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EVIDENCE THAT T-KININ DOES NOT MEDIATE PAW SWELLING INDUCED BY
CARRAGEENIN: STUDIES WITH PLASMA HIGH MOLECULAR WEIGHT KININOGEN AND
LOW MOLECULAR WEIGHT KININOGEN DEFICIENT RATS

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Paw swelling induced by carrageenin was compared in Brown Norway Kitasato rats (normal) and Brown Norway Katholiek rats, deficient in HMW and LMW kininogens. The increase in the paw volume of the former after the carrageenin injection was about 50-70% of the initial volume, whereas that of the latter was about 10%. Pretreatment with captopril, a kininase inhibitor, enhanced the edema significantly in the former but had no effect in the latter. The T-kininogen level in the plasmas of both strains increased gradually and peaked on the 2nd day at 10 times of the control level. But the paw swelling peaked at 4-5 hours after the carrageenin injection. These results indicate that bradykinin but not T-kinin induces paw swelling by plasma exudation.

KEYWORDS—— HMW-kininogen; T-kininogen; captopril; T-kinin; paw edema; carrageenin

T-kinin, isoleucyl-seryl-bradykinin, was identified as a peptide released by trypsin from a distinct kininogen of rats.¹⁾ The kininogen was named T-kininogen²⁾ and was differentiated from the classical HMW and LMW kininogens, since it did not release any kinin by the action of either plasma kallikrein or glandular kallikrein.^{3,4)} It has been reported that the plasma level of T-kininogen increases following inflammatory stimuli or injection of bacterial lipopolysaccharide (LPS).⁵⁻⁷⁾ The content of the mRNA encoding T-kininogen in the liver also increased after rats received LPS.⁸⁾

This is proof that neither T-kinin nor T-kininogen play a role in the paw swelling of rats induced by the intradermal injection of carrageenin in rats congenitally deficient in plasma HMW and LMW kininogens.⁹⁾

Inbred strains of male Brown Norway Kitasato (B/N-Ki) rats, 12-15 weeks old with normal plasma kininogens, and Brown Norway Katholiek (B/N-Ka) rats deficient in plasma HMW and LMW kininogens, but with normal levels of T-kininogen,^{3,4)} were injected in the right paw with 0.1 ml sterile saline solution of 1% carrageenin (lambda-carrageenin, Sigma). The paw volume was measured with a mercury

plethysmograph before and at 1, 2, 3, 5, 24 h after the carrageenin injection.¹⁰⁾ The B/N-Ka and B/N-Ki rats were each divided into two groups; one group of each strain received intravenously 10 mg/kg captopril (kindly supplied from Sankyo co.,Ltd.). The other groups received saline 30 min before the carrageenin injection. Blood samples were drawn from the subclavian vein before the carrageenin injection, and rats from each group were exsanguinated at the indicated times after the carrageenin injection. Plasma was obtained from the citrated blood as previously described.⁴⁾ T-kininogen and HMW-kininogen were measured by radioimmunoassay using ¹²⁵I-labeled kininogen as previously described.⁶⁾

Table I clearly indicates that the B/N-Ka rats show less swelling and thus HMW kininogen has an important role in the induction of plasma leakage. This coincides with our previous result of carrageenin-induced pleurisy,¹¹⁾ in that B/N-Ka rat showed less pleural fluid accumulation than the normal strain, B/N-Ki and Sprague-Dawley rats. The result also agrees with Damas *et al.*¹²⁾ who report a role of the plasma kallikrein-kinin system in urate crystal-induced paw edema in kininogen-deficient rats. These results also demonstrate that T-kininogen may not have any role in the paw swelling, since the level of T-kininogen in B/N-Ka rat plasma is the same as that in B/N-Ki rat plasma, and it increased in the same way it did in B/N-Ki rats as shown in Fig. 1. Pretreatment with captopril in B/N-Ki rats enhanced paw swelling at 1-2 hr, indicating that bradykinin may be released from HMW-kininogen by plasma kallikrein activation, as previously reported for sprague-Dawley rat.¹¹⁾ Pretreatment with captopril may inhibit kininase and cause bradykinin to stay longer at the site to induce more swelling. As shown in Fig. 2, the plasma levels of HMW kininogen in B/N-Ka rats

Table I. Percentage increase in paw volumes in Brown Norway Kitasato (B/N-Ki) and Brown Norway Katholiek (B/N-Ka) rats after carrageenin injection with or without pretreatment of captopril (10 mg/kg)

	Hours after carrageenin injection				
	1	2	3	5	24
B/N-Ki (vehicle) (n = 12)	30.4 ± 3.2	45.5 ± 4.7	50.2 ± 5.1	48.8 ± 3.4	21.7 ± 2.1
B/N-Ki (captopril)* (n = 10)	69.0 ± 5.8	61.9 ± 6.7	59.9 ± 7.5	56.5 ± 4.7	21.7 ± 2.6
B/N-Ka (vehicle) (n = 12)	10.8 ± 1.7	6.8 ± 1.1	8.0 ± 1.2	11.8 ± 2.1	9.3 ± 1.2
B/N-Ka (captopril) (n = 11)	9.0 ± 2.0	6.2 ± 1.5	7.5 ± 2.2	8.1 ± 1.2	5.7 ± 1.4

Data are means with standard errors. n: number of rats used. *: statistically significant at 5%.

were 1/30 - 1/50 as high as those in B/N-Ki rats and showed no change during inflammation. The B/N-Ka rats showed no enhancement with captopril, indicating that T-kinin may not be released from T-kininogen at the paw site. If T-kinin has been released the effect would be enhanced, since exogenous T-kinin caused the same degree of vascular permeability increase in rats as bradykinin does.¹¹⁾ T-kinin was also inactivated with rat plasma as bradykinin and captopril partly prevented the inactivation (Data not shown).

Furthermore, the plasma level of T-kininogen increased at 24 h after the carrageenin injection, when the paw swelling had already decreased. The peak swelling was at about 5 h and at that time the T-kininogen level had not yet increased. The time lag in the increase of T-kininogen also indicates no role of T-kinin, if any release, for the increased vascular permeability during paw swelling. The profiles and increased levels of T-kininogen in both strains were almost the same, also indicating that the different degree of swelling did not reflect the level of T-kininogen but HMW kininogen (Fig. 2), since the level of HMW kininogen in B/N-Ka rats was less than 1/30 of that of normal rats. All of these results indicate that neither T-kinin nor T-kininogen has any direct role in the paw swelling, while bradykinin may be released from the HMW kininogen to induce vascular permeability increase in the paw. This is quite different from the report of Barlas *et al.*¹³⁾, who found that in rats, T-kinin is released in the granuloma induced by carrageenin. Thus the question remains : What is the role of the increased level of T-kininogen ?

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REFERENCES

- 1) H. Okamoto and L.M. Greenbaum, *Biochem. Biophys. Res. Commun.*, **112**, 701 (1983)
- 2) H. Okamoto and L.M. Greenbaum, *Life Sci.*, **32**, 2007 (1983)
- 3) I. Hayashi, T. Ino, H. Kato, S. Iwanaga, T. Nakano and S. Oh-ishi, *Thromb. Res.*, **36**, 509 (1984)
- 4) I. Hayashi, S. Oh-ishi, H. Kato, K. Enjyoji, S. Iwanaga and T. Nakano, *Thromb. Res.*, **39**, 313 (1985)
- 5) A. Barlas, H. Okamoto and L.M. Greenbaum, *Biochem. Biophys. Res. Commun.*, **129**, 280 (1985)
- 6) I. Hayashi, S. Oh-ishi, K. Enjyoji, H. Kato and S. Iwanaga, *Chem. Pharm. Bull.*, **34**, 3502 (1986)
- 7) J. Damas and A. Adam, *Mol. Phys.*, **8**, 307 (1985)
- 8) R. Kageyama, N. Kitamura, H. Ohkubo and S. Nakanishi, *J. Biol. Chem.*, **260**, 12060 (1985)
- 9) J. Damas and A. Adam, *Experientia* **36**, 586 (1980)
- 10) S. Oh-ishi and A. Sakuma, *Japan. J. Pharmacol.*, **20**, 337 (1970)
- 11) S. Oh-ishi, I. Hayashi, M. Hayashi, K. Yamaki, A. Yamasu, T. Nakano. I. Utsunomiya and Y. Nagashima, *Agents Actions*, **18**, 450 (1986)
- 12) J. Damas, G. Remacle-Volon and A. Adam, *Naunyn-schmied. Arch. Pharm.*, **325**, 76 (1984)
- 13) A. Barlas, K. Sugio and L.M. Greenbaum, *FEBS Lett.*, **190**, 268 (1985)

Fig. 1. Plasma T-kininogen levels in Brown Norway Katholiek (B/N-Ka)(A) and Brown Norway Kitasato (B/N-Ki)(B) rats following the carrageenin injection.

Intravenous injection of captopril, 10 mg/kg (○), or vehicle (●) were administered 30 min prior to the carrageenin injection.

Fig. 2. Plasma HMW-kininogen levels in B/N-Ka (A) and B/N-Ki (B) rats following carrageenin injection into the paw.

Symbols are the same as in Fig. 1.

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