Quantitative Assay of Lentinan in Human Blood with the Limulus Colorimetric Test

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Yajima, Y., Satoh, J., Fukuda, I., Kikuchi, T. and Toyota, T. Quantitative Assay of Lentinan in Human Blood with the Limulus Colorimetric Test. Tohoku J. Exp. Med., 1989, 157 (2), 145-151 — A conventional limulus test detects not only endotoxin but also β(1→3) glucan. Therefore, using a quantitative limulus test (the limulus colorimetric test) we studied the pharmacokinetics of lentinan, an antitumor β(1→3) glucan, in the blood of 10 healthy volunteers and three patients with advanced gastric cancer. The calibration curve of lentinan in the human plasma was linear in the range of 0 to 100 ng/ml. When incubated with human plasma at 37°C in vitro, lentinan had the recovery of almost 100% as compared to the initial concentration even after 60-min incubation, indicating the stability of lentinan in human plasma. When 1 mg of lentinan was intravenously administered over a 2 hr period, lentinan concentration reached the maximum levels (50–80 ng/ml) at the end of the drip infusion and decreased gradually thereafter. In the near future, the more appropriate modes of lentinan administration will be determined by further investigation of its kinetics in the human body.

Lentinan is an antitumor polysaccharide extracted and purified from Lentinus edodes (Berk) sing. It is a high molecular weight glucan of a major chain of β(1→3) with a branch of β(1→6) binding (Chihara et al. 1970). Lentinan, one of the biological response modifiers (BRM), exerts T cell-oriented immunopotentiation in which macrophage functions take some part and shows tumor regression effects in various experimental tumors (Zakany et al. 1980; Suga et al. 1984). In clinical studies, similar effects as well as the prolongation of survival time have been confirmed in patients with advanced gastric cancer (Taguchi et al. 1985). However, the pharmacokinetics of lentinan in the human body remain unknown, because there has been no quantitative assay for lentinan in the blood with the exception of a study of radiolabeled lentinan administered to animals (Obara 1980).

The limulus test is an assay for endotoxin which has been used clinically to detect endotoxin in the blood (Levin et al. 1970). It was previously believed to...
be endotoxin-specific (Rojas-Corona et al. 1969). Recent studies, however, have shown that the limulus test is not specific for endotoxin. Iwanaga and co-workers found another pathway activated by $\beta (1\rightarrow 3)$ glucan in the limulus test, apart from one by endotoxin (Iwanaga et al. 1984).

In the present article, we report the quantitative determination of lentinan concentration in vitro and its pharmacokinetics in vivo using Limulus Colorimetric Test (LCT), a quantitative limulus test using synthetic chromogenic substrate, developed by Iwanaga and co-workers (1978).

**MATERIALS AND METHODS**

**Subjects**

Ten healthy volunteers and three patients with advanced gastric cancer complicated with cancerous peritonitis were chosen for this study.

**Preparation of endotoxin-free glassware**

All glassware was washed sufficiently, dried and heated at 250°C for at least 4 hr to eliminate endotoxin.

**Blood sample collection**

Venopuncture was performed after cleansing the skin with 90% ethanol. Two ml of blood was collected with 20 units of heparin (Novo industry A/S, Bagsverd, Denmark) in endotoxin-free disposable syringes. Plasma was separated by centrifugation at 3000 rpm for 15 min and stored at $-20^\circ$C.

**Deproteinization of plasma containing lentinan**

To eliminate protein factors which show positive or negative reactions in the limulus test, plasma containing lentinan was deproteinized with perchloric acid (PCA) according to Tamura et al. (1982). Two hundred $\mu$l of 0.32 M PCA solution was added to 100 $\mu$l of plasma samples in glass tubes and the mixture was incubated at 37°C for 20 min. After centrifugation at 3000 rpm for 15 min, 50 $\mu$l of the supernatant was neutralized with 50 $\mu$l of 0.18 N NaOH.

**Lentinan assay using Limulus Colorimetric Test (LCT)**

Toxicolor® and Endospecy® (both produced by Seikagaku-kogyo, Tokyo) were used. Both kits consist mainly of lysate (amebocyte lysate of Tachypleus tridentatus) and chromogenic substrate (Boc-Leu-Gly-Arg-pNA). Toxicolor® is a conventional LCT kit which can detect both endotoxin and $\beta$-glucan quantitatively (Obayashi 1984). On the other hand, Endospecy® is an endotoxin-specific LCT kit in which $\beta$-glucan sensitive factor (G-factor) is selectively eliminated from its lysate (Obayashi et al. 1985). Details of LCT are described elsewhere (Yajima et al. 1985).

**Administration of lentinan**

Lentinan (Yamanouchi Pharmaceuticals Co., Ltd., Tokyo) was dissolved in 500 ml of 5% glucose solution and administered intravenously for 2 hr.

**RESULTS**

**Calibration curve of lentinan**

One mg of lentinan dissolved in 500 ml of 5% glucose solution was diluted
with plasma of each of five volunteers to obtain lentinan concentrations of 100, 70, 40 and 10 ng/ml. One hundred μl of each concentration from each volunteer was subjected to deproteinization, followed by both conventional LCT and endotoxin-specific LCT to determine the activating potency of lentinan on the limulus enzymes and to check the contamination of endotoxin in lentinan. The conventional LCT provided an almost linear calibration curve in the range of 0 to 100 ng/ml. On the other hand, no endotoxin activity could be detected with the
endotoxin-specific LCT in any of the lentinan concentrations examined. The detection limit of the conventional LCT was 2 ng/ml because volunteer plasma showed absorbance equivalent up to 2 ng/ml of lentinan (Fig. 1).

**Recovery of lentinan from plasma with perchloric acid method**

One mg of lentinan was dissolved in 500 ml of 5% glucose solution. The solution was then diluted to 10 ng/ml with distilled water or saline and to 60 ng/ml with each of five volunteer plasma because deproteinization procedure decreases lentinan concentration by six-fold. Lentinan solutions with distilled water or saline were subjected directly to conventional LCT. On the other hand, plasma solutions of five volunteers were deproteinized and subjected to conventional LCT. Distilled water solution and saline solution of lentinan showed almost equal absorbances, whereas plasma solutions showed nearly 90% of absorbance of distilled water solution, indicating that the recovery rate of lentinan with PCA method is nearly 90% (Fig. 2).

**Stability of lentinan in human plasma**

Five volunteer plasma containing 60 ng/ml of lentinan were kept at 37°C and lentinan concentration was determined at 0, 20, 40 and 60 min of incubation. Lentinan concentrations were almost equal at each incubation time and lentinan was thought to be stable in the human plasma (Fig. 3).

**Change in lentinan concentration in blood of healthy volunteers**

Ten volunteers were divided into two groups. In five volunteers, 2 mg of lentinan was administered and in another five ones 4 mg. Lentinan concentra-

![Fig. 3. Stability of lentinan in human plasma. Five volunteer plasma containing 60 ng/ml of lentinan were kept at 37°C. Vertical bars represent mean ± s.d. (n = 5).](image-url)
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Fig. 4. Pharmacokinetics of lentinan in healthy volunteers. Open circles (○) indicate 4 mg group and closed circles (●) 2 mg group. Vertical bars represent mean ± s.D. (n=5).

Fig. 5. Pharmacokinetics of lentinan in patients with advanced gastric cancer.

Lentinan concentrations were assayed by the method mentioned above. Lentinan concentrations at 24 hr after administration were 71±21 ng/ml in 4 mg group and 53±11 ng/ml in 2-mg group (mean±s.D.), following gradual decrease to 7th day where lentinan concentrations were around the detection limit (Fig. 4).

Change in lentinan concentration in blood of patients with advanced gastric cancer

In 3 patients with advanced gastric cancer, 1 mg of lentinan was administered. Lentinan concentrations increased linearly during a 2-hr drip infusion and reached 51, 73 and 71 ng/ml in cases 1, 2 and 3, respectively, followed by a relatively rapid decrease until 24 hr after the administration and a gradual decrease thereafter. In cases 2 and 3, 5 ng/ml of lentinan was detected in the
blood even a week after the administration (Fig. 5).

**DISCUSSION**

Currently, various biological response modifiers (BRM) are used in clinical cancer therapy. However, measurement of these BRM (such as polysaccharides and crude extracts of bacteria) in human blood is generally difficult. Therefore, the kinetics of lentinan in the human body have been unknown. This is the first study in which the pharmacokinetics of lentinan administered to the human body have been quantitatively established. The kinetics of lentinan in the human body as clarified by the LCT was similar to those obtained with rats intravenously administered ³H-lentinan (Obara 1980).

Stability of lentinan in the plasma was evaluated because it is known that there is a rapid decrease of endotoxin recovery when incubated in plasma at 37°C (Yajima et al. 1986). Fortunately, however, lentinan exhibited no such a behavior and was stable in the plasma. Consequently, the decrease of lentinan concentration may be caused by the uptake or degradation by some cells, probably by phagocytosis of the Kupffer cells of the liver (Obara 1980).

In the near future, a relation between the concentration of lentinan in the blood and its anti-tumor activities as a BRM will be clarified. More appropriate modes of lentinan administration will be also determined by investigating its kinetics in the human body.

**References**

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