Hiroshima Journal of Medical Sciences
Vol. 31, No. 2, June, 1982
HIJM 31-20

Effect of β-Glucuronidase Inhibitor from *Mycobacterium tuberculosis* Against Microbicidal Activity in Phagocytes of Guinea Pigs*)

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(Received Feburuary 12, 1982)

Key words: Mycobacterium tuberculosis, β-Glucuronidase inhibitor, Phagocyte

ABSTRACT

β-Glucuronidase inhibitor extracted and purified from culture filtrate of *Mycobacterium tuberculosis* H37Rv significantly reduced the microbicidal activity of guinea pig peritoneal macrophages against *Candida parapsilosis*. It did not have any effect on the antimicrobial action of polymorphonuclear cells against *Staphylococcus aureus* 209P.

A lot of studies have been made on biologically active substances in cell constituents of *Mycobacterium tuberculosis*, especially on lipid fractions. However, little is known of substances excreted from the cells with the exception of the purified protein derivative[1]. When mycobacteria are cultured in liquid media, proteinaceous substances are secreted into the media, and the amount is greater with *M. tuberculosis* than with mycobacteria other than tubercle bacilli.

We attempted to separate any biologically active substances in the culture filtrate of *M. tuberculosis, and* found a substance possessing an inhibitory activity against several lysosomal enzymes[9]. Recently, we isolated another proteinaceous substance with a molecular weight of approximately 25,000[4]. The inhibitory action is non-competitive against β-glucuronidase obtained from polymorphonuclear leukocytes (PMNs) and peritoneal macrophages (PMφs) of guinea pigs. Of interest is the fact that the activity is optimum at pH of about 4.5 which is almost the same as the pH within the phagolysosome. The present short communication describes the effect of this particular β-glucuronidase inhibitor on survival of microbes ingested by cultured phagocytes.

The inhibitor was isolated from unheated culture filtrates of *M. tubercilosis* H37Rv by the method described previously[4].

PMNs were prepared according to the method of Simmons et al.[8] using a male guinea pig, Hartley strain, weighing approximately 400 g. Eighteen hr after intraperitoneal injection with 12% casein-saline (pH 7.4) in a dose of 12 ml/kg, peritoneal exudate cells (PMNs) were collected, washed, and adjusted to 5×10^6 cell/ml in Hanks balanced salt solution containing 0.1 % gelatin (gelatin-Hanks).

The bactericidal activity was determined according to the methods of Cohn et al.[2] and Quie et al.[7] Briefly, a 50 μl of 2×10^8 cocci/ml suspension of *Staphylococcus aureus* FAD 209P JC-1 freshly cultivated in Tryptosoy broth(Eiken Chemical, Tokyo) was mixed with an aliquot of the inhibitor, to which was added 4-fold diluted fresh guinea pig serum in gelatin-Hanks solution. After 10 min opsonization in a shaking water bath at 37°C, the mixture was added with 500 μl of the PMNs suspension, and was reincubated for 0, 30, 60 and 120 min with shaking. At the indicated incubation period, a portion of 10 μl of the mixture was withdrawn. Sedimented PMNs were washed with saline and added with 0.1% Triton X-100 to make cell lysate, which was used for measuring β-glucuronidase activity of the cells. Bacillary counts were carried out on the cell lysate as well as the supernatant of the mixture.

*) 田坂博信，清谷克寛，松尾吉恭：結核菌由来の β-glucuronidase inhibitor のモルモット食細胞の殺菌作用に対する影響について

PMφs were obtained by the method of Oren et al[6]. A guinea pig was injected intraperitoneally with 30 ml of 1.2% casein-saline (pH 7.4). Peritoneal exudate cells were harvested 4 to 5 days later and washed with 199 medium containing 10 μg/ml of kanamycin and 10% fetal calf serum. The adherent cells(PMφ)were separated by the method of Nozawa et al[5]. A 0.2 ml of the suspension containing 2×10^4 PMφs was introduced in each well of a Coaster microplate (96 wells). Each well received 50 μl of the β-glucuronidase inhibitor and incubated for 6 hr. A 10 μl of serial diluted yeast suspension of *Candida parapsilosis* was added in each well and the maximum number of microbes killed (MNMK) was assessed by the method of Nozawa et al.[5] 72 hr after incubation at 37°C in a CO_2 incubator.

As shown in Fig. 1, the number of viable *S. aureus* within the PMNs decreased with the lapse of time, and no effect of the β-glucuronidase inhibitor was observed on the intracellular killing action of PMNs against *S. aureus*. The number of extracellular cocoi remained almost constant during the incubation time from 30 to 120 min; that is, the inhibitor showed no effect on phagocytosis of PMNs (Fig. 2). However, β-glucuronidase activity of PMNs ingested *S. aureus* was approximately 10% lower than that of the control cells (Fig. 3).

In contrast, the inhibitor reduced the microbicidal activity of PMφs against intracellular *C. parapsilosis* to appoximately a quarter. The

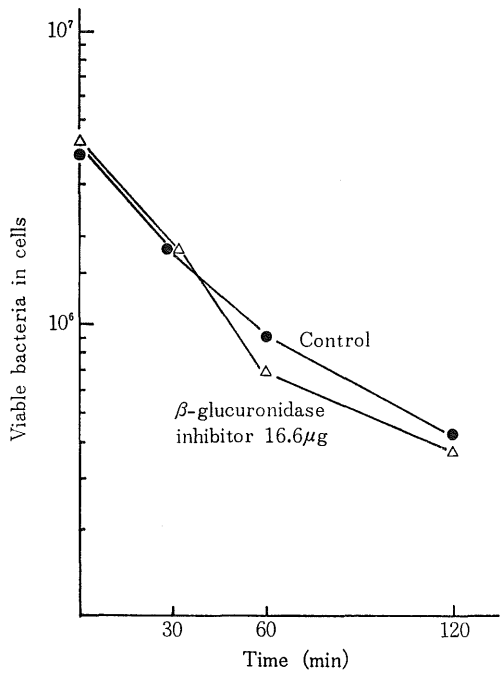

Fig. 1. Effect of β-glucuronidase inhibitor on the staphylococcicidal activity of guinea pig polymorphonuclear leukocytes (PMNs)

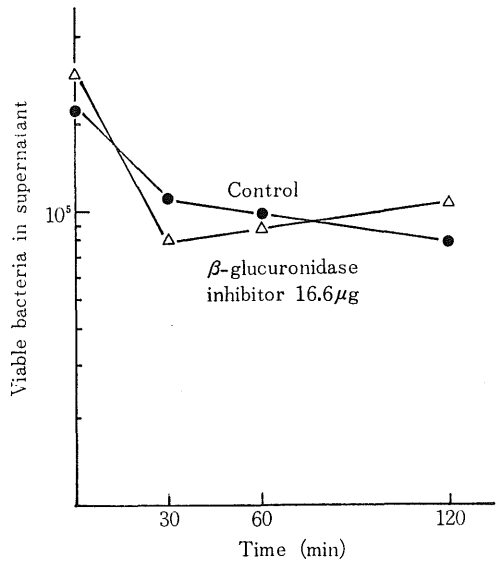

Fig. 2. Effect β-glucuronidase inhibitor on the phagocytosis of *Staphylococcus aureus* of guinea pig PMNs

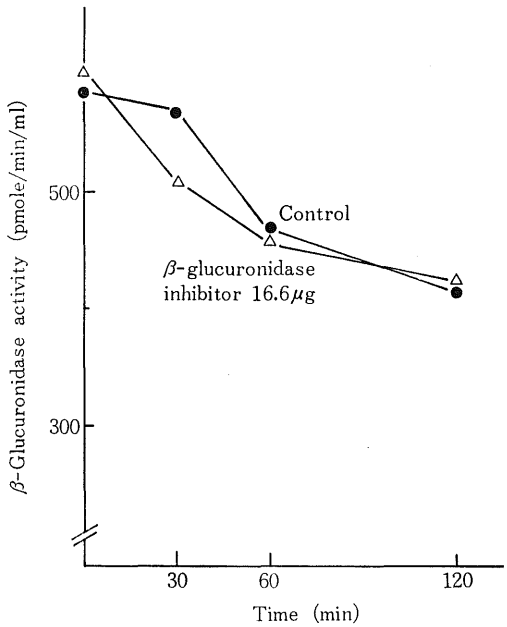

Fig. 3. β-Glucuronidase activity of guinea pig PMNs after ingestion of *Staphylococcus aurers*

Table 1. Effect of β-glucuronidase inhibitor on the candidicidal activity of guinea pig peritoneal macrophages

Control	β-glucuronidase inhibitor		
	12.5 μg	25.0 μg	50.0 μg
148*	37	37	37
37	9.3	9.3	9.3
37	4.6	9.3	9.3

* Maximum number microbes killed

dose response of the inhibitor was not clear (Table 1).

Although phagocytes are known to have intracellular killing potential, the extent of direct involvement of lysosomal hydrolases has not been elucidated. Kanai and Kondo[3] suggested that lysosomal hydrolase might serves to decompose static and/or dead microorganisms. As far as the present results are concerned, the β-glucuronidase inhibitor interferes with the killing activity of PMφs against *C. parapsilosis*. It seems probable that lysosomal carbohydrolase acts on the components of microbial cell wall to make the intracellular microbe susceptible to subsequent killing mechanism. The effect of the inhibitor on the survival of intracellular mycobacteria will be further investigated. The discrepancy of protective effect of the inhibitor between on *S. aureus* and *C. parapsilosis* in phagocytes may be attributed to the fact that the former is quickly processed by the primary oxidative killing system of the cells.

REFERENCES

1. **Barksdale, L. and Kim, K. S.** 1977. *Mycobacterium*. Bacteriol. Rev. **41** : 217-372.
2. **Cohn, Z. A. and Morse, S. I.** 1959. Interactions between rabbit polymorphonuclear leukocytes and staphylococci. J. Exp. Med. **110** : 419-443.
3. **Kanai, K. and Kondo, E.** 1968. Further studies on the antimycobacterial principle of mouse lysosomal components. Japan. J. Med. Sci. Biol. **21** : 405-414.
4. **Kiyotani, K.** 1981. Isolation and characterization of β-glucuronidase inhibitor from culture filtrate of *Mycobacterium tuberculosis* H37Rv. Hiroshima M. J. **29** : 861-880.
5. **Nozawa, R., Sekiguchi, R. and Yokota, T.,** 1980. Stimulation by conditioned medium of L-929 fibloblasts, *E. coli* lipopolysacchride and muramyl dipeptide of candidacidal activity of mouse macrophages. Cell Immunol. **53** : 116-124.
6. **Oren, R., Farnham, A. E., Saito, K., Milofsky, E. M. and Karnovsky, M. L.** 1963. Metabolic patterns in three types of phagocytizing cells. J. Cell Biol. **17** : 487-501.
7. **Quie, P. G., White, J. G., Holmes, B. and Good, R. A.** 1967. In vitro bactericidal capacity of human polymorphonuclear leukocytes: diminished activity in chronic granulomatous disease of childhood, J. Clin. Invest. **46** : 668-679.
8. **Simmons, S. R. and Karnovsky, K. L.** 1973. Iodinative ability of various leukocytes and their bactericidal activity. J. Exp. Med. **138** : 44-63.
9. **Tasaka, H., Kato, M. Kiyotani, K. and Matsuo, Y.** 1979. Inhibitory effect of tuberculoglycopretein on lysosomal enzyme activity. Kekkaku **50** : 621-264.