한국환경과학회지 제7권(제4호), 501~504, 1998
J. of the Korean Environmental Sciences Society

Importance of Iron in the Toxicity of Vibrio vulnificus

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(Manuscript received 27 November 1997)

The role of iron as a possible pathogenic factor in the infection of V. vulnificus was examined in this paper. The effects of iron and CCl_4 on the growth of V. vulnificus in human and rabbit sera were also done. Injection of iron to mice resulted in a lowering of the 50% lethal dose and in a reduction in the time of death postinfection. Serum iron levels were also elevated by damaged livers with infections of CCl_4. The inoculum size required to kill these mice was directly correlated with serum iron levels. Iron appeared to be the limiting factor in the ability of this organism to survive or grow in mammalian sera. These results, both in vitro and in vivo, provided strong evidence that iron may play a major role in the pathogenesis of V. vulnificus.

Key words : iron, toxicity, pathogenic factor

1. Introduction

Marine Vibrio vulnificus was found in estuarine environments of coastal waters and perhaps the most invasive of vibrio species. Unlike other vibrios, it was associated with a high incidence of sepsis, necrosis of surrounding tissue, and septicemia correlated with a high degree of mortality (Blake et al., 1980). Blake et al.(1980) provided a comprehensive clinical presentation of infections produced by this bacterium. 75% of infections resulting in septicemia were associated with liver damages or other chronic diseases - hemochromatisis, thalassemia, and chronic cirrhosis, et al. - that could result in iron overload.

The importance of iron for microorganisms has long been recognized, and the ability of iron has been proposed as a contributing factor in a number of experiments on the bacterial infections (Bezkorovainy, 1980; Bullen et al., 1975; Weinberg, 1978). Iron within the mammalian host was bound to various proteins and was, therefore, not readily available for bacterial acquisition(Schade et al., 1946). For example, the bactericidal properties of serum were attributed to the sequestering of iron. These properties could be abolished by increasing the saturation of transferrin with iron.

In considering the high correlation of V. vulnificus infections with diseases involving increased iron level, the effects of iron and iron-chelating agents on the pathogenicity and on the ability of this organism to survive were examined in this paper.

2. Materials and Methods

2.1 Organism

V. vulnificus was used for injections into 6- to 8-week-old male mice(ICR) or for inoculations into sera. Inocula were obtained from early stationary-phase growth in brain heart infusion and washed three times in phosphate-buffered saline(PBS) before use.

2.2 Iron injection

50% lethal dose(LD_{50}) was calculated by the method of Reed and Muench(1938), using intraperitoneal(i.p.) injection(0.5mL) of V. vulnificus and concurrent i.p. injection(0.2mL) of sterilized ferric ammonium citrate(80μg) in PBS. Control mice received only iron injection.

2.3 CCl_4 injection

Mice were fasted 12h and then CCl_4 was administered(0.5mL, i.p.) as a 20% solution in olive oil as described by Gomez et al.(1975). Inocula of V. vulnificus were injected into mice 24, 48, and 72h after injections of either CCl_4 in olive oil or olive oil alone. Control animals received only CCl_4 injections.

2.4 Serum iron level

Blood samples were obtained from mice by cardiac puncture at intervals of 1 to 72h. Serum sam-

Table 1. Effect of iron on 50% lethal dose

inoculum(log)	No. dead (n=5)	
	PBS	Fe
8	5	-
7	4	-
6	1	-
5	0	5
4	0	5
3	0	5
2	-	5
1	-	4
0	-	4
-1	-	0
LD_{50}	5×10^6	1×10^0

ples from at least four mice were pooled and serum iron levels were measured by the micromethod of Henry as described by Weissman and Pileggio(1974). Only samples free of hemolysis were used.

2.5 Growth in sera

Pooled sera were obtained aseptically from either four humans or three rabbits and stored at 4 °C before use. V. vulnificus was inoculated into 10mL of either rabbit serum(RS), human serum (HS), HS with 0.01mg or 0.10mg of ferric ammonium citrate per mL. All additions to sera were of 0.1mL volumes and cultures were incubated at 37 °C. Growth was determined by plate counts on marine medium.

3. Results and Discussion

3.1 Iron effect

To investigate experimentally whether or not iron availability could be a factor in the pathogenicity of V. vulnificus, we examined the effect of injected iron on the LD_{50} in mice. Ferric ammonium citrate has been reported to be toxic in mice at levels of 12.5μg/g(Holbein et al., 1979) and the concentration used in our experiments (4μg/g) was never lethal. Table 1 showed that ferric ammonium citrate produced a lowering of the LD_{50} from 5×10^6 to 1×10^0. It should be noted

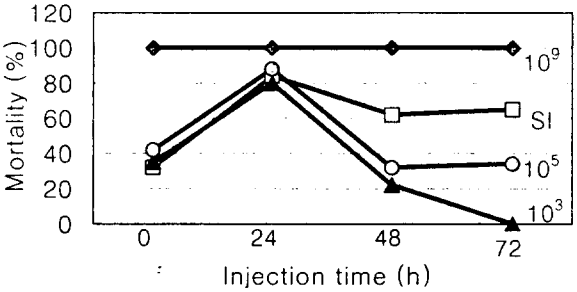

Fig. 1. Effect of serum iron level on mortality after CCl_4 treatment. SI: serum iron level

that inocula which were sublethal in mice receiving PBS produced 100% mortality in iron-treated mice. Observations were also made on the host survival time with or without iron treatment. Iron treatment greatly reduced the host survival time after V. vulnificus infections at inocula below 10^9.

3.2 CCl_4 effect

Serum iron values for CCl_4-treated mice were presented in Fig. 1. Values obtained on serum from untreated mice within a 24h period ranged from 170 to 200%. An increase in serum iron levels was seen between 6 and 24h after CCl_4 treatment. These levels began to decline at 48h. Injections of V. vulnificus into mice at the time of increased iron levels revealed an increase in mortality as shown in Table 2. Fig. 1 illustrated that increased mortality correlated with increased serum iron levels. Since one mode of transmission of septicemia was by ingestion of contaminated seafood, mice were also given oral doses of V. vulnificus. When given 10^9 organisms, approximately 50% of the mice died; no deaths occurred at 10^3(Table 2). When V. vulnificus was given orally 24h after CCl_4 treatment, all mice died with the lowest inoculum tested(10^3).

3.3 Serum effect

Human serum(HS) is an iron-limiting environment as most of the available iron is bound to transferrin, which is normally about 30% saturated with iron(II). Rabbit serum is more fa-

Table 2. Effect of CCl_4 on mortality

V. vulnificus inoculum	route	mortality(No. dead/No. tested)	
		untreated	treated
2.0×10^9	i.p.	6/10	10/10
2.0×10^5	i.p.	2/10	9/10
2.0×10^3	i.p.	0/10	9/10
2.0×10^9	oral	6/10	10/10
2.0×10^3	oral	0/10	10/10

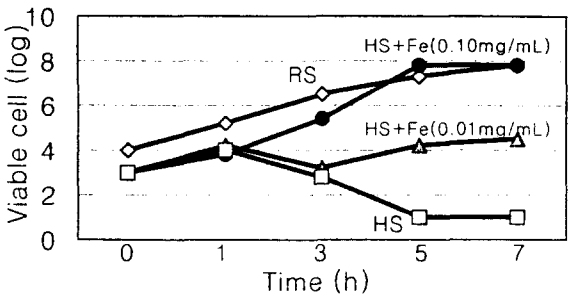

Fig. 2. Effect of serum on growth of V. vulnificus. RS: rabbit serum, HS: human serum

vorable environment for bacterial growth since rabbit transferrin is about 60% saturated with iron(II). Fig. 2 demonstrated that rabbit serum was an excellent medium for V. vulnificus. HS, on the other hand, was bactericidal for this organism. The lethal effects of HS was reversed by the addition of ferric ammonium citrate and enhancement of growth was related to the concentration of added iron(Fig. 2).

The withholding of iron from bacteria by host proteins has been reported to play an important role in resistance to infection and has been designated as a type of nutritional immunity. Increasing the availability of this nutrilite can induce the increase of microbial growth and pathogenicity. The ingestion of iron in mice increased both the lethality and rapidity of V. vulnificus infections. Increasing iron availability by ingestion of CCl_4 produced a similar effect with this organism. CCl_4 is believed to produce a hepatocellular necrosis which liberates iron stores and increases serum iron(Reissmann et al., 1954). Increased serum iron levels induced by this method were correlated directly with increased lethality in mice by V. vulnificus. It was difficult to determine in vivo whether or not iron facilitated growth of the pathogen or interfered with host defense mechanism. Therefore, the effects of iron on in vitro growth of V. vulnificus were examined.

It seemed likely that the determining factor for the killing of V. vulnificus by serum was iron deprivation. The effects of iron in experimental V. vulnificus infections corresponded vey well with experimental evidence for the importance of iron in other bacterial infections. The high correlation of V. vulnificus septicemia with clinical iron-overload in humans was perhaps even more significant to the study of the role of iron in infection. Although the experimental evidence for the importance of iron in microbial infections was rapidly expanding, the relationship between iron status in a clinical situation and the susceptibility to systemic infections was still a matter of controversy. The study presented here provided an experimental basis for clinical observations and suggested that V. vulnificus may be a valuable model for releasing iron to microbial infection.

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Vibrio vulnificus의 독성에 있어서 Iron의 중요성

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(1997년 11월 27일 접수)

Vibrio vulnificus가 병원성을 일으키는 한가지 요인으로서 iron의 역할을 본 연구에서 알아보고 V. vulnificus의 성장에 미치는 사람과 토끼 혈청에서의 iron과 CCl_4의 영향도 조사하였다. 쥐에 iron을 주사한 결과 50% 치사량이 감소하였고 감염 후 치사 시간도 감소하였으며 CCl_4로 감염된 간에 의해서 혈청 중 iron 준위는 증가하였다. 쥐를 치사시키는데 필요한 접종량은 혈청 중 iron 준위와 직접적으로 상호 관련성이 있었다. Iron은 이 미생물이 포유류 혈청에서 살아남거나 성장하는 능력에 있어서 제한 요인으로 나타났다. 시험관 내에서와 생체 내에서 이들 결과는 iron이 V. vulnificus의 병원성에 있어서 중요한 역할을 한다는 강한 증거를 제공해주었다.