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Health Promoting Effects of Lactoferrin from Milk

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ABSTRACT

The ubiquitous presence of lactoferrin(LF) receptor in human as reported by the research group of Prof. Bo Lonnerdal, Univ. California (Suzuki, Y. A., 2001) encouraged us to search for the unknown physiological roles of LF. Under the collaboration with Prof. Etsumori Harada, Tottori Univ., and his research group, we have found two novel biological activities of LF as the control of the lipid metabolism and the effect on the central nervous system. Relating to the lipid metabolism, LF could, in animal experiments, reduce triglyceride and total cholesterol both in blood and liver (Takeuchi, T. *et al.*, 2003). LF increased plasma HDL-C and lowered LDL-C. In the central nervous system, LF showed anti-nociceptive activity mediated by μ-opioid receptor in the rat spinal cord (Hayashida, K. *et al.*, 2003). LF enhanced analgesic action of morphine synergistically via nitric oxide synthesis (Hayashida, K., *et al.*, 2003) LF showed opioid-mediated suppressive effect on distress induced by maternal separation in rat pups (Takeuchi, T., *et al.*, 2003).

(**Key words** : lactoferrin(LF) receptor, central nervous system, anti-nociceptive activity, analgesic action, opioid mediated suppressive effect)

I. Introduction

In new-born babies, LF and other components of mother's milk go directly through the stomach into the intestinal lumen. In adults, orally administered LF is susceptible to the peptic digestion in the stomach. An enteric-coated LF tablet has been developed for the delivery of an intact molecule of LF onto the receptor in the intestine. In this formulation LF molecules are protected from the proteolytic digestion in the stomach since the tablet is coated by an acid-resistant material, which dissolves easily in a neutral pH condition in the intestine. Each tablet contains 50~100 mg of bovine LF. The tablet is stable for more than 2 years at room temperature since LF powder is formulated under an extremely dry condition and enteric-coated.

Thus, an intact LF molecule can bind to the receptor to show the multi-potent biological activities. The precise mechanism of action after the binding of LF to the receptor is still to be investigated. As is shown later, LF molecules could be absorbed effectively into the systemic circulation when administered orally in the enteric-coated formulation via receptor-mediated transcytosis to reach to each target organ to show various biological activities by a so-called "Swiss Knife Model" as proposed by Prof. Shimazaki. An alternative mechanism in which LF signals on the intestinal immune system in a so-called "Billiard Model" might also be possible.

LF enhances an anti-infective activity of antibiotics. In the experiment shown in Fig. 1, 5 mice out of 6 mice infected by *Klebsiella pneumonia* were dead without a

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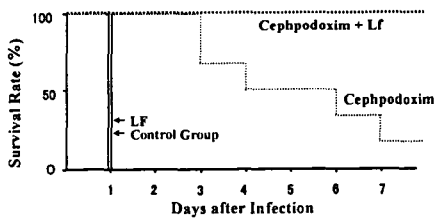


ICR mouse : ♂ , 4weeks, 6mice/group, Klebsiella pneumonia 3k25strain,
Antibiotics : Cephpodoxim 5mg/mouse,
Lf : 0.4mg/mouse. [S.Miyazaki et. al., Chemotherapy, 39:829-835(1991)]

Fig. 1. Augmentation of anti-infective activity by lactoferrin.

Table 1. Minimum effective dose of LF

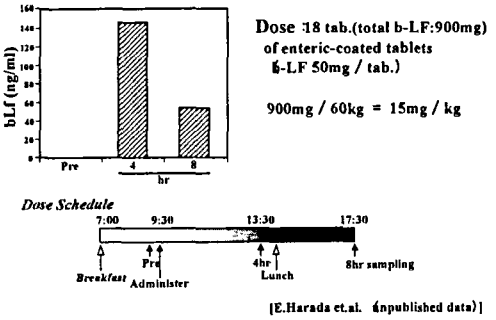
Lf mg/mouse	ED_{50}
50	0.124 (0.098~0.156)
5	0.124 (0.085~0.180)
0.5	0.124 (0.085~0.180)
0.05	0.124 (0.081~0.189)
0.005	0.312 (0.229~0.426)
Control	0.394 (0.294~0.527)

Minimum effective dose : 2.5mg/kg, ca.150mg/60kg-bw human
[S. Miyazaki *et al.*, Chemotheraapy 39:829-835(1991)]

treatment by Cephpodoxim. When LF was co-administered with the antibiotics, all mice survived even at the non-effective dose of LF used alone. We could determine the minimum effective dose of LF using this infection model(Miyazaki, S., *et al.*, 1991).

The effective dose of Cephpodoxim for the 50% survival was decreased in the presence of LF (Table 1). The minimum effective dose of LF was 0.05mg per mouse. In human, therefore, the minimum effective dose was calculated to be 150mg for the 60kg body weight.

Dr. Harada *et al.* have reported the transport of colostral components into cerebrospinal fluid via serum in neonatal pigs(Harada, E., *et al.*, 1999) and of latoferrin from the intestinal lumen into the bile via the blood in piglets (Harada, E., *et al.*, 1999) But in human, it has been unsuccessful to detect LF in the blood after the oral administration of even 20 grams of bovine LF as reported by Dr. Tsuda (personal communication).



[E.Harada et.al. unpublished data)]

Fig. 2. Absorption of orally administered b-LF from human intestine.

Recently, Prof. Harada could detect bovine LF in human blood for the first time using the enteric-coated LF tablets after the oral administration of only 900mg of b-LF (Fig. 2).

Recently, Dr. Hiroshi Kimoto of the Nagatsukai Saitoh Hospital, Chiba, Japan observed preliminary, but very interesting clinical results on the control of the lipid metabolism by an oral administration of the enteric-coated bovine lactoferrin tablets(Kimoto, H., 2003).

The beneficial effects observed clinically by Dr. Kimoto are as follows:

(1) Rapid decrease of serum triglyceride in blood
(2) Significant hypocholesterolemic activity
(3) Stimulation of basal metabolic rate
(4) Reduction of body weight

The level of triglyceride in serum goes high up to the level of more than 500mg/dl after excess drinking (Fig. 3). The level of triglyceride decreased gradually to the level of still higher than the normal range even after 7 days. By taking 3 tablets at once, three times a day (total 450mg of LF), the level decreased rapidly and reached to the normal range after 1~2 days.

Although the mechanism of LF action on the lipid metabolism needs further investigation, this rapid decrease of triglyceride level led us to speculate that LF switches on

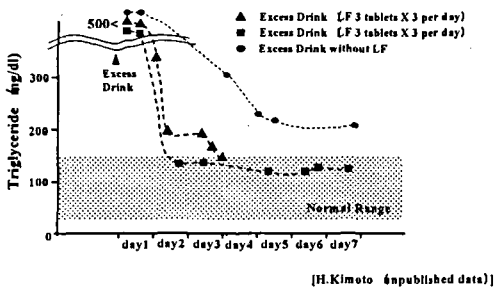


Fig. 3. Effect of LF on triglyceride level.

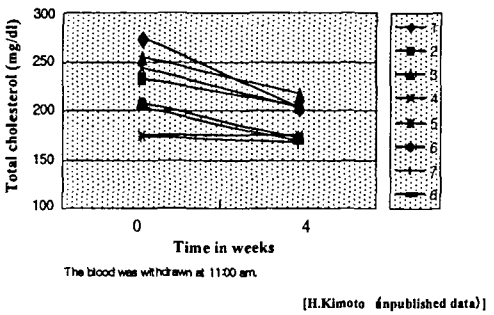


Fig. 4. Change in total cholesterol level.

fat burning, leading to the stimulation of the basal metabolic rate asshown later.

The remarkable effect of the enteric-coated LF tablet as shown in Fig. 3 led Dr. Kimoto to study other clinical efficacies using the staffs of his hospital, who are mainly nurses, as volunteers. After taking 9 tablets a day for 4 weeks, the total cholesterol decreased in 7 cases out of 8 volunteers, suggesting hypercholesterolemia could be improved by this treatment (Fig. 4).

Dr. Kimoto studied on the possible stimulation of the basal metabolic rate by the enteric-coated LF tablet. The basal body temperature at rising seemed to shift slightly higher, and the body temperature 1 hour after meal also seemed to shift higher, suggesting the stimulation of the basal metabolic rate by taking the enteric-coated LF tablet (Fig. 5).

If the basal metabolic rate is really stimulated by LF, the enteric-coated LF tablet may help dieting without any special guidance for foods and exercise. As is shown in Fig.

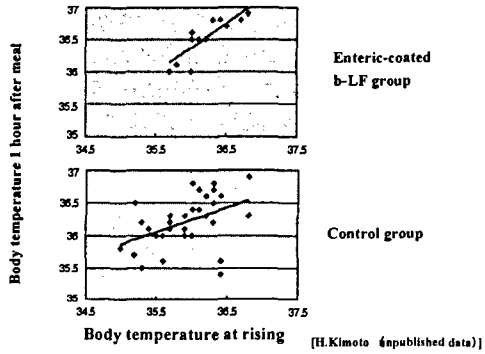


Fig. 5. Stimulation of basal metabolic rate.

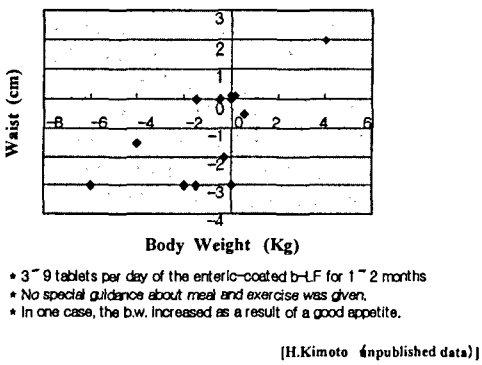


Fig. 6. Change of body weight and waist.

6, the weight loss of 4~6 kgs in a short period of 1~2 months was observed in some cases by taking 3~9 tablets a day. The reduction of 2~3 cm of the waist was seen in 5 cases out of 13 volunteers.

The results provide a speculation that lactoferrin may stimulate the uncoupled oxidation of fatty acids, resulting in the reduction of the body fat deposit. The hypocholesterolemic activity is probably due to the interruption of the enterohepatic circulation of bile acids by lactoferrin.

Although the enteric-coated LF tablet may be quite promising as a health supplement, but the potential of LF may lead to the development as a therapeutic agent for hyperlipidemia, hypercholesterolemia and obesity. There is no drug at present which can reduce both triglyceride and cholesterol like LF.

The effect of lactoferrin on central nervous system will

also be discussed based on our recenct findings.

Ⅱ. References

1. Suzuki, Y. A., Shin, K. and Lonnerdal, B. 2001. Molecular cloning and functional expression of a human intestinal lactoferrin receptor. Biochemistry 40: 15771-15779.
2. Takeuchi, T., Shimizu, H., Ando, K. and Harada, E. 2003. Bovine lactoferrin reduces plasma triglyceride and free fatty acid accompanied by decreased hepatic cholesterol and triglyceride contents in mice. Br. J. Nutr.(submitted).
3. Hayashida, K., Takeuchi, T., Shimizu, H., Ando, K. and Harada, E. 2003. Novel function of bovine milk derived lactoferrin on antinociception mediated by μ opioid receptor in the rat spinal cord. Brain Research 965: 239-245.
4. Hayashida, K., Takeuchi, T., Shimizu, H., Ando, K. and Harada, E. 2003. Lactoferrin enhances opioid-mediated analgesia via nitric oxide in the rat spinal cord. Am. J. Physiol. Regul. Integr. Comp. Physiol. 285 (in press).
5. Takeuchi, T., Hayashida, K., Inagaki, H., Kumahara, M., Tsubone, H., and Harada, E. 2003. Opioid mediated suppressive effect of milk-derived lactoferrin on distress induced by maternal separation in rat pups. Brain Research (in press).
6. Miyazaki, S., Harada, Y., Tsuji, A. and Goto, S. 1991. *In vivo* combined effects of lactoferrin and drugs on bacterial infections in mice (in Japanese). Chemotherapy 39: 829-835.
7. Harada, E., Sugiyama, A., Takeuchi, T., Sitizyo, K., Syuto, B., Yajima, T. and Kuwata, T. 1999. Characteristic transfer of colostral components into cerebrospinal fluid via serum in neonatal pigs. Biol. Neonate 76: 33-43.
8. Harada, E., Itoh, Y., Sitizyo, K., Takeuchi, T., Araki, Y. and Kitagawa, H. 1999. Characteristic transport of lactoferrin from the intestinal lumen into the bile via the blood in piglets. Comparative Biochemistry and Physiology Part A 124: 321-327.
9. Kimoto, H. 2003. Case report of 4 patients on the improvement of serum lipids by the enteric-coated LF tablet (in Japanese). Progress in Medicine 23: 1519-1523.
10. Togawa, J., Nagase, H., Tanaka, K., Inamori, M., Umezawa, T., Nakajima, A., Naito, M., Sato, S., Saito, T. and Sekihara, H. 2002. Lactoferrin reduces colitis in rats via modulation of the immune system and correction of cytokine imbalance. Am. J. Physiol. Gastrointest. Liver Physiol. 283: G197-G195.