Vesnarinone: a differentiation-inducing anti-cancer drug

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Vesnarinone has been shown to be a unique anti-proliferating, differentiation-inducing and apoptosis-inducing drug against several human malignancies, including leukemia and several solid tumors. Furthermore, vesnarinone potentiates the effect of conventional cytotoxic chemotherapy or radiation therapy. Combination of differentiation-inducing therapy by vesnarinone with conventional chemotherapy or radiation therapy might be second- or third-line therapy in patients with advanced cancer. Analysis of the molecular mechanisms of the tumor differentiation therapy by vesnarinone might provide selective and targeted molecules for novel tumor dormancy therapy. *Anti-Cancer Drugs* 14:391–395 © 2003 Lippincott Williams & Wilkins.

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Introduction

The goal of cancer therapy is to completely kill or resect the cancer cells; however, a new concept for cancer therapy termed 'tumor dormancy therapy' has recently been proposed [1]. The concept of this therapy is to prolong the survival time of cancer patients while retaining their quality of life. Differentiation-inducing therapy, apoptosis-inducing therapy, anti-angiogenic therapy and anti-metastasis therapy are examples of tumor dormancy therapy [1]. Differentiation-inducing therapy for leukemia, such as administration of all-*trans*-retinoic acid on acute premyelocytic leukemia, is widely recognized [2–4]. However, differentiation-inducing therapy for solid tumors is still underdeveloped. We have been studying the mechanisms of salivary gland differentiation [5,6] and have devised a differentiation-inducing therapy for salivary gland cancer [7]. Here, we focus on the action of the differentiation-inducing drug vesnarinone (Otsuka Pharmaceutical Company, Tokyo, Japan) on several human malignancies and discuss the molecular mechanisms of its effect.

Molecular structure and biological activity

Vesnarinone (OPC-8212; 3,4-dihydro-6-[4-(3,4-dimethoxybenzoyl)-1-piperazinyl]-2(1H)-quinolinone; Fig. 1), a synthetic quinolinone derivative with inotropic effects, was originally developed to treat cardiac failure [8] and has been clinically used for the treatment of patients with chronic congestive heart failure [9,10]. However, vesnarinone has been reported to demonstrate a unique spectrum of intriguing biological effects. It exhibits immunomodulatory activities, such as suppression of tumor necrosis factor-induced activation of NF-κB [11], the suppression of NK cell activity [12,13], endotoxin-induced production of inflammatory cytokines [14–19], nitric oxide synthase from macrophage and cardiac myocytes [20,21], and abrogation of E-selectin expression in endothelial cells [15]. Vesnarinone also causes agranulocytosis by impairing stromal functions and cytokine inhibition [22,23], and inhibits immune-mediated hepatic injury [24]. In addition, it inhibits the production of HIV-1 [25] and reduces the lethality of endotoxemia [26].

Affect on the growth of solid tumor cells *in vitro*

In addition to the above immunomodulatory effects, vesnarinone has been found to inhibit the growth of a wide variety of solid tumor cells *in vitro*. We demonstrated that vesnarinone inhibited the growth of a human salivary gland cancer cell line TYS *in vitro*, it apparently induced G_1 arrest in TYS cells but did not show any cytocidal effect on the cells [5,27]. We also showed that vesnarinone induced p21^{waf1} and transforming growth factor (TGF)-β1 gene expression in TYS cells [5,27]. Furthermore, we recently reported that vesnarinone directly activated the p21^{waf1} promoter via the activation of Sp1 and Sp3 transcription factors and histone hyperacetylation in TYS cells [28,29]. However, contrary to our results with TYS cells, Yoneda *et al.* reported that

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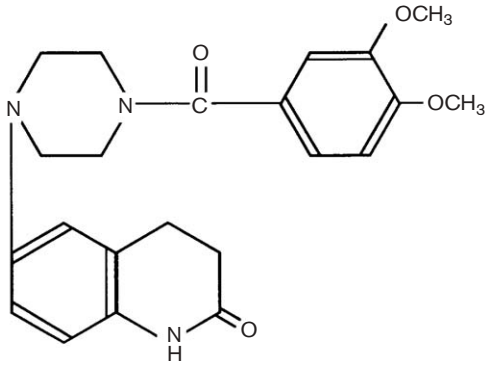
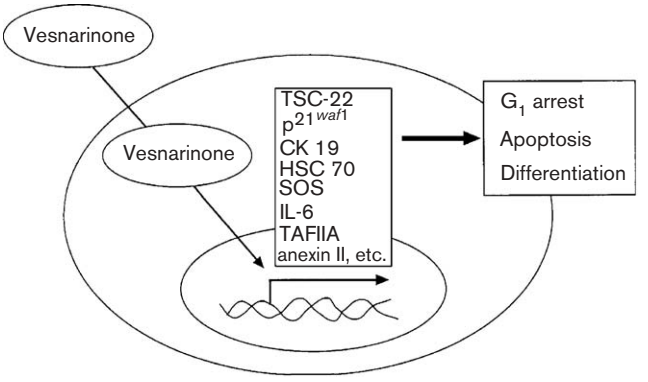
Fig. 1



Chemical structure of vesnarinone.

Fig. 2



Schematic representation of the action of vesnarinone on cancer cells.

vesnarinone did not directly activate the $p21^{waf1}$ promoter in oral squamous cell carcinoma, but stabilized its mRNA [30]. Thus, the mechanisms of induction of $p21^{waf1}$ protein by vesnarinone might vary between different cell types. Vesnarinone is reported to regulate the expression of several factors which are associated with growth and differentiation, and induces G_1 arrest, apoptosis and differentiation in cancer cells (Fig. 2).

With regard to the effect of vesnarinone on other types of cancer cells *in vitro*, Isaka *et al.* reported that vesnarinone induced apoptosis in a human choriocarcinoma cell line by the induction of the c-*myc* gene [31]. Yokozki *et al.* noted that vesnarinone induced G_0/G_1 arrest without apoptosis in a gastric cancer cell line concomitant with the reduction of the expression of cyclin A, D1 and E, and of CDK2 [32]. Fujita *et al.* reported that the combination of vesnarinone with cisplatin or VP-16 showed a synergic or additive inhibitory effect on human lung cancer cell lines [33]. Fujita *et al.* also reported that vesnarinone improved the sensitivity to irradiation of human lung cancer cell lines [34]. Furthermore, Kubo *et al.* showed that vesnarinone could act as a biochemical modulator of anti-cancer agents on hepatocellular carcinoma cells [35].

Effect on the growth of solid tumor *in vivo*

Several investigators demonstrated the anti-tumor effect of vesnarinone in *in vivo* experiments. Sato *et al.* noted that vesnarinone inhibited the growth of TYS-nude mouse tumors and that it induced the differentiation of the tumor cells [36]. Honma *et al.* reported that vesnarinone induced G_1 arrest in cooperation with glucocorticoids in human non-small cell lung carcinoma cells grown in nude mice [37]. Tanaka *et al.* demonstrated the apoptosis-inducing and growth-suppressing activity of vesnarinone on heterotopically or orthotopically transplanted human glioma cells in nude mice [38]. Kawai *et al.* showed the enhanced anti-cancer effects of vesnarinone when combined with radiation and conventional anti-cancer agents on human gastric tissue xenografts in nude mice [39]. Nio *et al.* showed the anti-invasive effect of vesnarinone *in vitro* and the anti-proliferative effect on human pancreatic cancer cells in nude mice [40].

Effect on the growth of leukemia cells

Kondo *et al.* reported that vesnarinone increased the intracellular content of a pro-apoptotic lipid mediator, ceramid, and that it induced apoptosis in myeloid HL-60 cells [41]. They also showed that the vesnarinone-induced apoptosis of HL-60 cells was mediated by increased oxidative damage via ceramid-induced inhibition of catalase function [41]. Fujiwara *et al.* showed the anti-proliferative effect of vesnarinone on primary myeloid leukemia cells from nine patients; three cases of M1, two of M2, three of M3, one of M4 and one of M6 [42]. However, they also showed that growth of bone marrow mononuclear cells from a healthy control was not affected by vesnarinone treatment [42]. The anti-proliferative effect of vesnarinone was also demonstrated on hairy leukemia cells [43], leukemic blast progenitors in acute myelogenous leukemia patients [44] and human erythroleukemia HEL cells [45].

Isolation of a differentiation-inducing gene, TSC-22, as a vesnarinone-inducible gene

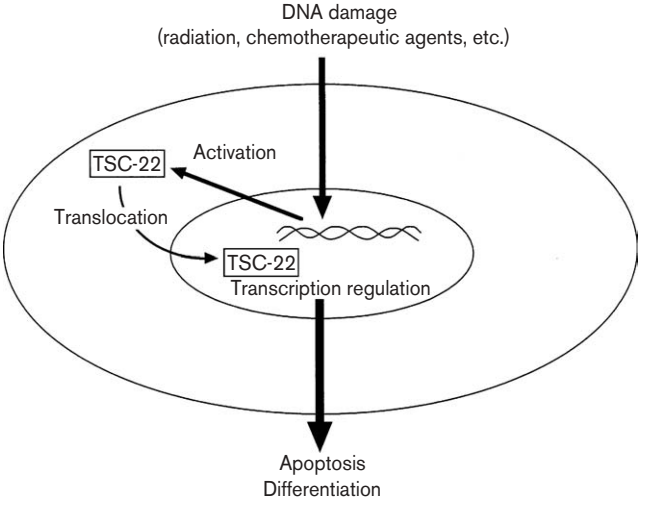
In order to clarify the molecular mechanisms of the growth-inhibitory effect of vesnarinone on TYS, we isolated several genes as vesnarinone-inducible genes from a cDNA library constructed from vesnarinone-treated TYS cells [5]. Among these genes, we focused on TSC-22 (TGF-β-stimulated clone 22), and analyzed the role of TSC-22 protein on the growth of salivary grand cancer cells [5,6,46–50], and structure and function of

TSC-22 gene [51]. The expression of TSC-22 was up-regulated within a few hours after treatment with vesnarinone and was continued for 3 days [5]. The level of TSC-22 mRNA in TYS cells was increased continuously until the cells reached confluency [5]. Antisense oligonucleotide against TSC-22 suppressed the anti-proliferative effect of vesnarinone [5]. We transfected TYS cells with the sense or antisense TSC-22 expression vector, and examined the *in vitro* growth and the tumorigenicity in nude mice of the transfectants [6]. Contrary to our expectation, up-regulation of TSC-22 protein did not affect either the *in vitro* or *in vivo* growth of TYS cells [6]. However, down-regulation of TSC-22 markedly enhanced the growth of TYS cells *in vitro* and *in vivo* [6].

TSC-22 was originally reported as a TGF-β-inducible gene in mice osteoblastic cells, MC3T3E1 [52]. TSC-22 was shown to encode a putative transcriptional regulator containing a leucine zipper-like structure [5,52,53]. Recently, we reported that over-expression of TSC-22 enhanced chemosensitivity [46] and radiation sensitivity by inducing apoptosis in the cancer cells [48]. In living cells, TSC-22 was clearly localized to the cytoplasm; however, in the apoptotic cells, the TSC-22 was translocated from the cytoplasm to the nucleus [49]. Thus, after receiving the apoptotic stimuli, TSC-22 translocates from the cytoplasm to the nucleus and shows transcription-regulatory activity (Fig. 3) [49].

Taking previous reports concerning the anti-cancer effect of vesnarinone together with our molecular biological examination of the function of TSC-22, we conclude that the anti-proliferative effect of vesnarinone is, at least in part, mediated by the TSC-22 signaling pathway.

Fig. 3



A possible mechanism of TSC-22 for inducing apoptosis and differentiation.

Clinical study of vesnarinone as an anti-cancer agents

Vesnarinone is currently used as a chemotherapeutic agent for several cancers combined with radiation and/or conventional chemotherapeutic agents in Japan [54,55], the US [56] and India (unpublished data, Otsuka Pharmaceutical Company). Sato *et al.* reported that vesnarinone markedly regressed the advanced submandibular adenoid cystic carcinoma by inducing tumor cell differentiation [54]. They also showed the differentiation-inducing effect of vesnarinone on oral squamous cell carcinoma [55]. In the US, phase I and pharmacokinetic studies of vesnarinone in combination with gemcitabine in patients with advanced cancer were conducted [56]. Twenty-six patients with breast cancer (10 patients), non-small cell lung cancer (six patients), prostate cancer (three patients), carcinoma of unknown primary (two patients), bladder cancer (two patients), small cell lung cancer (one patient), pancreas cancer (one patient) and osteosarcoma (one patient) were treated with 92 courses of vesnarinone/gemcitabine. The principal toxicities of the regimen consisted of neutropenia and thrombocytopenia. Pharmacokinetic studies of vesnarinone revealed significant interpatient variability at any given dose level. Two partial responses occurred in patients with refractory breast and non-small cell lung carcinoma. One patient with breast carcinoma involving lymph nodes, bone and liver metastasis achieved complete resolution of her visceral and nodal metastases accompanied by a decreased intensity of radioisotope uptake in metastatic lesions on the bone scan after two courses of vesnarinone and gemcitabine. The partial response lasted 4 months. Another patient with non-small cell lung carcinoma involving brain and lymph node metastasis showed 74% reduction in extent of nodal disease after two courses of the treatment. Stable disease lasting from 2 to 8 months was observed in 11 patients with a variety of other solid tumors.

Conclusion and the future direction

As vesnarinone potentiates the effect of conventional cytotoxic chemotherapy or radiation therapy, combination of differentiation-inducing therapy by vesnarinone with conventional chemotherapy or radiation therapy might be used as a second- or third-line therapy in patients with advanced cancer. Analysis of the molecular mechanisms of the tumor differentiation therapy by vesnarinone might provide selective and targeted molecules for novel tumor dormancy therapy.

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