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Full Length Research Paper

Effects of experimental *Neisseria meningitis* W135 infection on serotoninergic parameters in mice

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This study investigated the effects of *Neisseria menigitidis* W135 infection via intraperitoneal route on plasma free tryptophan concentration, brain serotonin and 5-hydroxyindole acetic acid (5-HIAA) levels in albino mice fed normal and tryptophan-enriched diets. The kinetics of appearance of viable bacteria in the blood, brain and liver following infection were also investigated. The serotoninergic parameters were determined by colorimetric and HPLC methods while colony counts were measured by plate count technique. Compared to normal diet, the tryptophan-enriched diet resulted in significantly ($P<0.05$) higher level of plasma free tryptophan level irrespective of infection but with time-point increase in death score (8.3–66.7%) in the infected mice. Despite dietary tryptophan enrichment, the infected mice were also observed to elicit a time-dependent significant ($P < 0.05$) decrease in brain serotonin (253.9–131.4 vs. 262.4–283.7 ng/g tissue) but increase in 5-HIAA (71.8–174.5 ng/g vs. 70.4–79.6 ng/g tissue) compared to uninfected animals. Establishment of *N. meningitidis* W135 meningitis in mice and subsequent brain serotonin depletion was further found to display a dose-dependent effect at 6–12 h post infection when inoculation with 10^4 and 10^6–10^7 cfu/ml were compared. Viable bacteria also appeared at different time-points in the blood, brain and liver of the infected mice with growths in blood and brain exhibiting similar kinetics but a disparity of 0.2–1.2 logarithmic cfu/ml. The results of this study strongly support the involvement of altered serotoninergic activity in meningococcal infection due to *N. meningitidis*.

Key words: Neisseria meningitidis W135 infection, serotoninergic pathway, tryptophan - enriched diet, mice.

INTRODUCTION

Meningococcal infection due to *Neisseria meningitidis* remains a public health burden in many countries of the world where it cumulatively accounts for about 500,000 clinical cases annually with 50% of such cases and up to 27,000 deaths occurring in sub-Saharan Africa (Tikhomirov et al., 1997). *N. meningitidis* epidemics within the meningitis belt in sub-Saharan Africa is even more calamitous, characterized by clonal diversity of aetiology agents, increased number of cases and case fatality rates (CFRs) in the last two decades (Haimanot et al., 1990; Mohammed et al., 2000). The emergence and predominance of *N. meningitidis* W135 in the early 21st century as a significant epidemic serogroup has further complicated the management of meningococcal infection in developing countries resulting in a multitude of poor outcomes and survivors with long-term neurological sequelae (Hodgson et al., 2001). The latter is a consequence of neuronal damage inflicted by *Neisseria* colonization of the central nervous system and host/bact-

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erium inflammatory events (Nau, 2003).

Reports emanating from several animal models of experimental meningitis and clinical studies in children have validated infections to result in the disruption of blood-brain barrier, haemodynamic collapse and loss of cerebral blood flow auto-regulation following the invasion of sub-arachnoid space coupled with receptor mediated activation of cells of the reticuloendothelial system in the brain (Moller et al., 2000; Kongstad and Graeme, 2001; Park et al., 2003; Nau, 2003). Whereas adjunctive treatment of meningitis with dexamethasone prior to antibiotic regimen has produced contrasting clinical outcomes (de Gans and van de Beek, 2002; Molyneux et al., 2002), the use of norepinephrine and DOPA (dihydroxy-L-phenylalanine) as a clinical strategy of maintaining cerebral blood flow to meet brain increased oxygen and energy demand at the expense of increased intracranial pressure due to infection has revealed tremendous therapeutic benefits in animals and humans (Park et al., 2003; Nau, 2003). Previous studies have implicated impairment of sympathetic–adrenal system characterized by secretion of catecholamine and depletion of adrenal catecholamine store of patients with meningitis dead or alive (Glotova and Sirina, 1976; Sirina et al., 1978). However, data are lacking to implicate catecholamine metabolic dysregulation, which occurs in the pathogenesis of meningococcal infection as a prognostic marker of neurological deficits: seizures, learning and memory impairment and depression often observed among survivors (Hodgson et al., 2001).

Serotonin (5-hydroxytryptamine), a neurotransmitter of the serotoninergic neurons regulates mood, memory and learning behaviours (Fernstrom and Wurtman, 1971; Fernstrom, 1994). The ability of serotonin to modulate behaviours has been correlated with the level of the neurotransmitter in the plasma and brain (Fernstrom and Wurtman, 1972) and lower levels of this neurotransmitter have been reported in patients with neurodegenerative diseases, depression and those lacking stress coping ability (Rosenthal et al., 1989; Maes and Meltzer, 1995; Markus et al., 1998). We hypothesize that an understanding of the role(s) played by the serotoninergic pathways in the pathogenic disposition of *N. meningitis* and host response to infection would improve adjunctive management of meningococcal disease and reduce the risk of neurological-sequelae among survivors. The present study was designed using a mouse model of experimental meningitis due to hyper-invasive ST11 complex *N. meningitidis* W135 strain, a CFS isolate obtained during the 2003 epidemic in Jigawa State (Nigeria) in which 230 cases and 133 deaths (Case Fatality Rate [CFR] = 57.8%) were reported (Uwah et al., personal communication). The effects of infection on plasma tryptophan, brain serotonin levels as well as concentration of metabolite 5-hydroxyindole acetic acid (5-HIAA) were investigated.

MATERIALS AND METHODS

Animals

Adult inbred albino mice weighing 25–28 g (mean weight = 26.4 g) were used for this study. The animals were obtained form the Animal House of the Nigerian Institute of Medical Research, Lagos, Nigeria. Prior to the experiments, the animals were fed normal diet (Ladokun Farms, Ibadan, Nigeria) and water *ad libitum*. They were kept in metabolic cages and observed under 12 h/12 h light/dark cycle in a well-ventilated room at 26–27°C. Experiments were performed according to the National Institute of Health Guidelines for Care of Laboratory animals (NIH, 1978).

Diets

To investigate the effect of dietary tryptophan supplementation on the course of *N. meningitidis* W135 pathogenesis, serotoninergic response and survival in mice, a tryptophan enriched diet with additional 1.24 g/100 g feed was formulated. The normal diet consists of corn starch 64.8%, casein 18.6%, crude fibre 3.2%, corn oil 4.5%, vitamin mix 4.0%, mineral mix 4.0%, bile salt 0.1%, DL-methionine 0.30% and L-cysteine 0.36%. The enriched diet was normal diet plus 1.24 g/100 g tryptophan (Table 1).

Bacteriology

A *N. meningitidis* W135 strain recovered from CSF sample during meningitis epidemic in Jigawa State in 2003, stored in Trans-Isolate (T-I) medium (Ajello et al., 1984) containing 1% VCN (vancomycin-colistin-nystatin) inhibitor (BBL) in vented bottles at 32-35°C and transported to the Central Public Health Laboratory (CPHL), Lagos, were used for inducing experimental meningitis in mice in this study. The viability of the organism was tested by growth on chocolate agar plates at 37°C for 24 h in 5% CO_2 atmosphere. Speciation was confirmed based on oxidase reaction, O-nitrophenyl-β-D-galactopyranoside test, sugar fermentation (Cruishank et al., 1975) and β-lactamase test (Bush and Syke, 1980). Serotyping, subtyping and electrophoretic typing of the isolate was carried by dot blot analysis (Wedege et al., 1990) and multilocus electrophoresis (Selander et al., 1986) at the Central Laboratory of Norwegian Institute of Public Health, Oslo, Norway. For colony forming units (cfu) counts, blood was withdrawn from infected mice at different time-points by cardiac puncture before sacrifice and added to a tube containing 3.8% of sodium citrate. Brains and livers were excised and homogenized in 2 ml of sterile PBS (phosphate buffered saline). Bacterial counts in blood, brain and liver were performed by plating 10-fold dilutions onto blood-agar plates. The limit of detection was 50 cfu/ml.

Sample preparation

Infected and control mice were lightly anaesthesized with chloroform and then sacrificed by cervical decapitation. For brain serotonin and 5-hydroxy indole acetic acid (5-HIAA) determination, whole brain tissue of the animal was immediately excised and homogenized by sonication with 0.5 – 1.0 ml of 0.1 M perchloric acid for 30 s. The resulting homogenate was centrifuged at 6500 rpm at 4°C for 15 min followed by collection of supernatant into a sterile capped vial bottle. Plasma was prepared by centrifugation at 3500 rpm for 10 min at 20°C of citrated tail blood of the animals and also collected into sterile bottle for immediate use.

Table 1. Composition of normal and tryptophan-enriched diets for animal feeding.

Composition (g/100 g feed)		
Nutrient	**Normal Diet**	**Tryptophan – enriched diet**
Casein Protein	18.6	18.6
Corn Oil	4.5	4.5
Corn Starch	64.8	63.6
Crude fibre	3.2	3.2
Amino acid supplement		
Methionine	0.40	0.40
Tryptophan	-	1.24
Cysteine	0.36	0.36
Mineral mix[a]	4.0	4.0
Vitamin mix[b]	4.0	4.0
Bile salt	0.1	0.1
Total	100.0	100
Energy Value (Kj/mol)	3266	3264

[a]Vitamin – thiamin 21.74%, riboflavin 19.26%, pyridoxine 12.82%, calcium panthothenate 8.64% and nicotinic acid 24.8%.
[b]Minerals – Ca $(C_2H_5O_4)_2$. $5H_2O$, 35%; $CaCO_3$, 5.06%; CaH_2PO_4, 14.6%; $KHPO_4$, 6.46%; $NaHPO_4$, H_2O, 18.76%; NaCl, 9.34%; $MgSO_4$, 7.18%; $ZnSO_4$, 0.035%; $CuSO_4$, 0.039%; KI, 0.039%; Fe-Citrate, 3.2% and $MnSO_4$. H_2O, 0.33%.

Determination of serotoninergic parameters

The serotoninergic parameters estimated were plasma concentration of tryptophan, brain serotonin and 5-hydroxyindole acetic acid (5-HIAA) levels. Plasma tryptophan was determined colorimetrically following reaction with p-dimethylaminobenzaldehyde (p-DAB) under acidic conditions according to Doppini et al. (1959). Brian serotonin and 5-HIAA were determined by high performance liquid chromatography (HPLC). 20 µl of sample (supernatant of brain homogenate) was injected into HPLC column (Perkin Elmer, 12460, USA) and sample constituents were mobilized with 0.163 M citric acid buffer (pH 3.0) containing 0.02 mM EDTA, 0.69 mM sodium octane sulfonic acid, an ion pairing reagent, 4% (v/v) acetonitrile and 1.7% (v/v) tetrahydrofurane at 900 psi pressure. Serotonin and 5-HIAA were electrochemically detected by oxidant at 0.85 V using carbon/Ag-AgCl electrode circuit detector (DXETR-45 Kyoto, Japan) at emission wave length of 320 nm. The amounts of serotonin and 5-HIAA were calculated by comparing their elution times (1.80 and 2.15 min, respectively) with those of standards: serotonin creatinine phosphate (100 – 700 ng) and 5-hydroxyindole acetate-Na (200 – 500 ng). Both parameters were measured in ng/g brain tissue (Vasconcelos et al., 2004).

Statistical analysis

Data were expressed as mean ± SEM and percentages (%). Disparities between mean values were analyzed by one-way analysis of variance (ANOVA) while differences between percentages were evaluated using chi-square (χ^2) test. Probability (P) values less than 0.05 were considered significant.

RESULTS

Effects of experimental meningitis with *N. meningitidis* W135 infection at 10^4 cfu/ml, and then 10^5 - 10^7 cfu/ml on serotoninergic parameters in mice have been investigated. Based on infection at 10^4 cfu/ml, data presented in Table 2 revealed a generally higher plasma tryptophan concentration in infected and uninfected animals on tryptophan-enriched diet compared to animals fed normal diet over the 48 h study period. The observed elevated plasma tryptophan concentration was further found to be time-dependent and elicited significant ($P<0.05$) disparity in infected and uninfected dietary groups from 24 h post-infection and 9 h post-feeding respectively. At 9–48 h post infection, plasma tryptophan concentration in uninfected animals fed tryptophan diet (31.6–36.1 ± 2.5 – 3.6 mg/dl) was observed to be significantly ($P<0.05$) higher than 28.1–33.7 ± 1.3–1.5 mg/dl found in the infected mice.

Time-dependent increase in brain serotonin level (262.7–283.7 ng/g tissue) was also observed in uninfected mice fed tryptophan-enriched diet. This was contrary to the time-dependent reductions in infected mice fed with normal (253.7–128.3 ng/g tissue) and tryptophan-enriched diets (253.9–131.4 ng/g tissue) (Table 3). Whereas, in uninfected group significant disparity ($P<0.05$) in brain serotonin level began in 24 h post feeding. This was manifested in all the time points over the 48 h study period between uninfected and infected animals fed with normal and tryptophan-enriched diet (Table 3).

Results shown in Table 4 revealed increased levels of 5-HIAA with time from 70.4 – 72.3 ng/g tissue to 79.6 – 186.4 ng/g tissue in uninfected and infected mice fed tryptophan diet over the 48 h study period, respectively.

Table 2. Effect of dietary tryptophan enrichment on plasma tryptophan concentration in normal and *Neisseria meningitidis* W135 (10^4 cfu/ml) infected mice.

Plasma tryptophan concentration (mg/dl)						
Time (h)	Control (uninfected)		Infected			
	Normal diet	Tryptophan diet	Normal diet	Tryptophan diet		
N	8	8	12	12	χ^2	P
6	28.4 ± 2.1 (0)	29.2 ± 3.6^{b} (0)	26.8 ± 2.4 (8.3)	27.4 ± 2.6 (16.7)^{b}	0.38	0.54
9	26.7 ± 1.8 (0)	31.6 ± 2.5^{ac} (0)	27.0 ± 0.8 (16.7)	28.1± 1.3 (17.7)^{b}	-	-
12	28.5 ± 3.6 (0)	32.7 ± 1.5^{ac} (0)	26.8 ± 2.4 (33.3)	28.9± 1.8 (33.3)^{b}	-	-
24	30.2 ± 3.4 (0)	34.3 ± 2.1^{ac} (0)	28.1± 1.2 (41.7)	30.6 ± 3.1 (33.3)^{a}	0.89	0.35
36	28.7 ± 2.1 (0)	34.8 ± 3.3^{ac} (0)	28.3 ± 2.1 (50.0)	31.5 ± 1.7 (41.7)^{a}	0.69	0.41
48	28.1 ± 2.4 (0)	36.1 ± 3.6^{ac} (0)	28.4 ± 1.1 (66.7)	33.7 ± 1.5 (50.0)^{a}	0.69	0.41

N = number of animals per group. Data are presented as mean ± SEM and analyzed by one-way ANOVA. Figures in parentheses are death scores (%) in the experimental animals. Disparity between death scores was analyzed by chi-square (χ^2) test. $^{a}P<0.05$; $^{b}P>0.05$ (Normal diet vs. Tryptophan diet). $^{c}P<0.05$ (Uninfected vs. Infected).

Table 3. Effect of dietary tryptophan enrichment on brain serotonin level in normal and *Neisseria meningitidis* W135 infected mice.

Brain serotonin level (ng/g tissue)				
Time (h)	Control (uninfected)		Infected	
	Normal diet	Tryptophan diet	Normal diet	Tryptophan diet
N	8	8	12	12
6	261.1 ± 23.7	262.7 ± 18.3^{bc}	253.7 ± 19.4	253.9 ± 22.1^{b}
9	268.4 ± 21.5	270.1 ± 24.6^{bc}	210.4 ± 17.6	212.7± 21.5^{b}
12	266.7 ± 36.7	270.4 ± 27.5^{bc}	200.5 ± 22.7	201.4 ± 36.2^{b}
24	270.6 ± 31.3	278.4 ± 21.5^{ac}	182.4 ± 31.4	183.7 ± 23.7^{b}
36	269.3 ± 23.7	278.6 ± 34.6^{ac}	158.6 ± 27.2	161.7 ± 26.5^{b}
48	271.4 ± 28.3	283.7 ± 42.7^{ac}	128.3 ± 17.5	131.4 ± 21.3^{b}

Data are presented as mean ± SEM and analyzed by one-way ANOVA. N = Number of animals per group. $^{a}P<0.05$; $^{b}P>0.05$. (Normal diet vs. Tryptophan diet). $^{c}P<0.05$ (Uninfected vs. Infected).

Table 4. Effect of dietary tryptophan enrichment on brain 5 – hydroxyindole acetic acid (5-HIAA) level in normal and *Neisseria meningitidis* W135 infected mice.

Brain 5- HIAA level (ng/g tissue)				
Time (h)	Control (uninfected)		Infected	
	Normal diet	Tryptophan diet	Normal diet	Tryptophan diet
N	8	8	12	12
6	68.4 ± 4.6	70.4 ± 3.3^{b}	72.3 ± 4.1	71.8 ± 1.8^{bc}
9	70.6 ± 3.1	71.3 ± 2.8 b	86.4 ± 2.8	81.5± 1.4^{bc}
12	70.8 ± 3.5	73.7 ± 1.8^{b}	114.3 ± 5.2	112.7± 3.6^{bc}
24	71.5 ± 2.8	76.4 ± 1.7^{b}	120.4 ± 4.3	117.3 ± 3.1^{bc}
36	70.5 ± 3.3	78.3 ± 2.1^{a}	158.6 ± 7.2	160.5 ± 4.2^{bc}
48	71.4 ± 2.7	79.6 ± 3.4^{a}	186.4 ± 4.7	174.5 ± 3.4^{ac}

Data are presented as mean ± SEM and analyzed by one-way ANOVA. N = number of animals per group. $^{a}P<0.05$; $^{b}P>0.05$. (Normal diet vs. Tryptophan diet). $^{c}P<0.05$ (Uninfected vs. Infected).

However, the observed variation in brain 5-HIAA levels became significant (P<0.05) between uninfected dietary groups and infected counterparts from 36 h post-feeding (70.5 – 71.4 vs. 78.6 ng/g tissue) and at 48 h post-infection (186.4 vs. 174.5 ng/g tissue). The level of this metabolite was further found to be significantly (P<0.05)

Table 5. Effect of *Neisseria meningitidis* W135 load on brain serotonin level in infected mice.

Brain serotonin level (ng/g)					
Time (h)	**Control (N = 6)**	**Infected$^{\Psi}$ (N = 6 – 12)**			
		10^4	**10^5**	**10^6**	**10^7**
6	262.7 ± 28.5	253.4 ± 17.6	251.4 ± 21.7^b	247.9 ± 18.8^b	241.2 ± 34.1^a
9	267.6 ± 23.7	212.5 ± 18.4	210.4 ± 17.6^b	206.3 ± 17.8^b	202.5 ± 15.7^a
12	268.2 ± 31.3	201.7 ± 18.5	198.6 ± 16.5^b	197.6± 26.5^b	190.4 ± 27.1^a
24	270.2 ± 27.4	182.6 ± 28.6	181.3 ± 28.6^b	180.5 ± 18.4^b	178.4 ± 28.6^a
36	270.4 ± 22.5	158.4 ± 34.6	156.2 ± 27.8^b	155.7 ± 30.1^b	153.5 ± 26.2^a
48	271.2 ± 24.5	128.1 ± 23.1	126.3 ± 20.5^b	125.7 ± 23.7^b	122.6 ± 18.4^a

Data are presented as mean ± SEM and analyzed by one-way ANOVA. N = number of animals per group. $^{\Psi}$Animals were intraperitoneally inoculated with different doses of *N. meningitidis* W135 strain ^{a}P<0.05; ^{b}P>0.05 (10^4 vs. 10^5 – 10^7).

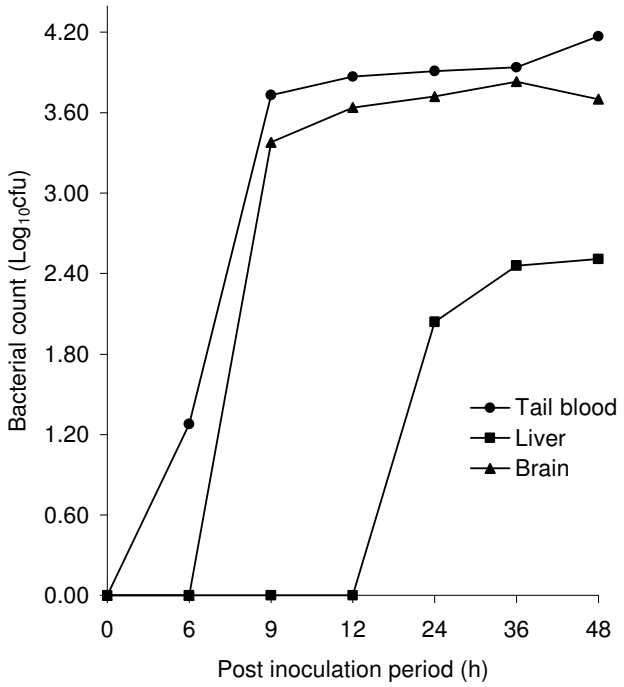

Figure 1. Bacterial count variation at three body sites in Neisseria meningitidis W135 infected mice. Each data point is a logarithmic (Log10) transformed colony forming unit (cfu) of *Neisseria menigitis* W135 cells.

higher in infected tryptophan fed mice than their uninfected counterparts.

Increment of infective dose of *N. meningitidis* W135 from 10^4 cfu/ml to 10^5–10^7 cfu/ml in the animals was found to result in significant (P<0.05) reduction in brain serotonin only at 9 h post-infection in 10^6 cfu/ml inoculated mice and at 6–12 h in 10^7 cfu/ml inoculated animals when compared to mice infected with 10^4 cfu/ml dose of the bacterial suspension. Infection with 10^5 cfu/ml did not elicit significance (P > 0.05) in brain serotonin level compared to 10^4 cfu/ml-induced infection (Table 5). Viable bacteria began to appear at 6, 9 and 36 h post-infection in the blood, brain and liver of the animals infected with 10^4 cfu/ml of *N. meningitidis* W135 strain, respectively. The kinetics of appearance of the bacterial strains in the blood and brain were observed to be similar but with difference of 0.2 – 1.2 logarithmic units between 9 and 36 h post-infection (Figure 1).

DISCUSSION

In addition to increasing morbidity and mortality in endemic and epidemic loci in the world, meningococcal infection due to the highly virulent *N. meningitidis* W 135 has further elevated the risk of neurological sequelae dominated by depression, hearing and cognitive impairment among survivors (Tikhomirov et al., 1997; Hodgson et al., 2001; Nau, 2003). Experimental meningitis infection induced via parenchyma, intercisternal, intranasal, subarachnoid and intraperitoneal routes in using rats, rabbits, guinea pig and mice as hosts have been employed in various studies to improve understanding of pathogenesis of meningitis, pathogens' virulence factors diversity and drug efficacy (Tsai et al., 1990; Tan et al., 1995; Tsao et al., 2002; Hirst et al., 2003; Chiavolini et al., 2004).

The present study has further revealed the alteration in serotoninergic pathway following experimental *N. meningitidis* W135 infection via the intraperitoneal route in mice. Although dietary tryptophan enrichment resulted in elevated plasma level of the indole amino acid in infected and uninfected animals compared to mice fed with normal diets, 8.3–66.9% of the infected animals died owing to the lethal infection of *N. meningitidis* W135. Previous studies (Fernstrom and Wurtman, 1971; Fernstrom et al., 1979) have revealed the dependence of plasma tryptophan concentration on dietary level with inc-

reased ratio of free tryptophan to large amino acids (LNAAs) contrary to most protein diets, which contain ≤ 1% tryptophan and ≤25% LNAAs (i.e. leucine, isoleucine, phenylalanine, valine and methionine) (Leiberman et al., 1986). It is therefore not surprising that plasma tryptophan concentration was elevated in our experimental mice fed with diet enriched with 1.24 g/100 g tryptophan compared to those fed with normal diet independent of *N. meningitidis* W135 infection. However, the lower level of plasma tryptophan concentration in infected animals compared to uninfected mice despite dietary tryptophan fortification may not be unconnected with disruption of blood-brain barrier permeability caused by *N. meningitidis* infection (Leib and Tauber, 1999; Nau, 2003). A study by Park et al. (2003), in which *E. coli* meningitis was induced via cisternal and intraperitoneal routes in newborn piglets, demonstrated pathologically the bacterial colonization of nervous tissues, increase in intracranial pressure and subsequent decrease cerebral blood flow in these animals. Although, the latter possibility was not investigated in this study, impaired cerebral blood flow auto-regulation as a consequence of increased intracranial pressure, and reduced cerebral blood flow as a response to reduced mean arterial pressure has been attributed to meningococcal infection and associated CNS disturbance in several animal and human studies (Goiten and Tamir, 1983; Tureen 1989; Tureen et al., 1990; Nau, 2003). Meanwhile, improve brain serotonin synthesis and secretion through trypto-phan pyrolase dependent pathway and membrane transport (Lovenberg et al., 1968) has been associated with increased plasma tryptophan concentration, trypto-phan:LNAAs ratio and flux of tryptophan from the plasma to the brain through the blood-brain barrier (Fenstrom and Fenstrom, 1999). Sharp et al. (1995) were able to achieve enhanced serotonin release from rat hippocam-pus and improved serotoninergic neuronal activity following systemic tryptophan administration. Therefore, our observed increased brain serotonin level in tryptophan diet fed mice is an attestation to these findings. However, that brain serotonin level reduced markedly in infected mice may also be due to *N. meningitidis* colonization of the nervous tissues, since disruption of blood-brain barrier integrity has been implicated in altered kinetics of tryptophan transport in several studies (Padridge and Oldendorf, 1975; Struider and Weicker, 2001). This can be said to be complemen-ted by observed elevated 5-HIAA levels in the brain homogenate of our infected mice, connoting increased serotonin turnover. Reduced brain serotonin level due to its biodegradation to its primary metabolite 5-HIAA has been blamed on hyperactivity of monoamine oxidase in patients with depression and neurodegenerative diseases (Carriers and Marco, 2004; Youdim et al., 2005). Strategies aimed at inhibiting the activity of this enzyme have been found to elicit favourable clinical outcome (Rascol et al., 2005). The adoption of this strategy as an adjunct in the management of meningococcal infection which demands investigation of the activity and kinetic of monoamine oxidase in meningitis is therefore suggested. However, it is not clear from the present study whether the observed reduced brain serotonin level is a generalized pathology or cell type dependent since whole brain homogenate was analyzed. This is so because previous experimental meningitis studies have demonstrated variations in the pattern of pathology and inflammatory responses by the dentate gyrus, neocortical and context - learning and memory controlled hippocampal regions of the brain (Nau, 2003).

Nevertheless, the appearance of viable bacteria in the blood, brain and liver of our infected mice provides a clue for possible variation in inter-organ pathology due to *N. meningitidis* W135 infection. The subsequent alteration of serotoninergic pathway emanating from this pathology in the brain may further be modulated by the degree of infection since significant time-dependent disparity in serotonin level was elicited by the pathogen at 10^4 cfu/ml and 10^5 - 10^7 cfu/ml in this study. A related study by Chiavolini et al. (2004) revealed colonization of nervous tissues and systemic dissemination of mice by *S. pneumoniae* serotypes with time-point variation in cfu counts following subarachnoid infection.

In conclusion, this study has revealed reduced brain serotonin level in surviving mice with *N. meningitidis* W135 infection, implicating altered serotoninergic activity as one of the mechanisms of neurological sequelae in meningococcal infection and may be the basis for depression episode found frequently among survivors of meningococcal infections.

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