牛の実験的肝膿瘍における血漿カリクレインの変動

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Plasma Kallikrein Elevation in Cattle Induced with Hepatic Abscess

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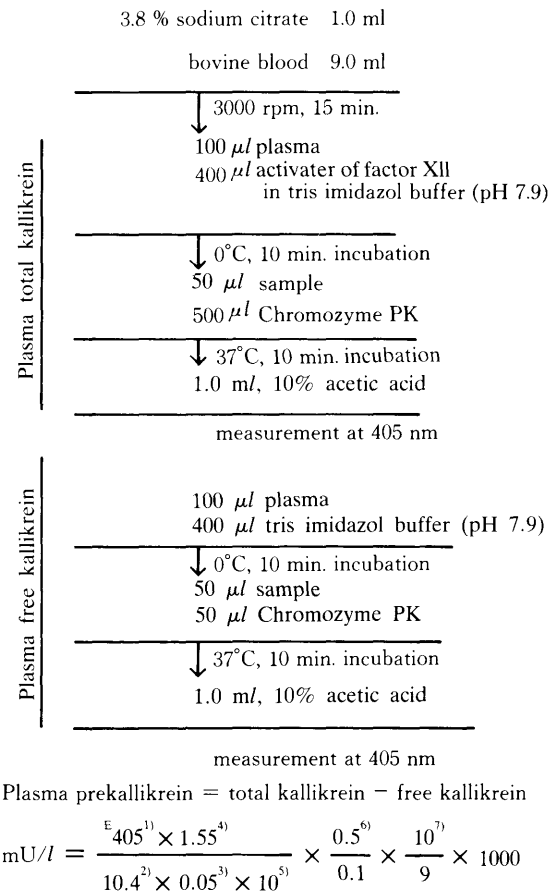
In the previous experiment, the authors [7] inoculated cattle with *Fusobacterium necrophorum* to produce experimental hepatic abscess. When these cattle were infected clinically, such acute phase reactants as sialic acid and mucoprotein contained in blood began to increase remarkably in them in the eary stage after inoculation. These results indicated that the inflammatory reaction of the hepatic tissue was reflected to the blood in the process of formation of hepatic abscess. On the other hand, process of inflammation is regulated by many chemical mediators. Above all, the kallikrein-kinin system is known as an important system for the production of mediators [6]. Plasma kallikrein is an enzyme which produces plasma kinin specifically. Plasma prekallikrein is activated mainly by the activated factor Xll into kallikrein (EC 3. 4. 21. 34). Kallikrein acts specifically upon high-molecular kininogen contained in blood, so that bradykinin may be freed. The bradykinin is known to induce inflammatory reaction, such as systemic hypotension, dilation of peripheral blood vessels, and action to enhance permeability [8, 10]. The relationship between kinin and disease had not so frequently been reported. It was pointed out that the kallikrein-kinin system in blood related in human beings with acute pancreatitis, edema [4, 5], pneumonia and acute hepatitis [2, 3]. It has not been reported, however, that this system participates in any inflammatory disease in domestic animals. It is unknown whether or not this system takes part in the abscess formation in the bovine liver.

The present studies were carried out to examine bovine hepatic abscess from an inflammatory biochemical point of view. An attempt was made to clarify whether kallikrein participated in the formation of hepatic abscess in cattle. The cattle used in this attempt were those employed in the previous experiment [7]. They were 11 healthy Holsteins 2 months to 6 years old weighing 70–530 kg. Of them, eight were inoculated with bacterial suspension to induce the hepatic abscess, and three with physiological saline to serve as controls.

The bacteria, the method of bacterial culture, and the preparation of a bacterial suspension used were the same as reported by Takeuchi et al. [11]. The bacterial suspension and physiological saline were injected into the *vena ruminalis* of 10 cattle in the same manner as mentioned in the previous report [7]. In the remaining animal (No. 6), the bacterial suspension was injected into the *vena omaso-abomasica*. Blood samples were collected from the jugular vein in all the cattle at a certain time after inoculation. Autopsy was conducted in the following sequence: 2 animals (Nos. 1 and 2) 14 days after inoculation, 3 animals (Nos. 3 to 5) 21 days, 1 animal (No. 6) 32 days after inoculation, 2 animals (Nos. 7 and 8) 42 days, and the 3 controls (Nos. 9 to 11) 41 days. By means of their pathological findings, it was confirmed whether hepatic abscess had been produced or not. To collect plasma, nine volumes of blood were mixed with one volume of 3.8% sodium citrate. The mixture was centrifuged at 3000 rpm for the 15 minutes.Plasma kallikrein was estimated with Chromozyme PK (Bz-Pro-Phe-Arg-pNA) [Pentapharm, Ltd., Basel] as substrate. This synthetic substrate is high in specificity for the enzyme and free from the influence of any other enzyme contained in blood [1]. It was also reported that elaidic acid was good activater of factor Xll [9]. In the present work, a preparation containing elaidic acid [Actin, Dade diagnostic, Inc., Puerto Rico] was used to activate factor Xll. Plasma total, free and prekallikrein activities were measured by the same method as mentioned by Nakamura et al. [9] without sample volume used. Fig. 1 shows assay procedure and calculation formula of bovine plasma kallikrein.

Hepatic abscess was produced in six of eight cattle inoculated with bacteria. It was absent in the other two cattle, as well as in three controls.



Plasma prekallikrein = total kallikrein − free kallikrein

$$\text{mU}/l = \frac{{}^{E}405^{1)} \times 1.55^{4)}}{10.4^{2)} \times 0.05^{3)} \times 10^{5)}} \times \frac{0.5^{6)}}{0.1} \times \frac{10^{7)}}{9} \times 1000$$

Fig. 1. Assay procedure and calculation formula of bovine plasma kallikrein. 1) Absorbance at 405 nm(E_{405}), 2) Molecular extinction coefficient for p-nitroanilin at 405 nm(M^{-1}, cm^{-1}), 3) Sample volume, 4) Total volume, 5) incubation time, 6) Plasma dilution, 7) Blood dilution.

Fig. 2 shows free kallikrein (FK), prekallikrein (PK), and total kallikrein (TK) which were detected in the plasma of all the cattle used in the experiment. In the six cattle with abscess formed, PK and TK activity began to increase remarkably 10 days after the inoculation. But after that, they were maintained at a high level, presenting a progressive increase. In the two cattle with no abscess and the controls, PK and TK activity tended to increase a little about 13 days after the inoculation, showing a plateau almost constantly up to 30 days after the inoculation. FK activity was kept almost always constant level and about one-sixth of TK activity in the two cattle with no abscess and the controls. Contrarily, it began to exhibit a conspicuous increase in the cattle with abscess about 20 days after inoculation. In the present experiment no bradykinin was estimated. But judging from the specific function of kallikrein to bradykinin production, it seemed reasonable to presume that bradykinin might also have been generated in agreement with the enhancement in plasma kallikrein activity. Accordingly, it is most likery that kallikrein-kinin system as chemical mediator relates to occurrence of bovine hepatic abscess. Changes in blood sialic acid and mucoprotein in this work were reported in the previous paper [7]. There was a time lag between the changes in kallikrein and those in the sialic acid and mucoprotein. Kallikrein activities began to increase 4–5 days later than sialic acid and mucoprotein and remained still in a

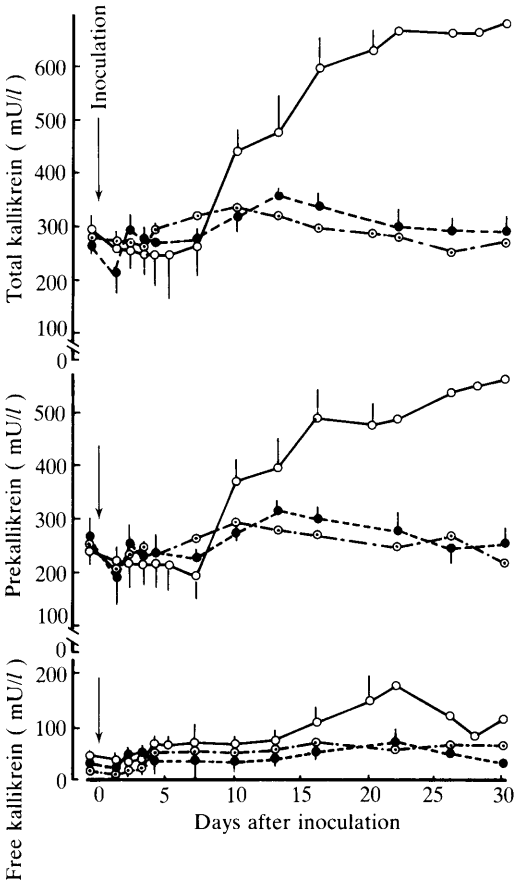


Fig. 2. Changes in activities of plasma kallikreins in cattle experimentally affected with hepatic abscess. ●: Controls (three cases), saline injection. ⊙: Cattle affected without hepatic abscess after the inoculation (two cases). ○: Cattle affected with hepatic abscess after the inoculation (six cases to the 13rd day, 4 to the 20th day, and 1 thereafter).

remarkable high levels, predominant in plasma prekallikrein when the sialic acid and mucoprotein began to decrease a littel. Since the origin and role of acute phase reactants and kallikrein in the process of formation of hepatic abscess are unknown at present, the interrelation between them in the aspect of the time lag is not drawn by conference from this experiment. It is at least presumed, however, that such difference may be derived from the difference in their role or response to inflammatoty reaction in some stage of disease. Futher studies should be made to clarify this role.

REFERENCES

1. Amundsen, E., and Svendsen, L. 1977. pp. 211–220. *In*: New methods for the analysis of coagulation using chromogenic substrates. (Witt, I. ed.), Walter de Gruyter, New York.
2. Diniz, C. R., and Carvaluo, I. F. 1963. *Ann. N. Y. Acad. Sci.* 104: 77–89.
3. Forell, M. M. 1957. *Schweiz. Med. Wschr.* 87: 828–833.
4. Frey, E. K., Kraut, H., and Shulta, F. 1930. *Arch. Exp. Path. Pharmk.* 158: 334–347.
5. Hollenberg, M., Kobold, E. E., PrUett, R., and Thal, A. P. 1962. *Surgical Forum.* 13: 302–304.
6. Miles, A. A., Wilhelm, D. L. 1955. *Br. J. Exp. Pathol.* 36: 71–81.

7. Motoi, Y., Takeuchi, S., and Nakajima, Y. 1985. *Jpn. J. Vet. Sci. 47:* 341–347.
8. Movat, H. Z. 1979. pp. 1–90 *In*: Handbook of experimental pharmacology. Springer, Berlin.
9. Nakamura, S., Takizawa, N., Nakanishi, K., and Igarashi, M. 1980. *Jpn. J. Clin. Pathol.* 28: 670–674. (in Japanese).
10. Oh-ishi, S. 1982. pp. 95–102. *In*: Inflammation and antiinflammatory therapy [Enshō to kō enshōryho]. (Mizusima, Y. and Shiokawa, Y. ed). Ishiyaku Shyuppan. Tokyo (in Japanese).
11. Takeuchi, S., Nakajima, Y., Ueda, H., Motoi, Y., Kobayashi, Y., and Morozumi, T., 1984. *Jpn. J. Vet. Sci.* 46: 339–344.

要　　約

牛の実験的肝膿瘍における血漿カリクレインの変動（短報）：元井葭子，竹内正太郎[1)]，中島靖之[2)]（農林水産省家畜衛生試験場，[1)]同，北陸支場，[2)]同，東北支場）——*Fusobacterium necrophorum*接種による肝膿瘍発症中の血漿総カリクレインとプレカリクレイン活性は，接種後10日から非発症中と生理食塩水注入対照中にくらべて著明に高値を示し，以後30日まで高い活性を継続した。