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Murine Models for pathogenicity of Human Neurotropic Stealth and Normal Hemophilus Influenzae B [HIB]

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Abstract

Human neurotropic stealth and normal intact Hemophilus influenzae b [HIB] were recovered from cases of subacute meningitis pateints.Tempts were made to reproduce the disease in murine models .Two infectious live doses as one and ten international units of the WHO opacity tube were prepared from both of the stealth and intact isolates.The dose volume was fixed as 0.1 ml.These infectious doses were applied to the murine models via intracranial and intravenous routes.The matching of gross neurogenic signs and symptomes of the infected mice were lasted up to ten days post-infection.At the dose 10 IU ,both stealth and intact HIB cause sudden death.While,1 IU doses from both stealth and intact HIB yield neurogenic symptomes from 7 to 10 days then the animals died. The 10 IU doses from both stealth and intact HIB via intravenous routes were leading to mild neurogenic symptome ranged from 2 to 24 hrs then vanished. While, 0.1 doses from both forms of the infectious agents showed symptomless state. The pathogenicity spectrum was as; symptomeless, mild neurogenic symptom, sheffering, laid down on one body site on the stratum then death and/or sudden death.Reisolation was fruitful in all infected animals. The direct impression stained smears of the infected mice brains showed landmarks of sub-acute meningitis. Intracranial mice model seems to be novel for simulation of human subacute meningitis disease.

Keywords: Animal; cell wall defective; dose; infectious; intact; models.

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1. Introduction

Hemophilus infleunzae b HIB is small gram negative encapsulated rods.It holds the position of one of three important human encapsulated pyrogens along with;Streptococcus pneumoniae and Neisseria meningidtis.HIB serotyping is based on the capsular polysaccharide with six typable and one untypable serotypes.Of the six serotypes,type b causes most of the severe invasive diseases such as meningitis and sepsis.Type b capsule is composed of polyribotol phospate PRP.HIB is the only human pathogen with no evident animal reservoir.The organism produce several virulence factors like; capsule,OMP,Pili,IgA proteases,peptidoglygcan,iron acquisition and phase variation genes[1,2,3] .The story of HIB pathogenesis and pathogenicity can be as;colonization,invasion,bacteremia then localization[4,5,6,7,8,9].The reported pathogenicity animal models ranges as;mice junbo mice,rat,infant rat and infant rabbit.Among which white mice was the dominant one.The infection routes were; intraperitoneal IP, intranasal IN , and intracranial IC in various models[5,11-15],Table-1.Though,in mice models neither IC nor intravenous IV routes had been tempted.In the present work,it was aimed at the induction of the CNS disease through IC and IV using two live dose intensities and fixed volume of the infecting doses.

Table 1: An Abroad Animal Models for pathogenicity of H.influenzae

Sequence	Species	model	Dose/route	Disease	references
1	H.influenzae HilT	Jubo mice	Intranasal	Midel ear infection	Hood et al. 2016[10] Cheesem and Hood 2017[11]
2	H.influenzae	mice	Intratrachial	Air way inflammation	Venoparasal et al 2016[12]
3	H.influenzae	5 day infant rate Chichilla rabbit	Localized	Invasive disease Local infection	Virji and Hill 2003[13].
4	H.infuenzae	mice	Oral or IP	Meningitis	Marks et al.1982[5]
5	H.influenzae	rabbit	Intacranial	Meningitis	Sulc et al 1992[14]
6	H.influenzae	mice	IP	Meningitis	Crucishank et al.1975[15]

2. Materials and Methods

2.1 The Pathogen

Hemophilus-like cell morphology of the noted organisms in association with lymphocytic –neutrophilic infiltrates in the stained CSF films of some of the clinically proven meningitis cases.The film showed pleomorphic gram negative rodes in two cases and sowellen short coccoid in two other cases of the 50 tested

patients.CSF samples were cultured onto enriched nutrient agar media for first two and into variant neutrient agar media[16] for the second both under microaerophilic conditions.Growth onto enriched media was normal intact hemophilus like colony morphotypes and the second,stealth CWD hemphilus-like colony morphotypes were noted.Pure culture isolates were prepared and kept till use for identification and pathogenicity studies.These human local CSF isolates were;Gram negative short bacilli or short sowllencoccobacilli,nonmotile,microaerophilic needs X and V factors for growth, non-hemolytic onto blood agar,forms satellatism and produce normal intact and stealth cell wall defective forms.They were;oxidase , catalase, and nitrate reductase positive.Solubilize bile salt and ferment glucose and xylose with acid production.But non-acid producer from;lactose,sucrose,mannitol and innulin by standard biochemical tests and EPI 20E.These charcteristics are consistant with H.influenzae.Serotyping with specific serotyping kit DiFco BD TM showed that they were type b[17,18].

2.2 Election of the Test Pathogen

There were set of criteria tempted in election of the test pathogen as;i-association with severe human infection form , ii-marked inflammatory reacion in association with the presence of the infectious agent in CSF filme and production of pure heavy growth onto primary plate cultures of the CSF[18].

2.3 Infecting Doses

For the intact form fresh growth in an enriched broth culture was made anf centrifuged at 5000 rpm for tem minutes. While ,for the stealthCWD form fresh growth in variant broth culture medium was done,centrifuged at 5000 rpm for ten minutes.Pellets were resuspended in sterile saline ratified to match one and ten units on the international opacity WHO tubes.The dose size was fixed as 0.1 ml. for both infection routes and infecting doses[17].

2.4 Mice

A balb/C white mice weighing 25-30 gms were kept in cages under at libidum conditions for husing,food and drinks as in the following ,Table - 2 .

Table 2: Laboratory mice model infection protocols [19]

HITB	Dose volume/Intensity	Route	Replicates
Stealth CWD	0.1 ml / 1 IU	Intracranial	2
	0.1 ml/ 10 IU	intracranial	2
Stealth CWD	0.2 ml/ 1 IU	Intravenous	2
	0.3 0.1 ml/ 10 IU	intravenous	2
Normal intact	0.1 ml/ 1 IU	Intracranial	2
	0.1 ml/10 IU	intracranial	2
Normal intact	0.1 ml/ 1 IU	Intavenous	2
	0.1 ml/ 10 IU	intravenous	2
Saline Control	0.1 ml.	intracranial	2
Saline control	0.1 ml.	intravenous	2

2.5 Tropism

The ability of a pathogen to invade and survive in nervous tissues is the neurotropism[20,21]. .

2.6 Reisolation

Microaerophilic normal and stealth pathogen culture settings were adopted [17,18].

3. Results

3.1 Pathogenicity Spectrum

3.1.1 Stealth CWD Pathogen

At a dose of 10 IU via intracranial route cause sudden death.Same dose through intravenous route cause sluggishness for two hrs then vanished.The dose IU intracranial cause;shffering,sluggishness lasted for seven days the mice died.Thus,both 1 IU and 10 IU doses intracranial route cause death either sudden for the 10IU or after seven days for 1IU.The dose 10IU through IV cause sluggishness for 2 hrs and the dose 1IU was asymptomatic,Table- 3.

Table 3: Pathogenicity of stealth CWD HIB in mice

Dose unit per volume	Dose size in ml.	Route	Nature of the symptomes	Death end point
10IU	0.1	Intracranial		Sudden death
10 IU	0.1	Intravenous	Sluggishness for 24 hours	
1 IU	0.1	Intracranial	Neurogenic symptomes of sluggishness,sheffering laste for 7 days	Death after 7 days
1 IU	0.1	Intraveous	asymptomatic	

3.1.2 Normal Intact Pathogen

At a dose of 10IU intracranial HIB cause sudden death.Same dose through intravenous cause neurogenic symptomes lasted for 24 hrs only.The dose 1 IU HIB intracranial cause neurogenic symptomes lasted for 10 days the death.Same 1 IU dose through intravenous was found as asymptomatic.Thus ,one and 10 IU doses intracranial cause death after ten days in 1 IU and sudden death in 10IU.Both 1 IU and 10 IU doses through intravenous routes were asymptomatic in 1IU and and neurogenic symptome lasted for 24 hr for 10 IU doses,Table 4

Table 4: Pathogenicity of normal intact HIB in mice

Dose unit per volume	Dose size in ml.	Route	Nature of symptomes	Death end ponit duration
10 IU	0.1	Intracranial		Sudden death
10 IU	0.1	Intravenous	Slugishness lasted for 10 days	Death at the 10th day postinfection
1IU	0.1	Intracranial	Sluggishness for 2 hrs	
1 IU	0.1	Intravenous	A symptomatic	

4. Comparative View

The infectious –inflammatory reaction in CSF of human have shown lymphocytic-neutrophilic inflammatory cell infiltrates associated with the causal.Direct stained impression smears of the infected mice brains showed infilammatory cell infiltrates of mixed lymphocytic-neutrophilic nature.The intensity of reactions were more evident in the intracranial routeThe causal HIB were re-isolated from the infected mice models iline with that isolated from the natural human cases.The neurotropic HIB intracranial infection showed more evident neurogenic symptomes than those infected intravenously in both of the dose streangth and dose size.Smaller dose intensity induce neuriogenic symptome only in intracranial route[19],Table – 5.

Table 5: Comparative pathogenicity of HIB in mice

Stealth CWD	ml. 1 IU	Intracranial	Sluggisness,sheffering ,laydown on one side on stratum lasted for 7 days Asymptomatic	Death at the day 7th
	0.1 ml.1 IU	intravenous		
Stealth CWD	0.1 ml.10 IU	Intacranial		Sudden death
		intavenous	Sluggishness for 2 hrs	
Normal intact	0.1 ml. 1 IU	Intracranial	Sluggishness,sheffering,laydown on one side on stratum lasted for 10 days asymptomatic	Deathend point at the 10th day
		intravenous		

6. Discussion

Our journey with the stealthCWD bacterial pathogens had been started at 2003[16],followed by the contribution of Shnawa and his colleagues. on developing a lapin model for stealth and intact S.aureus and E.coli arthropathogenicity[23].Then, reaching the work of Thewaini et.al.[24] that have been investigated the urogenital pathogenicity of stealth CWD Citrobacter fruendii in alapin model.Presently,several animal models for hemphilus influenzae have been documented.Though niether intacranial nor intravenous routes [5,11-15] tempted,Table – 1.This communiction was aimed at tempting to study pathogenicity spectrum of stealth CWD and normal intact HIB in mice.The specrum of pathogenicity of both forms of HIB in murine model was as ; asymptomatic ,mild sluggisness moderate to severe slughisnness,sheffering,laying down on one side of the body on the stratum then death or sudden death as in n high density infectious doses through intracranial routes,Tables_ 3 -5. Such spectrum diferences may be attributed to differences in the virulence factors between Stealth and intact HIB [16].

The study evident factors affecting virulence vogor were;physical nature of the infecting agent,dose intensity,dose size and the infection routes[18].The intracranial routes were rapider than intravenous routes,Table 1-3 a finding inline with[4,5, 14]. And in contradiction with that of IV route showed symptomless infection or carrier state[5].In this experimental settings, due to the apparent failure of the pathogen, in passing blood brain barrier BBB in IV infected group.Hence, the intracranial route mimicates the human natural infection routes where the infectious agent find port of entry through nasal sinus to naspharynx to paranasal neural space to nerve ending tissues and may succssed in passing blood brain barriers [2,3,6 ,22,25,26].

7. Suggestion

A set of criteria were suggested for scoring neurotropism in murine experimental models simulating that of human beings as;i – Presence of bateria in association with inflammatory cell infiltrate,ii-The extent of matching of inflammatory reactions in tissue sections of the infected animal to that reaction in the patients CSF

iii – yeild of pure growth in primary plate culture of CSF and the reisolation tempts of nervous tissues of infected animals , and iv – neurogenic symptomes of murine models that match that of human being meningitis patients .

8. Conclusion

This murine model experimental settings Tables 2-4 can be summed up as in the followings

i-Intracranial high density doses of both forms of HIB lead to sudden death.

ii-intracranial low density doses for both forms of HIB lead to death after 7 to ten days

iii-Both high and low dose densities via IV route for both forms of HIB were showing mild neurogenic symptomes.

iv-The pathogenicity spectrum was;asymptomatic,mild transient,marked neurogenic symptomes lasted for 7 to 10 days followed by death.

v- Factors affecting affecting pathogeniity in these murine models were ; physcial nature of infectious agent,dose intensity and infection routes

9. Limitation

This experimental settings needs to be confirmed by application of a similar setting onto non-human primat the monkeys models.Since monkeys are genetically more closer to human being than rabbits.On trying to extrapolate the results to human beings.

10. Conflict of Interest

Authors have no competing interest.

11. Ethical Issue

Care, housing,handinling and interventions on mice were done following the international acts regulating care,housing,and handling of laboratory animals.

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