fact that H_2O_2 , Ca^{+2} , Mg^{+2} ATPase, and GSH increased in the stomach tissue of animals that received oseltamivir or indomethacin alone or combined with *Salmonella Typhimurium*. Probably the nitric oxide (NO) appears to play a critical role in modulating gastric mucosal defense, and the administration of NO donors has been reported to protect the gastrointestinal mucosa against damage induced by several irritants with the authors suggesting this as an alternative path to treat

Tab. 4. Oxidative biomarkers in stomach and C Reactive Protein in serum of *Salmonella typhimurium*-infected (*S.Typh*.) rats treated with oseltamivir (Osel) and indomethacin (Indo).

Groups	H_2O_2 ($\mu M/g$ tissue)	Calcium (mg/dL)	Ca^{+2} , Mg^{+2} ATPase (μM Pi/g tissue/min)	GSH (nM/g tissue)	C Reactive Protein
Control (PBS)	0.79±0.02	4.83±0.19	116.33±43	31.80±17*	Negative
Osel (100 mg/kg)	0.087±0.01	4.92±0.26	106.01±46*	33.94±19*	Negative
Indo (67 $\mu g/kg$)	0.084±0.02	4.95±0.09	83.99±35*	74.68±49*	Negative
Osel (100 mg/kg)+indo (67 $\mu g/kg$)	0.103±0.01	4.81±0.08	76.95±31*	51.68±43*	Negative
<i>S.Typh.</i> + Control (PBS)	0.112±0.02*	4.82±0.15	131.40±35	153.35±55*	Positive
<i>S.Typh.</i> + Osel (100 mg/kg)	0.108±0.01*	4.88±0.45	153.94±57	185.15±27	Positive
<i>S.Typh.</i> + Indo (67 $\mu g/kg$)	0.111±0.02	4.94±0.19	217.87±82	192.73±13*	Positive
<i>S.Typh.</i> +Osel (100 mg/kg)+indo (67 $\mu g/kg$)	0.112±0.01*	4.95±0.45	147.20±44	173.65±31	Positive

Mean values \pm SD. Kruskal-Wallis test. Steel-Dwass comparisons. * $p < 0.05$

H_2O_2 : *S.Typh.* + Osel + Indo vs Osel, control, Indo * $p < 0.002$

S.Typh. + Osel vs Control, Osel * $p < 0.005$ and *S.Typh.* control vs control, Indo, Osel * $p < 0.02$

Calcium: Indo vs Osel + Indo * $p < 0.02$

Ca^{+2} , Mg^{+2} ATPase: Indo vs *S.Typh.* + Indo, *S.Typh.* + Osel, *S.Typh.* + Osel + Indo * $p < 0.01$

Osel + Indo vs *S.Typh.* + Osel + Indo, *S.Typh.* + Osel, *S.Typh.* + Indo, *S.Typh.* control * $p < 0.02$

Osel vs *S.Typh.* + Indo * $p < 0.02$

GSH: Control vs *S.Typh.* + Indo, *S.Typh.* + Osel + Indo, *S.Typh.* + Osel, *S.Typh.* control * $p < 0.002$

Osel vs *S.Typh.* + Osel, *S.Typh.* + Osel + Indo * $p < 0.003$

Indo vs *S.Typh.* + Indo, *S.Typh.* + Osel, *S.Typh.* + Osel + Indo * $p < 0.001$

Osel + Indo vs *S.Typh.* + Osel, *S.Typh.* + Osel + Indo * $p < 0.001$

S.Typh. control vs *S.Typh.* + Indo, Osel + Indo, Osel, Indo * $p < 0.01$

S.Typh. + Indo vs Osel, Osel + Indo * $p < 0.003$

the universal problem of non-steroidal anti-inflammatory-drug-induced gastropathy (El-Demerdash *et al.* 2010). Therefore, in this study we induced inflammatory response with *Salmonella Typhimurium*, as suggested by the increased levels of C-reactive protein (Mézešová *et al.* 2013).

Findings of Uzkeser *et al.* (2012), suggest an increase in the level of GSH during the acute phase of inflammation. Results of these authors and our findings indicate that GSH may serve as a biomarker of inflammatory responses in animal models.

H₂O₂ levels increased in stomach tissue and cerebellum/medulla oblongata regions of animals that received *Salmonella Typhimurium*, as a consequence of hyperalgesia as suggested by Keeble *et al.* (2009), who demonstrated the notable effect of H₂O₂ in mediating inflammatory hyperalgesia, thereby highlighting H₂O₂ removal as a novel therapeutic target for anti-hyperalgesic drugs in the clinic.

With respect to calcium levels in this study there was a decrease in this biomarker in the stomach tissue of animals that received oseltamivir and indomethacin. Kordić *et al.* (2011) suggested that indomethacin pretreatment significantly decreased the magnitude and relaxation of calcium ion-induced tissue contractions, predominantly by potassium channels, and to a small extent, via β -adrenergic receptors or nitric oxide (NO)-dependent pathways (Kordić *et al.* 2011).

Total ATPase increased in cortex, hemispheres, cerebellum/medulla oblongata, and stomach tissue of animals treated with oseltamivir or indomethacin plus *Salmonella Typhimurium* probably as a consequence of changes in the affinity of the enzyme (Hoskins *et al.* 1985). This result is in accordance with the reports of Paul *et al.* (2014) who suggest that inflammation leading to the resultant increase in sodium influx must be countered to maintain osmotic homeostasis. These authors showed evidence that regulation of Na(+), K(+)-ATPase during major inflammatory disease states is critical for homeostatic protection of primary afferent neurons.

With these results we found that side effects of flu exposure simulation may also be associated with inflammatory response seen in oseltamivir or indomethacin consumption. Indeed, these results demonstrate the association between inflammatory response, oxidative stress, dopaminergic, and serotonergic metabolism in an experimental inflammatory animal model. However, more future experiments under this condition are needed to ascertain the validity of these results.

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REFERENCES

- Adham KG, Al-Humaidhi EM, Daghestani MH, Aleisa NA, Farhood MH (2012). Protective role of indomethacin on lipopolysaccharide-stimulated fever induction and cerebral catecholamine biosynthesis in Wistar rat. *Neuro Endocrinol Lett.* **33**: 713–21.
- Beck O, Palmkog G, Hultman E (1977). Quantitative determination of 5-hydroxyindole-3-acetic acid in body fluids by HPLC. *Clin Chim Acta.* **79**: 149–154.
- Beckman JS, Beckman TW, Chen J, Marshall PA, Freeman BA (1990). Apparent hydroxyl radical production by peroxynitrite: Implications for endothelial injury from nitric oxide and superoxides. *Proc Natl Acad Sci USA.* **87**: 1624–1629.
- Bediz CS, Baltaci AK, Mogulkoc R, Oztekin E (2006). Zinc supplementation ameliorates electromagnetic field-induced lipid peroxidation in the rat brain. *Tohoku J Exp Med.* **208**: 133–140.
- Benuck M, Banay-Schwartz M, DeGuzman T, Lajtha A (1995). Effect of food deprivation on glutathione and amino acid levels in brain and liver of young and aged rats. *Brain Res.* **678**: 259–264.
- Calderón-Guzmán D, Espitia-Vázquez I, López-Domínguez A, Hernández-García E, Huerta-Gertrudis B, Juárez-Olguín H (2005). Effect of toluene and nutritional status on serotonin, lipid peroxidation levels and Na⁺/K⁺-ATPase in adult rat brain. *Neurochem Res.* **5**: 619–624.
- Calderón GD, Osnaya BN, García AR, Hernández GE, Guillé PA, Juárez Olguín H (2008). Levels of glutathione and some biogenic amines in the human brain putamen after traumatic death. *Proc West Pharmacol Soc.* **51**: 25–32.
- Castilla-Serna L: Manual Práctico de Estadística para las Ciencias de la Salud. Editorial Trillas. 1^o Edición. México, D.F. 2011.
- Corsi L (2010). Effects of the novel non-peptidyl low molecular weight radical scavenger IAC in different models of inflammation: a new perspective in anti-inflammatory therapy. *Curr Med Chem.* **17**: 3918–324.
- Driver AS, Kodavanti PR, Mundy WR (2000). Age-related changes in reactive oxygen species production in rat brain homogenates. *Neurotoxicol Teratol.* **22**: 175–181.
- Dunn AJ (1992). Endotoxin-induced activation of cerebral catecholamine and serotonin metabolism: comparison with interleukin-1. *J Pharmacol Exp Ther.* **261**: 964–969.
- El-Abhar HS (2010). Coenzyme Q10: a novel gastroprotective effect via modulation of vascular permeability, prostaglandin E₂, nitric oxide and redox status in indomethacin-induced gastric ulcer model. *Eur J Pharmacol.* **649**: 314–319.
- El-Demerdash E, El-Mesallamy HO, Abu-Zaid NM, Gad MZ (2010). The potential therapeutic effect of nitric oxide modulators in experimentally-induced gastric ulcers. *Drug Discov Ther.* **4**: 276–284.
- Fiske CH, Subbarow Y (1925). The colorimetric determination of phosphorus. *J Biol Chem.* **66**: 375–400.
- Hernández RJ (1982). A serotonin agonist-antagonist reversible effect on Na⁺/K⁺-ATPase activity in the developing rat brain. *Dev Neurosci.* **5**: 326–331.
- Hissin PJ, Hilf R (1976). A fluorometric method for determination of oxidized and reduced glutathione in tissue. *Anal Biochem.* **74**: 214–226.
- Hoskins B, Ho IK, Meydrech EF (1985). Effects of aging and morphine administration on calmodulin and calmodulin-regulated enzymes in striata of mice. *J Neurochem.* **44**: 1069–1073.
- Keeble JE, Bodkin JV, Liang L (2009). Hydrogen peroxide is a novel mediator of inflammatory hyperalgesia, acting via transient receptor potential vanilloid 1-dependent and independent mechanisms. *Pain* **141**: 135–142.

- 19 Kordić-Bojinović J, Oreščanin-Dušić Z, Slavić M, Radojičić R, Spasić M, Milovanović SR, et al. (2011). Effect of indometacin pretreatment on protamine sulfate-mediated relaxation of the isolated rat uterus: the role of the antioxidative defense system. *Pharmacol Rep.* **63**: 1019–1028.
- 20 Martín-de-Saavedra MD, Budni J, Cunha MP, Gómez-Rangel V, Lorrio S, Del Barrio L, et al. (2013). Nrf2 participates in depressive disorders through an anti-inflammatory mechanism. *Psychoneuroendocrinology* **38**: 2010–2022.
- 21 Mehta A, Singh S, Ganguly NK (1999). Effect of *Salmonella typhimurium* enterotoxin (S-LT) on lipid peroxidation and cell viability levels of isolated rat enterocytes. *Mol Cell Biochem.* **196**: 175–181.
- 22 Mézešová L, Jendruchová-Javorková V, Vlkovičová J, Okruhlicová L, Frimmel K, Navarová J, et al. (2013). Supplementation with n-3 polyunsaturated fatty acids to lipopolysaccharide-induced rats improved inflammation and functional properties of renal Na,K-ATPase. *Nutr Res.* **33**:772–779.
- 23 Neault JF, Benkiran A, Malonga H, Tajmir-Riahi HA (2001). The effects of anions on the solution structure of Na,K-ATPase. *J Biomol Struct Dyn.* **19**: 95–102.
- 24 Noworyta-Sokołowska K, Górska A, Gołombiowska K (2013). LPS-induced oxidative stress and inflammatory reaction in the rat striatum. *Pharmacol Rep.* **65**: 863–869.
- 25 Nozu T, Takakusaki K, Okumura T (2014). Urocortin 2 blocks the suppression of gastric antral contractions induced by lipopolysaccharide in freely moving conscious rats. *Regul Pept.* **190–191C**: 12–17.
- 26 Oshima S, Nemoto E, Kuramochi M, Saitoh Y, Kobayashi D (2009). Penetration of oseltamivir and its active metabolite into the brain after lipopolysaccharide-induced inflammation in mice. *J Pharm Pharmacol.* **61**: 1397–1400.
- 27 Paul D, Soignier RD, Minor L, Tau H, Songu-Mize E, Gould HJ (2014). Regulation and pharmacological blockade of sodium-potassium ATPase: a novel pathway to neuropathy. *J NeuroSci.* **340**: 139–143.
- 28 Sinha AK (1972). Colorimetric assay of catalase. *Anal Biochem.* **47**: 389–394.
- 29 Swapna I, Sathya KV, Murthy CR (2005). Membrane alterations and fluidity changes in cerebral cortex during ammonia intoxication *Neuro Toxicol.* **335**: 700–704.
- 30 Thygesen P, Brandt L, Jørgensen T, Christensen HB, Hougen HP, Jensen ET, et al. (1994). Immunity to experimental *Salmonella typhimurium* infections in rats. Transfer of immunity with primed CD4+CD25^{high} and CD4+CD25^{low} lymphocytes. *APMIS.* **102**: 489–494.
- 31 Uzkeser H, Cadirci E, Halici Z, Odabasoglu F, Polat B, Yuksel TN, et al. (2012). Anti-inflammatory and antinociceptive effects of salbutamol on acute and chronic models of inflammation in rats: involvement of an antioxidant mechanism. *Mediators Inflamm* **2012**: 438912.
- 32 Yoshino T, Nisijima K, Shioda K, Yui K, Kato S (2008). Oseltamivir (Tamiflu) increases dopamine levels in the rat medial prefrontal cortex. *Neurosci Lett.* **438**: 67–69.