CARVACROL MAY AID INFECTION PROGRESSION OF BACILLUS CEREUS-MEDIATED ENDOPHTHALMITIS IN MICE

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Background

Bacillus cereus is a Gram positive, spore-forming bacterium estimated to cause more than 27,000 food-associated illnesses annually in the United States (1). *B. cereus* is found in soil largely as spores, and proliferates when brought into contact with organic matter or a host (2). The spores produced by *B. cereus* are heat-resistant and highly adhesive, allowing bacteria to opportunistically infiltrate food production environments and disseminate as viable spores or vegetative cells (2,1). This bacterium is also known to contaminate postsurgical or traumatic wounds and cause opportunistic infections, including endophthalmitis (1).

Endophthalmitis, when caused by *B. cereus*, creates irreversible tissue damage within 24 hours. *B. cereus* is one of the most common causes of both posttraumatic and endogenous forms of endophthalmitis (1). On average, 70% of *Bacillus* endophthalmitis cases result in total vision loss, with nearly half of those resulting in evisceration (3). In addition to the damage caused by the *B. cereus* toxins, an inflammatory response also occurs, causing subsequent damage to retinal tissues (3). It has been suggested that both an anti-inflammatory and an antimicrobial must be used together to treat the infection properly (3). As of now, in the majority of *Bacillus* endophthalmitis cases, vision loss occurs regardless of therapeutic or surgical intervention (3). Therefore, a clinical need exists to learn more about the ability of toxigenic *B. cereus* to infiltrate ocular tissue and perhaps go systemic during endophthalmitis.

Materials and Methods

Growth Conditions: *B. cereus* ATCC 14579 (VWR, West Chester, PA) was cultured under aeration at 150rpm and 32°C in Tryptic Soy Broth (TSB) (Weber Scientific, Hamilton, NJ USA).

Treatment Administration: Five groups of BALB/c mice (4 mice per group), were treated with either saline eyedrops, saline and minimum inhibitory concentration (MIC) of carvacrol, $7x10^7$ cfu sample of *B. cereus*, or the bacterial suspension followed by the sub-inhibitory concentration (SIC) or MIC of carvacrol four hours later. The carvacrol SIC was 1mM and MIC was 2mM. Each treatment was suspended in 15 µL of saline and administered by aseptic eyedrops in both eyes. Treatments were given while mice were anesthetized (Ketamine, Xylazine). Analgesic (Buprenorphine) was administered as needed.

Data Collection: Daily observations were made, recording any weight gain/loss, and behavioral changes. At the endpoint of the study (14 days), the immune effectors tumor necrosis factor alpha (TNF-α), and interleukin-6 (IL-6) were quantified from peripheral blood using both enzyme-linked immunosorbent assays (ELISA, eBioscience, San Diego, CA) and RT-PCR. Total RNA was extracted from serum of each group using a Ribozol (Amresco, Solon, OH). Real-time reverse-transcriptase PCR was conducted on purified RNA templates using the MasterAmp RT-PCR system (Epicentre Biotechnologies, Madison, WI), utilizing SYBR Green-I as the fluorescent chemistry. Serum collected was also

inoculated onto Mannitol Egg Yolk Polymyxin B Agar (Remel, Lenexa, KS) to enumerate *B. cereus* that may have entered peripheral blood.

Group Number	Treatment
Group 1	MIC + B. cereus
Group 2	SIC + B. cereus
Group 3	*B. cereus*
Group 4	MIC only
Group 5	Saline

Table 1: Experimental Groups

	5'→ 3'	Tm (°C)	G+C (%)	Reference
TNFα Fwd.	ATGAGCACAGAAAG CATGATC	53.2	42.8	-4
TNFα Rev.	TACAGGCTTGTCAC TCGAATT	54	42.8	-4
IL-6 Fwd.	GGAGAGCATTGGAA ATTGGGG	56.3	52.3	-5
IL-6 Rev.	CGGAGAGGAGACTT CACAGAGGA	59.5	56.5	-5
β-Actin Fwd.	GTGGGCCGCTCTAG GCACCA	64.8	70	N/A
β-Actin Rev.	CGGTTGGCCTTAGG GTTCAGGGG	64.1	65.2	N/A

Table 2: RT-PCR Primers, sequences, used in this study

Results

PCR Data For β- Actin		
	Ct Value	Tm (°C)
Group 1	23.17	82
Group 2	28.98	82
Group 3	28.29	84
Group 4	23.64	87
Group 5	24.69	88

Table 3A

PCR Data For IL-6		
	Ct Value	Tm (°C)
Group 1	20.84	77
Group 2	27.67	76
Group 3	24.60	76
Group 4	21.26	79
Group 5	17.13	81

Table 3B

PCR Data For TNFα		
	Ct Value	Tm (°C)
Group 1	22.50	79
Group 2	32.43	78
Group 3	26.11	78
Group 4	25.15	80
Group 5	23.08	83

Table 3C

Table 3A shows the values for β-Actin RT-PCR. **Table 3B** includes the values obtained during IL-6 RT-PCR. **Table 3C** includes the values generated from TNF-α RT-PCR.

Conclusions

Daily Observations: There was no significant change in the weights of the mice during the 14 day period (p>0.05). The activity level and appearance of the mice that received only the carvacrol or saline treatment remained consistent. By 14

days, the groups that received the *B. cereus* treatment followed by carvacrol had the most visible signs of infection, with the highest concentration of carvacrol causing the worst symptoms of infection. Infection was defined as red and/or swollen eyes and lethargy. The *B. cereus* control group (no carvacrol treatment) had fewer infection symptoms than the *B. cereus* groups that received carvacrol (data not shown). These results suggest that carvacrol aided infection progression locally, although these observations are qualitative.

Data Collection: There was no significant difference between the concentrations of IL-6 and TNF-α between any of the groups based on the ELISA results ($p>0.05$). Based on the RT-PCR results there was no significant difference in expression of TNF-α or IL-6. ELISA-based detection of the *B. cereus* nonhemolytic enterotoxin (NHE) complex also revealed no detectable signal, and no *B. cereus* bacteria were recovered from serum at the 14d endpoint on MYP agar plates. Based on these findings, we conclude that the ocular infections in every mouse group did not result in a measureable systemic response. Current work is focusing on histological examination of ocular tissue to quantify damage to retinal barrier epithelial (RBE) cells, and to visualize infiltration by important immune cells such as granulocytes.

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