Leustroducsin B, a New Cytokine Inducer Derived from an Actinomycetes, Induces Thrombocytosis in Mice

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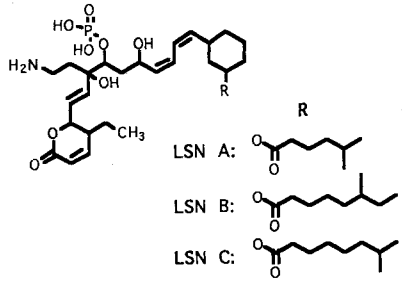
Leustroducsin B (LSN-B), a novel colony-stimulating factor (CSF) inducer produced by an actinomycetes, has previously been shown to induce CSF production in bone marrow stromal cells. To determine the biological activity of LSN-B on hematopoiesis *in vivo*, LSN-B was administered intraperitoneally to mice every day for three to six days. Peripheral platelet counts were markedly elevated on days 4 through to 6 compared with the control mice injected with vehicle. Serum IL-6 levels were low (0.8 ng/ml) or virtually undetectable in the drug treated groups. This cytokine profile suggests that LSN-B induction of thrombocytosis is mechanistically distinct from other cytokine inducers such as IL-1 or FK-565.

Recently a number of human hematopoietic growth factors have been genetically cloned and recombinant proteins produced[1]. Some of them are extremely useful in the treatment of hematopoietic disorders. However, the regulatory mechanisms for the production or the movement of these factors between cellular compartments remain unclear. Bone marrow stromal cells may be one of the key elements responsible for the control of hematopoiesis *in vivo*[2], and therefore, the regulation of CSF production by these cells is of interest.

The regulatory mechanism of CSF production in stromal cells was investigated using the clonal stromal cell line KM-102[3], to discover three novel CSF inducers named leustroducsin A, B and C[4,5]. These compounds have a common structural motif[1] (Fig. 1) which is distinct from other known inducers. Leustroducsin B (LSN-B) induced cytokines both *in vitro* and *in vivo*, and when administered to mice, they augmented host resistance in lethal infection of *E. coli*[6].

Fig 1. Chemical structure of LSNs.



In this paper, we describe the hematological profiles of mice administrated LSN-B and discuss other related biological activities. In the same discussion, a comparison is made with known cytokine inducers such as IL-1[7] and FK-565[8].

Materials and Methods

Mice

Seven-week-old specific pathogen-free C57BL/6 male mice, purchased from Japan Charles Rivers, were used for all experiments.

Chemicals

LSN-B was purified from the culture broth of *S. platensis* in accordance with a previously reported method[1]. This preparation was negative (<21 pg/mg) for endotoxin contamination in the Limes lysate assay. For administration, LSN-B was dissolved in ethanol and then diluted to less than 0.5% with saline. The saline containing an equal volume of ethanol was used as vehicle control.

In Vivo Study Design

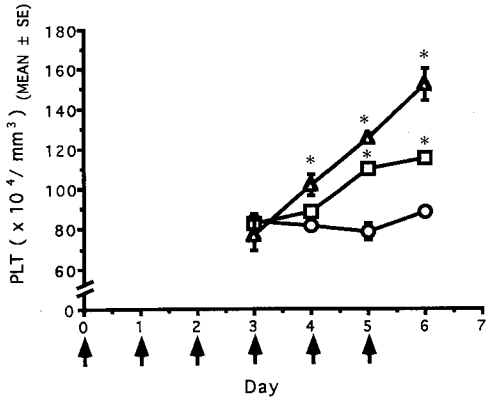
LSN-B was administered intraperitoneally to mice once daily at a dose of either 0.1 or 0.3 mg/kg in 0.05 ml saline for 6 days. Day 1 was designated as the day after initial injection.

Hematological Examinations

Blood samples were drawn into syringes initially containing EDTA-2K from the abdominal aorta under ether anesthesia. A complete blood count was performed using an automated hematologic analyzer (System-9000,

Fig. 2. Effect of LSN-B injection on platelet number in mice.

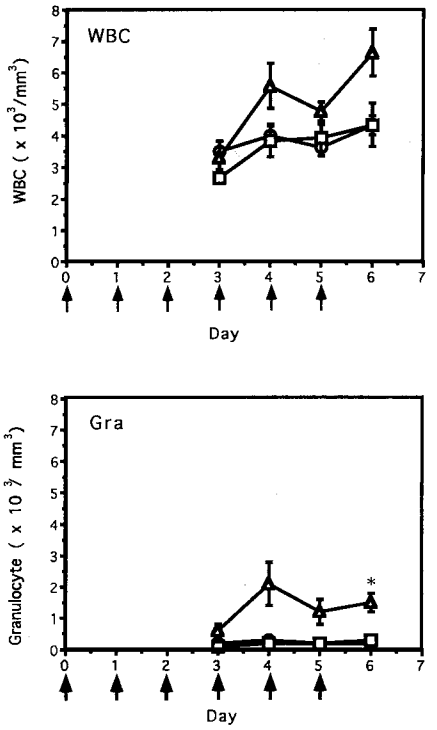
○: Vehicle, □: 0.1 mg/kg/day, △: 0.3 mg/kg/day.



LSN-B was administered intraperitoneally for 6 days. Platelet counts were given as means ± S.E. from 6~7 mice at each dose. * $P<0.01$ *vs.* vehicle group.

Fig. 3. Hematological changes after intraperitoneal injections of LSN-B.

○: Vehicle, □: 0.1 mg/kg/day, △: 0.3 mg/kg/day. WBC: white blood cell counts, Gra: granulocyte counts.



Hematological parameters other than platelet were shown. Data were given as means ± S.E. from 6~7 mice at each dose. * $P<0.01$ *vs.* vehicle group.

Japan Technicon, Japan). Differential counts of white blood cells were performed using an automated blood cell analyzer (Microx HEG-70A, Omron, Japan).

Results

Effect of LSN-B on Platelet Counts

To assess the biological activity of LSN-B towards platelet production, 0.1 or 0.3 mg/kg of LSN-B was administered intraperitoneally to mice daily for 6 days. As shown in Fig. 2, LSN-B increased platelet counts in a dose-dependent manner. Daily administrations of 0.3 mg/kg of LSN-B induced significant thrombocytosis on days 4 through to 6 compared with the control mice injected with vehicle. On day 6 at the 0.3 mg/kg dosage level, platelet count attained a level of 172% of control mice which equalled or exceeded that observed by IL-6 treatments[9]. Administrations of 0.1 mg/kg of LSN-B also induced a significant thrombocytosis on days 5 through to 6 and the platelet count level attained was 130% of control mice on day 6.

Effect of LSN-B on Other Hematological Parameters

Figure 3 shows the effect of LSN-B *in vivo* administration on other hematological parameters. Daily administrations of 0.3 mg/kg of LSN-B increased total white blood cell counts on days 4 through to 6. The granulocyte population increased by this treatment in turn accounts for most of the increase in total white blood cell counts. Interestingly, administrations of 0.1 mg/kg of LSN-B did not change granulocyte counts while platelet counts were changed markedly. LSN-B administration did not change parameters concerning red blood cells. Other hematological parameters (lymphocytes, monocytes, eosinophils, basophils, reticulocytes) were also unchanged by LSN-B treatment (data not shown).

Effect of LSN-B on Body Weight Change

No significant differences were observed among the groups in the body weight change. Furthermore during experiment, no behavioral alterations were noted in any of the groups.

Discussion

In a preliminary paper, we reported that LSN-B has a strong activity in protecting hosts against infection by *E. coli*[6]. Furthermore it was reported also that serum IL-6 levels were upregulated by treatment with 0.3 mg/kg of LSN-B[6]. Since IL-6 has a potent thrombopoietic activity *in vivo*, we expected that LSN-B treatment would cause thrombocytosis in mice. As shown in Fig. 2, administrations of LSN-B caused a marked increase in platelet counts and the increased levels of platelet equalled or exceeded those observed by IL-6 treatments[9].

To the best of our knowledge, this work represents the first report of potent thrombopoietic activity being elicited by a member of the leustroducsin related group of compounds.

ISHIBASHI *et al.* reported that a high serum IL-6 concentration (96.5 ng/ml) was observed when IL-6 administration induces thrombocytosis in mice[9]. Furthermore, IL-1[7] and FK-565[8] which are cytokine inducers like LSN-B, also promote high levels of IL-6 elicitation (28 ng/ml and more than 62.5 ng/ml, respectively) while concomitantly causing thrombocytosis *in vivo*. Conversely also when IL-6 causes thrombocytosis, it probably requires a high serum concentration of IL-6.

From previous work[6] LSN-B was found to increase serum IL-6 concentrations but the levels of IL-6 were relatively low (about 0.8 ng/ml). Furthermore, administrations of 0.1 mg/kg of LSN-B did not induce detectable amounts of IL-6 (<15 pg/ml). From this study however, a clear picture is evident, we observed potent thrombocytosis by LSN-B administration (0.1 mg/kg) without increased level of serum IL-6. This suggests that the mechanism of thrombocytosis caused by LSN-B are apparently different from those of other inducers. Thus LSN-B has the possibility of being able to stimulate thrombopoiesis without an increased level of serum IL-6.

In vitro studies demonstrated that LSN-B stimulate G- and GM-CSF production by bone marrow stromal cell line KM-102[1,2,5]. LSN-B also induces the production of IL-6, IL-11, and LIF in KM-102 cells, although IL-11 was undetected by bioassay using T1165 cells *in vivo* (data not shown). These cytokines are well known to cause thrombopoiesis[9~11]. Therefore LSN-B might cause thrombopoiesis *via* induction of the above cytokines, albeit the serum IL-6 level is likely to be unaffected. There are two possibilities which we can propose to account for the above paradox.

One is the induction of another thrombopoietic cytokine; LIF[11] or oncostatin M[12] are the other cytokines which may be mediating the mechanism of thrombocytosis. The other possibility is the site-specific cytokine induction by LSN-B. LSN-B may cause increased levels of thrombopoietic cytokines only in bone marrow which is a major hematopoietic organ.

IL-1 is known to induce IL-6 in various cell types other than bone marrow stromal cells. FK-565 is known to stimulate the cytokine production by macrophage *in vitro*[8]. LSN-B has been shown to induce cytokines by bone marrow stromal cells[1,2]. Further investigations on tissue-specificities of the above inducers would assist in resolving the paradox.

It is well known that circulating IL-6 causes not only thrombopoiesis but also various adverse effects including fever, chills, weight loss, anemia and elevation of acute phase protein[13]. Although the precise mechanisms of thrombopoietic activity of LSN-B is yet to be ascertained, the biological activities elicited by LSN-B seem to be suitable for the treatment of thrombocytopenic disorders.

Recently, thrombopoietin was discovered as a physiological factor in normal thrombopoiesis[14~18]. However the effect of LSN-B on thrombopoietin production remains unknown. No doubt because leustroducsins have a novel structure being distinct from that of known inducers[1], their relationship between LSNs and the above thrombopoietic factors will prove to be an interesting area of study.

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