Pathogenicity of *Fusobacterium necrophorum* Biovar C in Mice by Intraperitoneal and Intraportal Injections

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(Received 25 April 1989/Accepted 1 August 1989)

ABSTRACT. Three strains of *Fusobacterium necrophorum* biovar C were injected into mice intraperitoneally and intraportally. All the mice survived. In one mouse out of 15 mice injected intraperitoneally, a few focal abscesses were formed in the liver. The microorganisms were recovered from the liver abscess and the tissue of liver with abscess. No changes were observed in the organs of other 14 mice and no bacteria were recovered from them. In the 15 mice injected intraportally, no liver abscesses and no macroscopic changes in the organs were formed. However, the inoculated bacteria were recovered from the liver of four mice. The pathogenicity of *F. necrophorum* biovar C was weaker than that of other two biovars.—**KEY WORDS:** biovar C, *Fusobacterium necrophorum*, mouse pathogenicity.

Jpn. J. Vet. Sci. 51(6): 1111–1114, 1989

Fusobacterium necrophorum is divided into three biovars A, B and C, according to hemagglutinating and hemolytic activities. Biovar A has both activities, biovar B shows only hemolytic activity and biovar C lacks both activities [2]. *F. necrophorum* biovar A strains are fatal to mice with severe diffuse liver necrosis and biovar B strains produce focal liver abscess in mice, but are not fatal to them [4, 5]. No experimental injection of biovar C in mice was carried out except for the experiment performed by Fiévez [2]. In this study we observed the pathogenicity of three strains of biovar C for mice by intraperitoneal and intraportal injections.

MATERIALS AND METHODS

Mice: ICR mice were originally purchased from the Kyudo Animal Farm (Kumamoto), and had been bred in our laboratory. Five or six mice were used in each strain and five mice were employed as control in each injection route.

Injected strains and bacterial number: Three strains, nos. Fn 520 (strain 410 of Dr.

Beerens, Institut Pasteur de Lille, Lille, France and bovine feces isolate), Fn 521 (strain 118 of Dr. Beerens and ovine pulmonary abscess isolate), and Fn 522 (strain 130 of Dr. Beerens and ovine pus isolate), were used. They were cultured twice in BPPY broth [3] at 37°C for 24 hr and 0.3 ml of cultures containing 1.2×10^8 to 4.3×10^8 colony forming units (CFU) per mouse was injected into mice by intraperitoneal route [4, 5]. Intraportal injection was carried out according to the method previously described elsewhere [6]. The number of viable bacteria inoculated was 2.5×10^7 to 4.3×10^9 CFU per mouse.

Recovery of strains injected: The mice were killed by cervical dislocation and their liver, spleen, kidney and lung were aseptically obtained. Each organ was stamped on GAM agar (Nissui Seiyaku) supplemented with 5% defibrinated horse blood. The plates were cultivated anaerobically at 37°C for 72 hr. Recovered strains were identified as *F. necrophorum* biovar C by colonial and cellular morphologies, and additional some biological properties.

RESULTS

Intraperitoneal injection: The results are shown in Table 1. A few focal abscesses were formed in the liver of no. Fn 522-5 mouse injected with strain Fn 522 after 14 days. The microorganisms were recovered from the liver abscess and its normal liver tissue by stamp culture. No changes were observed in the other organs, spleen, kidneys and lungs. In other mice inoculated with strains Fn 520 and Fn 521, and in control mice, neither liver abscesses nor macroscopic changes in the other organs were observed.

Intraportal injection: The results are shown in Table 2. The mice in this experiment formed neither liver abscesses nor macroscopic changes in other organs after 7 days. However, the inoculated bacteria were recovered from the liver of nos. Fn 521-2, Fn 522-1, -3, -6 mice inoculated with strains nos. Fn 521 and Fn 522, respectively. No abscesses and no macroscopic changes in

organs were produced in control mice.

DISCUSSION

It is reported that pathological changes caused by biovar A strains in mice were lethal, mostly acute, and diffuse abscess in the liver, while those caused by biovar B strains were nonlethal, sporadic and focal abscess in the liver. In the biovar A infections, bacteria were recovered not only from the liver abscess but also from the liver, spleen, kidneys and lungs, while in the biovar B infections, they recovered from the liver alone [4, 5]. Moreover, Abe *et al.* [1], Shinjo [4], and Shinjo *et al.* [5] described that abscess was only produced in liver and no changes were observed in other organs.

In this study, although the strains of biovar C were intraperitoneally and intraportally inoculated into mice, abscess was not always observed in the liver. The inoculated bacterial number was 2.5×10^7 to 4.3×10^9 CFU per mouse and this CFU was enough for the strains of biovars A and B to

Table 1. Pathogenicity of *F. necrophorum* biovar C strains after intraperitoneal injection into mice

Strain	Mouse no.	Body weight (g)	No. of bacteria per mouse	Liver abscess formation	Recovery from			
					Liver	Spleen	Kidney	Lung
Fn 520	1	30	4.3×10^8	— ^{a)}	— ^{b)}	—	—	—
	2	35	4.3×10^8	—	—	—	—	—
	3	38	4.3×10^8	—	—	—	—	—
	4	36	4.3×10^8	—	—	—	—	—
	5	35	4.3×10^8	—	—	—	—	—
Fn 521	1	36	3.9×10^8	—	—	—	—	—
	2	24	3.9×10^8	—	—	—	—	—
	3	34	3.9×10^8	—	—	—	—	—
	4	20	3.9×10^8	—	—	—	—	—
	5	24	3.9×10^8	—	—	—	—	—
Fn 522	1	32	1.2×10^8	—	—	—	—	—
	2	32	1.2×10^8	—	—	—	—	—
	3	32	1.2×10^8	—	—	—	—	—
	4	30	1.2×10^8	—	—	—	—	—
	5	31	1.2×10^8	+	+	—	—	—

a) No abscess formation; +, abscess formation.

b) Negative for bacteria; +, positive for bacteria.

Table 2. Pathogenicity of *F. necrophorum* biovar C strains after intraportal injection into mice

Strain	Mouse no.	Body weight (g)	No. of bacteria per mouse	Liver abscess formation	Recovery from			
					Liver	Spleen	Kidney	Lung
Fn 520	1	38	1.0×10^9	— ^{a)}	— ^{b)}	—	—	—
	2	30	7.6×10^7	—	—	—	—	—
	3	32	7.6×10^7	—	—	—	—	—
	4	29	7.6×10^7	—	—	—	—	—
	5	32	7.6×10^7	—	—	—	—	—
Fn 521	1	27	1.5×10^7	—	—	—	—	—
	2	26	1.5×10^7	—	+	—	—	—
	3	30	1.5×10^7	—	—	—	—	—
	4	30	2.0×10^7	—	—	—	—	—
	5	33	2.0×10^7	—	—	—	—	—
	6	34	2.0×10^7	—	—	—	—	—
Fn 522	1	32	4.3×10^9	—	+	—	—	—
	2	33	4.3×10^9	—	—	—	—	—
	3	38	4.3×10^9	—	+	—	—	—
	4	38	3.4×10^7	—	—	—	—	—
	5	38	3.4×10^7	—	—	—	—	—
	6	33	2.5×10^7	—	+	—	—	—

a) No abscess formation.

b) Negative for bacteria; +, positive for bacteria.

produce liver abscess in mice [4–6]. Therefore this made sure again that the strains of biovar C had weak or no pathogenicity for mice as the results of Fiévez [2]. The microorganisms were recovered at somewhat higher rate from mice inoculated intraportally than those inoculated intraperitoneally. It might be the reason that the organisms could arrive directly at the liver. If the experimental period had extended, these viable bacteria would be excluded from the organs. It is obvious that the strains of biovar C are different from the strains of other biovars in pathogenicity for mice. Further studies are necessary about the virulent factors among them.

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要 約

Fusobacterium necrophorum 生物型 C のマウス腹腔内および門脈内接種による病原性について：平岩和通・新城敏晴（宮崎大学農学部家畜微生物学教室）——*Fusobacterium necrophorum* 生物型 C の 3 株をマウスの腹腔内および門脈内に接種して病原性を調べた。全てのマウスが生残した。腹腔内接種した15匹のマウスは、1 匹に肝膿瘍が形成された。接種菌は、膿瘍とその肝組織から分離されたが、その他のマウスでは、病変はなく、接種菌も分離されなかった。門脈内に接種したマウスでは、肝膿瘍はなく、肉眼的な変化もなかったが、4 匹のマウスの肝臓から接種菌が回収された。*F. necrophorum* 生物型 C は他の 2 生物型に比べると病原性が弱い事が明らかとなった。