CHAPTER 11

Secondary Metabolites with New Medicinal Functions from Marine Organisms

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Contents

Abstract

We have focused on the identification of natural key compounds that possess biologically and medicinally intriguing functions. Some of bioactive naturally occurring compounds isolated from marine organisms were found to possess unique biological activities. Halichlorine from the marine sponge *Halichondria okadai* was shown to inhibit the activity of nuclear factor-κB in endothelial cells and block L-type Ca^{2+} channels. Thus, it may have therapeutic potentials for diseases such as atherosclerosis and hypertension.

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I. INTRODUCTION

Various natural products with extraordinary chemical structures and significant biological activities have been isolated and characterized from both marine and terrestrial creatures. In our previous research, isolation and characterization of many biologically and medicinally intriguing compounds from both the marine and terrestrial creatures have been achieved. Some of those isolated compounds have been shown to exhibit various biological activities and have good potential for clinical or biological use. For example, halichondrins are the remarkable antitumor compounds which had been originally isolated from the marine sponge *Halichondria okadai* Kadota (Hirata and Uemura, 1986; Uemura *et al.*, 1985). A synthetic analogue of halichondrin B, eribulin mesylate (Halaven^{TM}), was recently approved for the treatment of patients with metastatic breast cancer by the US Food and Drug Administration (Huyck *et al.*, 2011; Towle, *et al.*, 2001). In our continuing research, we have constructed unique screening systems and have searched among secondary metabolites of marine organisms for biologically active compounds (Uemura, 2006, 2010; Uemura *et al.*, 2009). We have isolated both novel and known compounds and their new biological and physiological activities were examined by collaborating researchers.

II. HALICHLORINE, A BIOACTIVE MARINE NATURAL COMPOUND ISOLATED FROM A MARINE SPONGE *H. OKADAI* KADOTA

Halichlorine (**1**) (Fig. 11.1) is an alkaloid which was isolated from the marine sponge *H. okadai* Kadota (Kuramoto *et al.*, 1996). This compound was revealed to be a novel alkaloid containing an azaspiro[4.5]decane skeleton clarified by detailed spectroscopic analyses. The absolute stereostructure was confirmed by many synthetic studies (Arimoto *et al.*, 1998; Clive *et al.*, 2005; Liu *et al.*, 2009; Trauner *et al.*, 1999). Halichlorine was shown to inhibit the induction of vascular cell adhesion molecule-1 (VCAM-1) in cultured human umbilical vein endothelial cells. Drugs that block the inflammatory stimuli-induced expression of VCAM-1 may be useful for treating atherosclerosis, coronary artery diseases, angina, and noncardiovascular inflammatory diseases (Kock *et al.*, 1995). We introduce here the recent aspects of the biological and physiological activities of halichlorine.

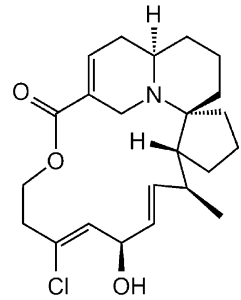

FIGURE 11.1 Structures of halichlorine (**1**).

A. Halichlorine inhibits LPS-induced NF-κb activation in endothelial cells

Various adhesion molecules are reported to be expressed on vascular endothelial cells at sites of inflammation (Libby, 2002). In fact, VCAM-1, expressed on the surface of vascular endothelial cells in response to inflammatory stimuli, is suggested to play an important role in leukocyte recruitment (Gerrity *et al.*, 1979). The adhesion of monocytes to vascular endothelial cells is the first critical step in the induction of atherosclerosis (Glass and Witztum, 2001). Supportively, the expression of VCAM-1 is reported to increase in atherosclerosis lesions (Cybulsky and Gimbrone, 1991). Intercellular adhesion molecule-1 (ICAM-1) and E-selectin are also expressed on the surface of vascular endothelial cells in response to inflammatory stimuli (Smith, 2008). VCAM-1 and ICAM-1 are adhesion molecules which are classified in the immunoglobulin superfamily, and E-selectin is a member of the selectin family. VCAM-1 is the receptor for VLA-4 (Elices *et al.*, 1990), whereas ICAM-1 is for LFA-1 and Mac-1 (Diamond *et al.*, 1990; Marlin and Springer, 1987; Rothlein *et al.*, 1986). E-selectin is the receptor for sialyl Lewis X (sLe^x) and sialyl Lewis A (sLe^a; Wittig *et al.*, 1996). The induction of ICAM-1, VCAM-1, and E-selectin by inflammatory stimuli such as tumor necrosis factor-α, interleukin-1β, and lipopolysaccharide (LPS) is associated with the functional activation of nuclear factor-κB (NF-κB) (Dustin *et al.*, 1986; Haraldsen *et al.*, 1996). NF-κB, mainly consisting of p65 and p50 proteins, is the transcription factor that binds to the κB sequence, which is found in the promoter regions of ICAM-1, VCAM-1, and E-selectin genes (Iademarco *et al.*, 1992; Karin and Greten, 2005; Sen and Baltimore, 1986; van de Stolpe *et al.*, 1994; Whelan *et al.*, 1991). These NF-κB-mediated gene expressions are significant for inflammation-related diseases including atherosclerosis, and thus, NF-κB is thought to be a good clinical target molecule. In fact, DHMEQ, a specific inhibitor of NF-κB, was reported to suppress induction of adhesion molecules and to reduce atherosclerosis in mice (Ariga *et al.*, 2002; Chiba *et al.*, 2006; Ohno *et al.*, 2005). Halichlorine

had been found to inhibit the induction of VCAM-1, downstream of NF-κB signaling (Kuramoto *et al.*, 1996). Recently, Tsubosaka *et al.* (2010b) investigated the effect of halichlorine on LPS-induced inflammatory responses in bovine aortic endothelial cells (BAECs) and reported that halichlorine inhibited NF-κB activation as follows.

In BAECs, halichlorine was revealed to inhibit LPS-induced mRNA expressions of VCAM-1, ICAM-1, and E-selectin (Fig. 11.2A). Further,

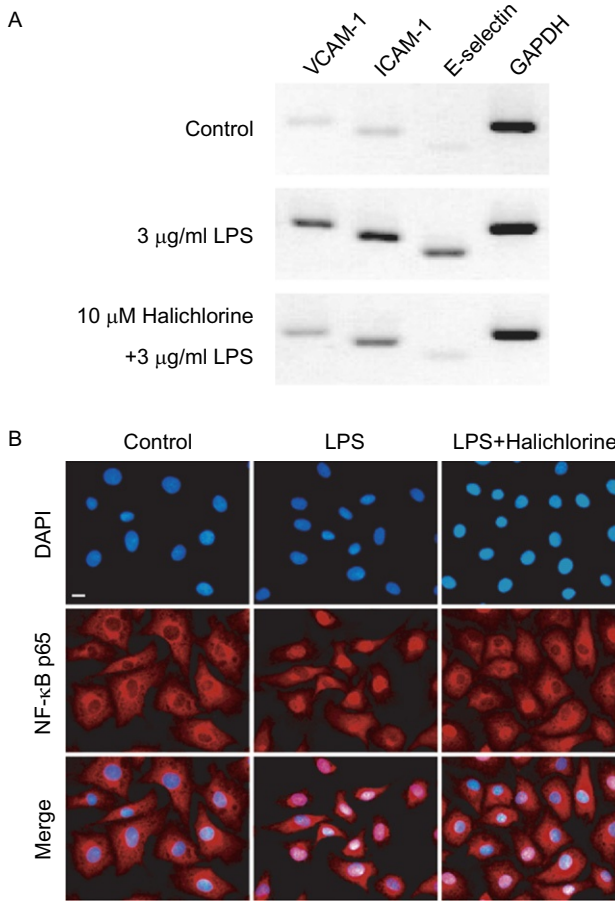

FIGURE 11.2 Inhibitory effect of halichlorine on NF-κB activation in BAECs. (A) BAECs were preincubated with or without halichlorine (10 μM) for 2 h and then exposed to LPS (3 μg/ml) for 3 h. Next, cells were collected and then analyzed by RT-PCR. Agarose gel electrophoresis demonstrates mRNA encoding GAPDH, VCAM-1, ICAM-1, and E-selectin. (B) BAECs were preincubated with or without halichlorine (10 μM) for 2 h and then exposed to LPS (3 μg/ml) for 1 h. The p65 subunit of NF-κB was immunostained with Alexa Flour 568-labeled antibody (red) and nuclei was stained with DAPI (blue). This figure is sited from Tsubosaka *et al.*, 2010b, and is permitted for use by the publisher.

halichlorine reduced U937 monocytes adhesion to LPS-stimulated BAECs. Then, phenomena upstream of adhesion molecules expressions were investigated, and halichlorine was demonstrated to inhibit LPS-induced nuclear accumulation of p65, a component of NF-κB (Fig. 11.2B). Because halichlorine did not inhibit the proliferation and viability of BAECs, its inhibitory effects against NF-κB were revealed to be not related to cytotoxicity. These data indicate that halichlorine is a unique inhibitor of NF-κB acting at the level of nuclear translocation. LPS is one of the major constituents of the outer membrane of Gram-negative bacteria and is recognized as a key molecule in the pathogenesis of inflammatory syndromes (Raetz and Whitfield, 2002). LPS induces the upregulation of adhesion molecules via the activation of NF-κB in endotherial cells in the same way as inflammatory cytokines, and inactivation of LPS leads to the suppression of the endothelial inflammation (Ohno *et al.*, 2004; Takahashi *et al.*, 2006). Various inflammatory cytokines are reported to be produced by activated macrophages and T cells in atherosclerosis (Glass and Witztum, 2001). Thus, LPS-induced adhesion between monocytes and BAECs can be supposed to reflect the situation that occurs in atherosclerosis. There may be other factors aside from inflammatory cytokines that induce cell adhesion to the endothelial cells in atherosclerosis. It was reported that native low-density lipoprotein (LDL) induced the expression of VCAM-1 and E-selectin in human aortic endothelial cells (Allen *et al.*, 1998). There is a possibility that halichlorine also inhibits the cell adhesion induced by LDL, if this adhesion is mediated by NF-κB.

As described above, halichlorine was shown to inhibit LPS-induced events in BAECs, including adhesion-molecule expression, adherence of monocytes, and NF-κB activation. Thus, halichlorine is thought to be an attractive candidate for the adjunctive therapy of diseases such as atherosclerosis.

B. Halichlorine inhibits L-type Ca^{2+} channels in vascular smooth muscle cells

Recently, Tsubosaka *et al.* (2010a) reported that halichlorine was also revealed to inhibit L-type Ca^{2+} channels, which leads to inhibit smooth muscle contraction. In their report, the direct effect of halichlorine on vascular contractility was investigated. Then, halichlorine was found to inhibit both high concentration of K^{+}- and phenylephrine-induced contractions in rat aorta dose dependently. The effect of halichlorine on high K^{+}-induced contraction was shown to be stronger than that on phenylephrine-induced contraction. Because known L-type Ca^{2+} channel blockers, verapamil and nifedipine, were observed to show the similar effect by them, it was suggested that halichlorine selectively inhibits L-type Ca^{2+} channels. Then, the effect of halichlorine on intracellular Ca^{2+} concentration in vascular smooth muscle tissue was examined using a fluorescent Ca^{2+} indicator, Fura-2.

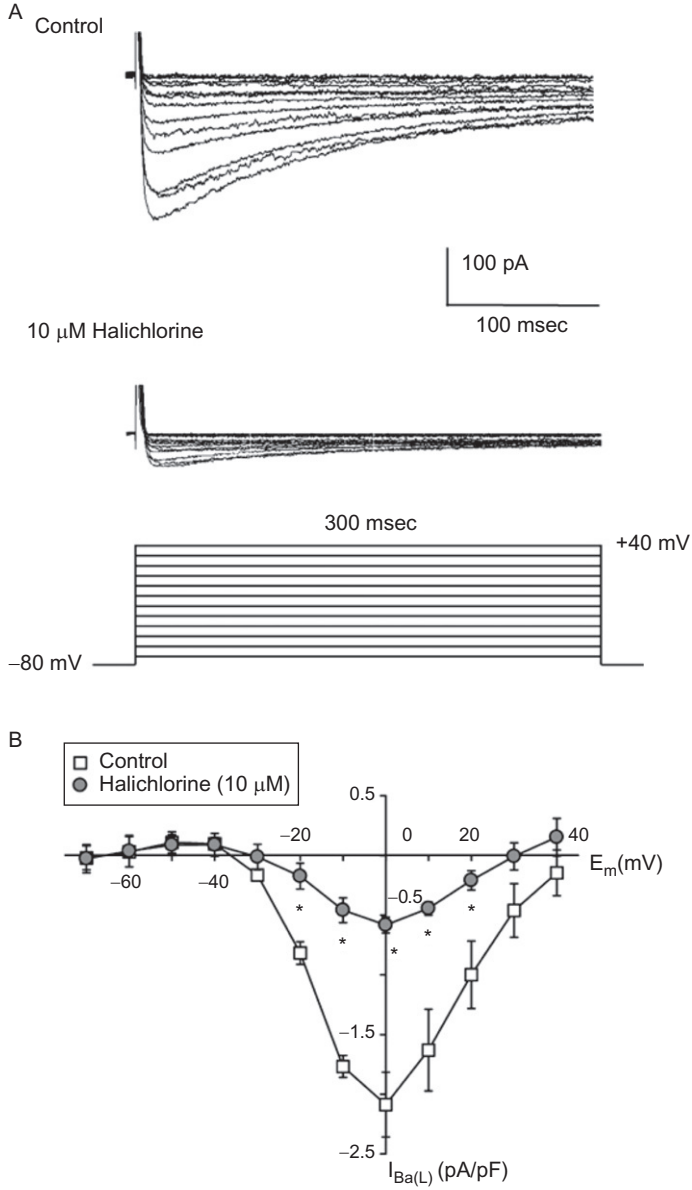

FIGURE 11.3 Effect of halichlorine on the voltage-dependent Ca^{2+} currents in rat aortic smooth muscle cells (A7r5 cells). (A) Typical traces of Ba^{2+} current for control (top trace) and after treatment of halichlorine (middle trace; 10 μM). A7r5 cells elicited by incremental 10 mV depolarizing steps from −70 mV to +40 mV. Holding potential was −80 mV. (B) Current–voltage relationships for the peak inward Ba^{2+} current density for control and after the addition of halichlorine (10 μM). $P<0.01$. This figure is sited from Tsubosaka *et al.*, 2010a, and is permitted for use by the publisher.

In this analysis, halichlorine was demonstrated to inhibit the Ca^{2+} influx induced by high K^+ significantly. High K^+-induced contraction in vascular smooth muscle is reported to be mediated mainly by Ca^{2+} influx through voltage-dependent L-type Ca^{2+} channels (Karaki *et al.*, 1997). Halichlorine was likely to inhibit L-type Ca^{2+} channel directly, and thus, this was examined using whole-cell patch-clamp analysis. As shown in Fig. 11.3A and B, halichlorine was revealed to reduce the Ca^{2+} channel current densities. These results suggested that halichlorine suppressed Ca^{2+} influx through the inhibition of voltage-dependent L-type Ca^{2+} channels. Thus, halichlorine was revealed to inhibit L-type Ca^{2+} channels selectively in vascular smooth muscle cells, which leads to the inhibition of intracellular Ca^{2+} influx and the reduction of vascular contractions. Although halichlorine-binding site in L-type Ca^{2+} channel has not been clarified, these findings may contribute to the development of halichlorine as a new Ca^{2+} channel blocker. Because many of L-type Ca^{2+} channel blockers have been applied for the treatment of patients with hypertension, there is a possibility that halichlorine has a potential as an antihypertensive agent.

III. CONCLUSION

As described above, several bioactive naturally occurring compounds from marine organisms and plants show unique biological activities. Halichlorine was shown to inhibit the activity of NF-κB in endothelial cells and to inhibit L-type Ca^{2+} channels. Although it needs to be examined more to clarify the mechanisms underlying its biological actions, halichlorine may have therapeutic potentials for diseases such as atherosclerosis and hypertension. We believe that these results and attempts will contribute to the development of novel key molecules with intriguing biological activities which are effective in preventing and curing diseases.

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