Twist Form of Teleocidin Derivatives is Active in in Vivo Tumor Promotion by (−)-Benzlactam-V-8-310

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Teleocidin derivatives and the core structure, (−)-indolactam-V (−)-IL-V, adopt two conformations in solution, the “twist” and the “sofa” forms. (−)-Benzlactam-V-8-310 (−)-BL-V-8-310, which specifically adopts the twist form in solution, has been reported to have a significant effect on HL-60 cells and protein kinase C affinity. In this paper, we describe the biological activity with regard to tumor promotion on mouse skin and the wide variety of biological activity of (−)-BL-V-8-310 and its derivatives. In both twist and sofa forms (−)-BL-V-8-310 inhibited specific 3H-12-O-tetradecanoylphorbol-13-acetate (TPA) binding to a particulate fraction of mouse skin more than (−)-IL-V. The doses for 50% inhibition (IC50) of (−)-IL-V, (−)-BL-V-8-310, and teleocidin B-4 were 1000, 400 and 12 nm, respectively. For the induction of tumor necrosis factor-α (TNF-α) release into the medium from HL-60 cells, the EC50 values, which are the concentrations of the compound required to achieve 200 pg/ml TNF-α in the medium, were 1700, 500 and 19 nm for (−)-IL-V, (−)-BL-V-8-310 and teleocidin B-4, respectively. The same amounts (5.5 amol per application) of (−)-BL-V-8-310 and teleocidin B-4, induced tumors on mouse skin initiated with 7,12-dimethylbenz(a)anthracene (DMBA) in 13.3% and 86.7% of tumor-bearing mice, respectively, in week 20. These results confirmed that the twist form of teleocidin derivatives is the active form as far as the induction of biological activity is concerned. Also (−)-BL-V-8-310 is a new synthetic tumor promoter designed from data obtained using the receptor cavity model of TPA-type tumor promoters.

Key words benzlactam; tumor promoter; teleocidin; twist form; tumor necrosis factor-α

Tumor promoters of the teleocidin type have a unique structure-function relationship. A number of teleocidins studied so far have different tumor-promoting activity in two-stage carcinogenesis experiments initiated with 7,12-dimethylbenz(a)anthracene (DMBA) on mouse skin. The most potent form of the teleocidins has the structure of (−)-indolactam-V (−)-IL-V, which is the common structure of teleocidin A and B, plus a hydrophobic domain: Teleocidin A has (−)-IL-V plus a linalyl group, and teleocidin B has (−)-IL-V plus an alkylated cyclohexene ring. These domains have been shown to fit a receptor cavity of some tumor promoters studied by three-dimensional computer graphics. The 9-membered lactam ring of (−)-IL-V in teleocidin A and B is in equilibrium between two conformational states, the twist and sofa forms. It is still not clearly understood which form is associated with the activity, except for some evidence obtained from a study of the synthesis of (−)-IL-V showing that the twist form seems to be involved. If a synthetic molecule could restrictively adopt either the twist or sofa form, it would allow us to solve this problem. Endo and his associates succeeded in synthesizing two derivatives of benzlactam-V: (±)-benzlactam-V-8-310 (±)-BL-V-8-310 (Fig. 1), containing a 9-membered lactam ring plus a C10H12-alkyl group, which can only adopt the twist form in solution, whereas (−)-benzlactam-V-9-310 (−)-BL-V-9-310, containing a 9-membered lactam ring plus a C10H21-alkyl group, can only adopt the sofa form. These two molecules have a common C10H21-alkyl group substituted on the benzene ring of benzolactam, which does not affect their conformation. Endo et al., reported that (±)-BL-V-8-310 induces HL-60 cell differentiation and inhibits specific 3H-12-O-tetradecanoylphorbol-13-acetate (TPA) binding to the regulatory domain of protein kinase C, whereas (±)-BL-V-9-310 is inactive. Furthermore, (−)-BL-V-8-310 was confirmed as active in biological tests, whereas the antipode, (±)-BL-V-8-310 was inactive. These results suggest that the active conformation of teleocidin derivatives is the twist rather than the sofa form.

Here we have studied the inhibition of specific 3H-TPA binding to a particulate fraction of mouse skin, induction of tumor necrosis factor-α (TNF-α) release into the medium from HL-60 cells, and tumor promotion on mouse skin using three compounds: (−)-BL-V-8-310, (−)-IL-V and teleocidin B-4. Our results showed that (−)-BL-V-8-310 associated with the twist form has tumor-promoting activity. Thus, we have

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confirmed that the twist form of (-)-IL-V is directly related to the active conformation of telocodin derivatives.

MATERIALS AND METHODS

Chemicals  Telocodin B-4 and (-)-IL-V were isolated from Streptomyces, a culture broth of Streptoverticillium,\(^7\) (-) and (+)-BL-V8-310 were synthesized by a previously described method.\(^9\) TPA was obtained from Chemsyn Science Lab., (Kansas, U.S.A.) and \(^3\)H-TPA (470 GBq/mmol) was purchased from New England Nuclear (MA, U.S.A.)

Animals  Female CD-1 mice were obtained from Charles River Japan Inc., Kanagawa.

Inhibition of Specific \(^3\)H-TPA Binding  A particulate fraction containing phorbol ester receptors was prepared from mouse skin as described previously.\(^8\) Specific \(^3\)H-TPA binding to the particulate fraction was measured by the cold acetone filter method described previously.\(^8\) Various concentrations of telocodin B-4, (-)-BL-V8-310 and (-)-IL-V were incubated with 4 nM \(^3\)H-TPA and 100 \(\mu\)g protein of a particulate fraction at 0 °C for 2 h.

Induction of TNF-\(\alpha\) Release from HL-60 Cells  A tumor promoter induces TNF-\(\alpha\) mRNA expression in target tissues, and TNF-\(\alpha\) release from the cells. Since TNF-\(\alpha\) acts as an endogenous tumor promoter, induction of TNF-\(\alpha\) release is a practical method to determine the tumor promoting activity of a compound.\(^9\)\(^10\) HL-60 cells (2×10\(^5\) cells/ml) grown in RPMI-1640 medium containing 10% fetal calf serum were incubated with various concentrations of (-) and (+)-BL-V8-310, telocodin B-4, and (-)-IL-V for 24 h. TNF-\(\alpha\) in the medium was determined by an ELISA Kit, as described previously.\(^9\)\(^10\)

Tumor Promotion in CD-1 Mouse Skin  A two-stage carcinogenesis experiment in mouse skin was performed by our standard experimental procedure.\(^1\)\(^6\) The skin on the back of a mouse was treated with a single application of 100 \(\mu\)g 7,12-dimethylbenz(a)anthracene (DMBA). From one week after this initiation treatment, repeated topical applications of (-)-BL-V8-310, telocodin B-4 and (-)-IL-V were made twice a week, until week 20. The amount of compound per application was 5.5 nmol for (-)-BL-V8-310 and telocodin B-4, and 40 nmol for (-)-IL-V dissolved in 0.1 ml acetone. Each experimental group consisted of 15 female CD-1 mice.

RESULTS AND DISCUSSION

Inhibition of Specific \(^3\)H-TPA Binding  Inhibition of specific \(^3\)H-TPA binding to a particulate fraction of mouse skin by three compounds was investigated. We used a particulate fraction of mouse skin as phorbol ester receptors for the experiments. Although, the particulate fraction did not exhibit any activation of protein kinase C by TPA, because the fraction contained inhibitors of protein kinase C, it did exhibit specific \(^3\)H-TPA binding.\(^8\) Figure 2 shows that telocodin B-4, (-)-BL-V8-310 and (-)-IL-V dose-dependently inhibited specific \(^3\)H-TPA binding and the IC\(_{50}\) values for 50% inhibition of telocodin B-4, (-)-BL-V8-310 and (-)-IL-V were 12, 400 and 1000 nM, respectively. (-)-BL-V8-310 was about 2.5 times more effective than (-)-IL-V, when their IC\(_{50}\) values were compared, suggesting that (-)-BL-V8-310 has slightly stronger tumor-promoting activity than (-)-IL-V. Activation of protein kinase C from mouse brain by these three compounds was also studied (data not shown). The order of their activating potency was similar to their inhibition of specific \(^3\)H-TPA binding.

Fig. 2. Inhibition of Specific \(^3\)H-TPA Binding to a Particulate Fraction of Mouse Skin by Three Telocodin Compounds  Telocodin B-4 (○), (-)-BL-V8-310 (●) and (-)-IL-V (△).

Fig. 3. Induction of TNF-\(\alpha\) Release from HL-60 Cells Treated with Four Compounds, Telocodin B-4 (○), (-)-BL-V8-310 (●), (-)-IL-V (△) and (+)-BL-V8-310 (×)

Induction of TNF-\(\alpha\) Release from HL-60 Cells  Based on our previous results showing that a tumor promoter induces TNF-\(\alpha\) release from target cells, mediated through both activation of protein kinase C by TPA and inhibition of protein phosphatases 1 and 2A by okadaic acid, TNF-\(\alpha\) release is a significant indication of a tumor promoter in an in vitro assay system.\(^9\)\(^10\) Consequently, we studied whether (-)-BL-V8-310 could induce TNF-\(\alpha\) release from one of the targets, HL-60 cells. Figure 3 shows that (-)-BL-V8-310 was active, whereas (+)-BL-V8-310 was virtually inactive. The EC\(_{50}\) value, the concentration of a compound required to release 200 pg/ml TNF-\(\alpha\) into medium, of telocodin B-4, (-)-BL-V8-310 and (-)-IL-V was 19, 500 and 1700 nm, respectively. These results correlate well with the inhibition of specific \(^3\)H-TPA binding (Table 1). Since TNF-\(\alpha\) stimulated transformation of BALB/3T3 cells initiated with 3-methylcholanthrene,\(^9\) we think that the potency of these compounds in inducing TNF-\(\alpha\) release is closely associated with the potency of their tumor-promoting activity.

Tumor Promotion in CD-1 Mice  Figure 4 shows the
percentage of tumor-bearing mice and the average number of tumors per mouse in the group treated with DMBA plus (−)-BL-V8-310, compared with those of two control groups treated with DMBA plus teleocidin B-4, and DMBA plus (−)-IL-V throughout the 20 week experiment. Table 1 shows the percentage of tumor-bearing mice and the average number of tumors per mouse in the three groups during week 20. The experimental group treated with DMBA plus (−)-BL-V8-310 showed tumors in 13.3% of mice. The groups treated with DMBA alone, teleocidin B-4 alone, (−)-BL-V8-310 alone, and (−)-IL-V alone did not produce any tumors (data not shown). The experiments showed that (−)-BL-V8-310 is a new but weak tumor promoter, and its tumor-promoting activity is compatible with its IC50 value for inhibition of specific 
$^3$H-TPA binding and its EC200 value for induction of TNF-α release.

Although (−)-IL-V was applied 7.2 times more liberally than (−)-BL-V8-310, the tumor-promoting activity of (−)-IL-V on mouse skin appears to be marginal (Table 1). Previously we reported that 41.8 nmol (−)-IL-V per application induced tumors in 29% of mice during week 30 of tumor promotion. When we compared the tumor-promoting activity of the compound at week 20 of tumor promotion, the percentage of tumor-bearing mice in the group treated with DMBA plus (−)-IL-V was only 6.7%.11 In contrast to (−)-IL-V, (−)-BL-V8-310 produced a slightly higher percentage of tumor-bearing mice than (−)-IL-V, indicating that (−)-BL-V8-310 has significant tumor-promoting activity in the two-stage carcinogenesis experiment on mouse skin.

The experiments showed that the three teleocidin compounds with the twist form (1) consistently bind to the phorbol ester receptors in a particulate fraction of mouse skin, (2) induce TNF-α release from HL-60 cells and (3) induce tumor-promoting activity on mouse skin. The potency of their activity seems to be related to a hydrophobic domain attached to (−)-IL-V.

When Endo and his associates succeeded in synthesizing (−)-BL-V8-310, they found that (−)-BL-V8-310 induced growth inhibition and differentiation of HL-60 cells 30 times more potently than (−)-IL-V.11 Hashimoto and Shudo reported a cytosolic-nuclear tumor promoter-specific binding protein (CN-TPBP), which moves into the nucleus after binding to TPA.11 They also showed that (−)-BL-V8-310 binds to CN-TPBP with a potency similar to that reported above. As for the restricted (±)-BL-V9-310, it was reported to be inactive in inducing growth inhibition and differentiation of HL-60 cells, and did not bind to CN-TPBP.12 (±)-BL-V9-310 was also much weaker than (−)-BL-V8-310 as far as inhibition of 
$^3$H-TPA binding to the protein kinase C regulatory domain and protein kinase C activation were concerned.13 Considering these results, we did not conduct a tumor-promotion experiment on mouse skin with DMBA plus (±)-BL-V9-310.

Based on recent evidence that direct interaction of phorbol-13-acetate with the cys2 domain of protein kinase Cζ took place in a crystalline complex, Itai and her associates also confirmed that the twist form of (−)-IL-V and (−)-BL-V8-310 fitted neatly into the same cavity with phorbol-13-acetate, indicating that the twist form of teleocidin derivatives exhibits hydrogen-binding to protein kinase C similar to that of TPA.13 However, the sofa form did not fit into the cavity.13 (−)-BL-V8-310 has a C10H21-alkyl group, teleocidin B-4 has an alkylated cyclohexene ring and (−)-IL-V has neither. Endo and his associates reported that the C10H21-alkyl group of (−)-BL-V8-310 was constructed based on data from a study of the receptor cavity model for TPA-type tumor promoters.6 Thus, a C10H21-alkyl group attached to the C-9 of benzoalactam is not the correct size for significant activity, compared with the lanyl group of teleocidin A or the alkylated cyclohexene group of teleocidin B. This study has shown that a new member of the TPA-type tumor promoters has been synthesized based on the stereochemistry of teleocidin A and B isomers, using evidence from the receptor cavity model. Although the activity of (−)-BL-V8-310 was not very marked, all the activities monitored indicated strongly that the twist form of teleocidin derivatives is the active one.

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