doi.org/10.36721/PJPS.2020.33.3.SUP.1271-1276.1

Effects of ceftazidime with and without imipramine and bromazepam on behavior and neuro-inflammatory parameters in rats with chronic suppurative otitis

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Abstract: The use of antibiotics in middle ear perforation due to chronic supprative otitis media (CSOM) is being widely employed across the world. The object of the present study was to evaluate the effect of ceftazidime with and without imipramine and bromazepam on behavior neuro-inflammatory parameters (serotonin, immunoglobulin and lymphotoxin beta) in CSOM induced rats. The rats were divided into five groups (six in each), G1; negative control (healthy rats maintained on normal saline), G2; positive control (diseased without any treatment), G3 (ceftazidime 15 mg/kg), G4 (ceftazidime 15 mg/kg with imipramine 1.15mg/kg) and G5 (ceftazidime 15 mg/kg with bromazepam 0.09mg/kg. All the drugs were administered intra-peritoneally for seven days. Behavior studies were conducted after treatment period and the serum was subjected for Elisa method for the estimation of inflammatory markers. Behavioral deficit and decreased serotonin and elevated IgE and LT-beta induced by CSOM was significantly reverted back to the normal levels in groups received imipramine or bromazepam with ceftazidime but not in group treated only with ceftazidiem. Therefore, the antidepressant or anxiolytic drugs may be helpful at low doses to attenuate the depression associated with chronic suppurative otitis media alongwith neuro-inflammatory markers (serotonin, IgE and LT-beta).

Keywords: Chronic suppurative otitis media, behavior, serotonin, IgE, lymphotoxin beta, depression.

INTRODUCTION

Chronic suppurative otitis media (CSOM) is one of the leading reasons of hearing loss across the world due to the perforation in tympanic membrane (Aubert-Khalfa *et al.,* 2010). The correlation between hearing loss and depression alongwith other risk factors such as low socioeconomical status and neurological disorders co-mobilities etc is well established. However, one study showed the association of depression, anxiety and stress with CSOM in local population of Pakistan. The sensory alteration or modification in auditory threshold may contribute the induction of depression in conductive and sensoneural hearing loss in patients of CSOM (Mehboob *et al.,* 2019).

Literature supports the role of different inflammatory markers in effusion and perforation of tympanic membrane which stimulate the uncontrolled inflammation in chronic infection of otitis media in patients. These components of immune system such as macrophages and monocytes expresses $5HT_{1E}$, 5 HT_{2A}, $5HT_3$, $5HT_4$, and $5HT_7$ to elicit their functions and the concentration of serotonin in the blood play important role to declare the stage of infection (Durk *et al.,* 2005, Passlick *et al.,* 1989, Serbina *et al.,* 2008). One of the inflammatory markers immunoglobulin E (IgE) is well known for its role innate and adoptive immunity and has been reported in higher concentration in CSOM patients (Winter, 2000). Another marker under consideration is lymphotoxin-beta, also known as TNF-γ is a novel member of TNF super family, therefore, contributes in inflammation. Although its role in tumor formation has been established but in CSOM induced rats it still need to be further investigated (Browning *et al.,* 1991).

The choice of drugs for the management of chronic suppurative otitis media depends on a number of factors among which the resistance of the pathogens against the specific antibiotic is the most important. Antibiotics especially quinolones are reported to be the most effective against certain organisms with lesser side effects. Different studies proved effectiveness of different antibiotics in chronic otorrhoea such as ciprofloxacin, ofloxacin, amoxicillin-clavulanic acid in combination with dexamethasone (Connor *et al.,* 2009, Verhoeff *et al.,* 2006). In addition to anti-infective property, ciprofloxacin and amoxicillin also possess anti-inflammatory activity which is helpful in chronic infection associated with inflammation (Sachsel *et al.,* 2008, Casellas *et al.,* 1998).

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One of the studies conducted in Pakistan showed that ceftazidnime possessed maximum sensitivity against causative agents in CSOM patients (Mansoor *et al.,* 2009).

Antidepressants such as imipramine (TCAs) which inhibits serotonin–noepine thsphrine reuptake inhibitors (SNRIs) enhance the level of neurotransmitters and anxiolytic such as bromazepam (at low doses) which works through enhancing the effects of neuro-transmitter gamma-aminobutyric acid (GABA) at the $GABA_A$ receptor, are commonly helpful in short term treatment of depression and anxiety (Gillman, 2007, Amphoux *et al.,* 1982).

In the presented study ceftazidime was used with and without imipramine and bromazepam to observe their impact on behavior defecit and neuro-inflammatory markers.

MATERIALS AND METHODS

Animals

Male Sprague-dawley rats weighing 191.5g ± 12 which locally bred and housed individually under 12 hour light dark cycle and controlled room temperature (22±2ºC) with the access of cubes of standard rodent diet and water under the recommendations of NIH for the Care and Use of Laboratory Animals as approved in the protocol of higher education locally (Saleem *et al.,* 2018).

Animal protocol

Rats were randomly selected to serve in five groups (six rats in each group) as G1: Control negative (healthy rats maintained on normal saline), G2; control positive (diseased without any treatment), G3 (ceftazidime 15 mg/kg), G4 (ceftazidime 15 mg/kg with imipramine 1.15mg/kg) and G5 (ceftazidime 15.3 mg/kg with bromazepam 0.09mg/kg).

Induction of CSOM: Animals were given anaesthesia I.P through ketamine (100mg/kg) and diazepam (0.1 mg/kg) and induced with 0.04 ml of 6.4 * 10^7 CFU of *P.aerugenosa* into tympanic bulla. All the animals were kept under the guidelines of NIH for two weeks (Bhutta *et al.,* 2012).

Behavioral studies

Open Field Test: The open field, consists of 76*76 cm of square area having 42 cm high walls, used to evaluate the activity by counting number of squares crossed by the animals with all four paws was counted for 15 minute.

Light And Dark Cage Activity Test: This apparatus consist of one light compartment of transparent plastic and another dark portion of black translucent plastic, each measure 26*26*26 cm with 12*12 cm passageway and provided a source of white light. The reading is observed when the animal is introduced in the light area of the activity cage and the total time spent in the light compartment is noticed for a cut off time of 10 minutes (Haleem, 2009).

Force Swimming Monitoring Test: The force swimming test was conducted in a glass cylinder (40 cm height, 17 cm diameter) containing water (21°C) to a height of 30 cm. The cut off time is 6 minutes to notice the immobility (after a 15 minutes training session conducted before 24 hours on day 1) (Porsolt *et al.,* 1977).

Traction/Static Rods Test: The process of recording the traction time on static rod (30-50 cm) taking time taken to orientate 180° from the starting position and the transit time was the time taken to travel to the end of the rod considering 120 seconds as cut off (Bogo *et al.,* 1981).

Neuro-inflammatory parameters analysis

5ml of the blood of rats were collected from decapitated necks in the tubes. The serum was obtained after the centrifugation for 30 min at 3000 rpm in eppendorff and stored at -80ºC until used. The estimation of LT-β, IgE and serotonin in the serum were performed using method ELISA solid-phase antibody method and absorbance were taken at the wave length of 450 nm (Zhu *et al.,* 2017).

STATISTICAL ANALYSIS

Results are presented as mean ±SD. One way ANOVA was applied for behavioural and neuro-inflammatory markers analysis and group comparisons (Post Hoc) were done by Tukey's test ($p \geq 0.05$).

RESULTS

Effects on locomotor activities and muscular co-ordination by open field, forced swim test and traction

Fig. 1 shows the effects of locomotor activities in open field in CSOM induced rats. One way ANOVA shows that value of F was 1198.024 and p value was significant ($p<0.05$) throughout the experimental period. Post Hoc (Tukey's test) shows significantly decreased in number of squares covered by positive control ($p<0.01$) but improved in ceftazidime treated group ($p>0.05$) and significantly increased ($p<0.01$) in groups treated with imipramine and bromazepam with ceftazidime (G4 and G5).

Fig. 2 shows the effects of mobility time in forced swim test in CSOM induced rats in different groups. One way ANOVA shows that value of F was 18.68 and p value was significant ($p<0.05$) throughout the experimental period. Post Hoc (Tukey's test) shows significantly decreased mobility by positive control ($p<0.01$) but improved in

ceftazidime treated group and significantly increased (p<0.01) in groups treated with imipramine and bromazepam with ceftazidime (G4 and G5).

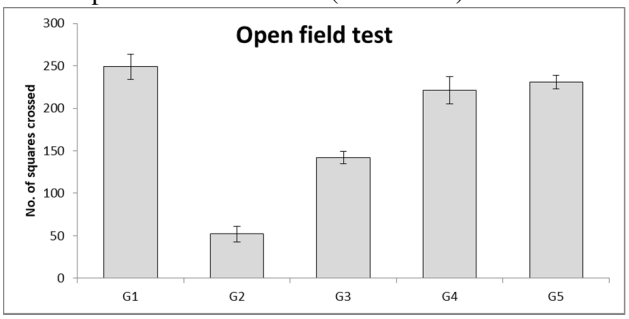

Fig. 1: Effects of ceftazidime with and without imipramine or bromazepam on locomotion in open field test presented byG1(negative control), G2 (positive control), G3 ceftazidime (15mg/kg), G4 (ceftazidime with imipramine 15mg/kg and 1.15mg/kg) and G5 (ceftazidime with bromazepam 15mg/kg and 0.09mg/kg. Insignificant difference of G4 and G5 from G1 by Tukey's test)

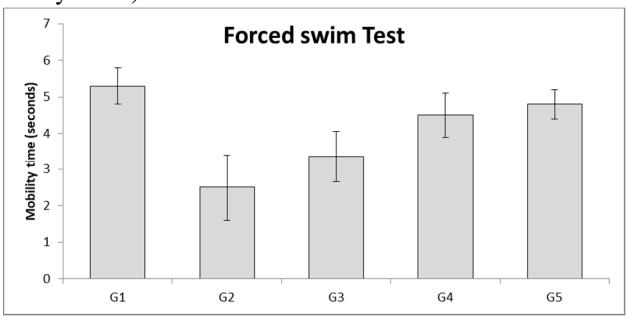

Fig. 2: Effects of ceftazidime with and without imipramine or bromazepam on locomotion in open field test presented by G1 (negative control), G2 (positive control), G3 ceftazidime (15mg/kg), G4 (ceftazidime with imipramine 15mg/kg and 1.15mg/kg) and G5 (ceftazidime with bromazepam 15mg/kg and 0.09mg/kg. Insignificant difference of G4 and G5 from G1 by Tukey's test)

Fig. 3 One way ANOVA shows that value of F was 589.468 and p value was significant (p<0.05).Post Hoc (Tukey's test) shows that positive control (G2) has significantly increased traction time than negative control (G1). Ceftazidime (G3) shows improvement in time but ceftazidime with imipramine (G4) or bromazepam (G5) present significantly decreased traction time very near to negative control (G1) (p<0.05).

Fig. 4 shows significant difference when one way ANOVA was applied with F-value 906.374 and p< 0.05.Post Hoc (Tukey's test)shows that transition time to the light in light and dark test, positive control (G2) have significantly decreased time than negative control (G1). Ceftazidime (G3) shows improvement and increased time but ceftazidime with imipramine (G4) or bromazepam (G5) present significantly increased transition time.

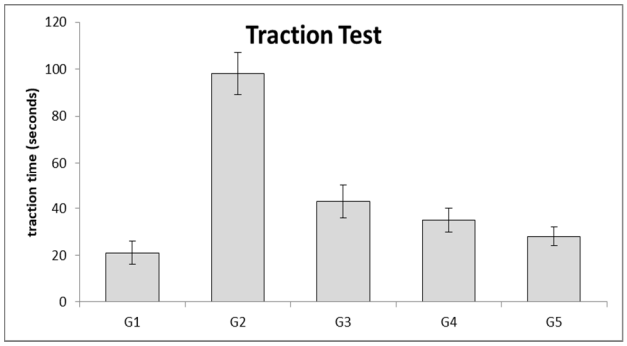

Fig. 3: Effects of ceftazidime with and without imipramine or bromazepam on locomotion in open field test presented byG1(negative control), G2 (positive control), G3 ceftazidime (15mg/kg), G4 (ceftazidime with imipramine 15mg/kg and 1.15mg/kg) and G5 (ceftazidime with bromazepam 15mg/kg and 0.09mg/kg. Insignificant difference of G4 and G5 from G1 by Tukey's test)

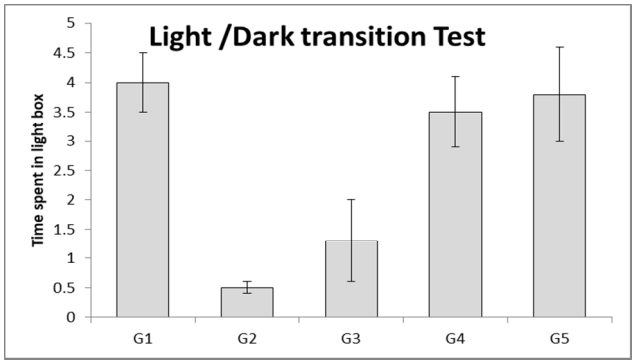

Fig. 4: Effects of ceftazidime with and without imipramine or bromazepam on locomotion in open field test presented by G1 (negative control), G2 (positive control), G3 ceftazidime (15mg/kg), G4 (ceftazidime with imipramine 15mg/kg and 1.15mg/kg) and G5 (ceftazidime with bromazepam 15mg/kg and 0.09mg/kg. Insignificant difference of G4 and G5 from G1 by Tukey's test)

Estimation of neuro-inflammatory parameters

During infection of CSOM in rats value of different markers (G2) were found significantly increased (p<0.01) such as IgE and LT beta from negative control (G1) and significantly decreased (p<0.01) such as serotonin from negative control group (G1) as shown in table 1.

DISCUSSION

Depression may be associated or induced by chronic suppurative otitis media infection (Mehboob *et al.,* 2019). Several studies showed that infection and inflammation triggering agents like bacteria, protozoa or virus can stimulate the catabolism of tryptophan (use to generate serotonin, a neurochemical which play very important role in depression) such as *Chlamydia psittaci*, *Toxoplasma gondii*, *Leishmania donovani*, and herpes

Table 1: Mean values of IgE, LT-β and5-HT in different groups of rats with ±S.D. One way ANOVA (Tukey's test) is applied to evaluate the effect of CSOM on IgE, LT-β and 5-HT (*p value is >0.05).

S. No.	Groups	Dose	IgE (IU/ml) Mean ±S.D	P value	5-HT(ng/ml) Mean ±S.D	P value	LT-β(ng/ml) Mean ±S.D	P value
1	G1 (negative control)	0.5ml	0.51± 0.42	0.00	10.36±0.61	.000	2.05±0.75	0.00
2	G2 (positive control)	_	1.48±0.18	0.00*	3.55±56	0.00*	5.13± 0.75	0.00*
3	G3 (ceftazidime)	15mg/kg	0.63±0.03	0.128	4.44±05	.001*	2.19±79	1.000
4	G4 (ceftazidime + imipramine)	15 mg/kg +1.15mg/kg	0.55±0.02	0.993	4.86±01	.135	2.47±45	0.965
5	G5 (ceftazidime +bromazepam)	15 mg/kg +0.09mg/kg	0.53±0.01	1.00	7.39±.49	.091	2.95±90	0.355

Groups maintained on ceftazidime (G3) showed significant decreased in IgE and LT-β levels as compare to G2, whereas, no significant differences were observed among test groups (G3, G4 and G6).
Co-administration of imipramine or bromazepam with ceftazidime shows improvement in the levels of serotonin as compare to the antibiotic treatment alone. However, this combination has no change on LT beta and IgE levels.

simplex virus (HSV)-2. Therefore, serotonin depletion may lead to the condition of depression as a consequence of infection (Khan *et al.,* 1996).

In open field test, forced swim test and traction test significantly decreased activities were observed in groups maintained on ceftazidime alone as compare to positive control (without treatment). However, insignificant decreased in activities were observed in the groups maintained on imipramine or bromazepam with antibiotic (G4 and G5) after the treatment period (fig .1 to fig.3). Similarly, in light and dark cage activity box (fig .4), only the groups maintained on imipramine or bromazepam with ceftazidime showed close transition time to the light to healthy rats (p>0.05). This showed that antidepressant or anxiolytic (imipramine and bromazepam) are responsible to recover the condition and to revert back normal activities from depression and anxiety (Tatsumi *et al.,* 1997).

It is possible that in the current study the decreased level of serotonin in serum and brain are responsible for the depression exerted due to the chronic infection in CSOM which resulted in the significantly altered activities in the open field, light and dark cage, force swimming test and traction timing. Hence, this study will suggest to eradicate not only source of depression which may or may not be infection but also to treated depression after monitoring it so that the subject under stress would get normal physical health but normal psychological status, too.

Higher IgE serum levels indicated over-reaction of the body in response of different stimuli eliciting the inflammatory responses of the body. IgE have also been reported to be increased in several infection conditions (Winter, 2000). In the presented study, elevated levels of IgE in positive control indicate the effects of CSOM on IgE and LT-β.

Different endotoxins are responsible to induce altered immune responses in CSOM which have capability to change the levels of tumor necrosis factor alpha in serum (Arguedas *et al.,* 2010) but the concentration of lymphotoxin beta (tumor necrosis factor gamma) in CSOM is unknown. Lymphotoxin beta is an important member of TNF family and contributes in chronicity of several infection (Browning *et al.,* 1991).and in tumor formation too. Significant elevated level of lymphotoxin beta in control positive showed its role in CSOM induced rats.

Previous studies proved the association of depression and anxiety in CSOM patients (Aubertet *et al.,* 2010). However; serotonin levels in the serum which are related to the mood and behavioral changes (Tamara and John, 2008). In the present study significant decreased in serotonin levels in control positive group will help to explore the association of depression with CSOM.

As far as anti-inflammatory actions of cefatzidime is concerned, data shows that it possess anti-inflammatory actions. The results of our experiment showed the significant decreased of IgE serum concentrations by ceftazidime.

The role of antibiotics in normalizing the levels of tumor necrosis factors has been reported in different diseases or chronic conditions (Gogos *et al.,* 2004) so as in present study.

Interesting results were obtained while evaluating the effects of ceftazidime with and without imipramine or bromazepam on serotonin levels The significant increased levels of serotonin in groups maintained on imipramine (G4) and bromazepam (G5) with insignificant differences from the group maintained on only ceftazidime only (G3). These results are supported by the studies conducted on imipramine or bromazepam exploring the role of these drugs to regain serotonin levels and frequently employed in depressive disorders (Delini-Stula *et al.,* 1995, Smiałowsk, 1991, Rastogi *et al.,* 1078). Therefore, addition of these antidepressants may help to revert the decreased serotonin serum levels. GABA receptors are mainly involved in behavior changes and produce anxiolytic effect (Sarwat *et al.,* 20018).

CONCLUSION

The combined therapy of ceftazidime with imipramine or bromazepam may help to attenuate behavioral deficit and restore the normal levels of neuro-inflammatory markers in CSOM induced rats. Further investigation on the role of these drugs should be conducted for the improvement of treatment protocol of CSOM at clinical level.

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