Cytostatic and pro-apoptotic effects of a novel phenylacetate–dextran derivative (NaPaC) on breast cancer cells in interactions with endothelial cells

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We have tested a novel hybrid molecule made of carboxymethylbenzylamide dextran (CMDB) and sodium phenylacetate (NaPa) groups, called the CMDB–NaPa ester (NaPaC), on the proliferation of breast cancer and endothelial cells as well as paracrine effects between these two cell types. Our results showed that NaPaC inhibited the proliferation of MDA-MB-231 cells and MCF-7 cells in a dose-dependent manner. NaPaC was 20-fold more active on highly invasive MDA-MB-231 cells than the NaPa parental molecule. On MCF-7 cells, which present a less aggressive phenotype, NaPaC was only 3-fold more active than the NaPa parental molecule. Furthermore, NaPaC had only a slight effect on the proliferation of primary cultured endothelial cells (HUVEC). A cytostatic effect of NaPaC on tumor cells was observed with cells accumulating in G_0/G_1 phase after 96 h of treatment. In addition, NaPaC induced a strong apoptotic effect on the two breast cancer cell lines. Conditioned media (CM) from tumor cells inhibited HUVEC proliferation, and this effect was enhanced in the presence of NaPaC (6 mM) and NaPa (10 mM). In addition to this cytostatic effect, CM from tumor cells induced a HUVEC early apoptosis which was increased, mainly, in the presence of NaPa (15 mM). Thus, this study shows that NaPaC is a more powerful anti-proliferative molecule than its parental NaPa molecule, with cytostatic and pro-apoptotic effects on MDA-MB-231 and MCF-7 tumor cells. Also, both molecules increased a pro-apoptotic effect of tumor cells on HUVEC. *Anti-Cancer Drugs* 15:975–981 © 2004 Lippincott Williams & Wilkins.

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Introduction

Sodium phenylacetate (NaPa), a physiological metabolite of phenylalanine, is normally found in human plasma at micromolar concentrations. At a higher concentration, NaPa induces cytostasis and reverses *in vitro* the malignant phenotype of different cancer cells [1–4]. We have previously shown that NaPa can induce apoptosis of MCF-7ras cells by a mechanism involving Bcl-2 down-regulation [5]. Furthermore, in a MCF-7ras tumor model, we showed that NaPa and tamoxifen combined together can act synergistically to inhibit tumor development, and prevent an escape from anti-estrogen inhibition [6].

Carboxymethylbenzylamide dextran (CMDB) derivatives are other compounds that we have previously described to inhibit breast cancer cell proliferation both *in vitro* and *in vivo* [7–11]. Their inhibitory effect was associated with a decrease in the S phase cell population and an accumulation in the G_0/G_1 phase, but not with apoptosis [7]. Indeed CMDB inhibited the activity of growth factors such as fibroblast growth factor (FGF) 2 and FGF4 as well as platelet-derived growth factor (PDGF)-BB and transforming growth factor (TGF)-β by changing their binding to their specific cognate receptors [9,10]. Our previous results showed that treatment with CMDB blocked the experimental development of tumors with FGF4-transformed HBL100 or MCF-7ras cells when they are xenografted in athymic mice [8,9]. Recently, we also demonstrated a synergistic effect of CMDB and NaPa on MCF-7ras cells *in vitro* and in animal models [12]. Consequently, we generated a novel hybrid molecule with a NaPa/CMDB ratio equivalent to that giving a synergistic effect when the drugs are used in combination, in order to obtain a molecule with both CMDB and NaPa properties (we call this CMDB–NaPa ester or NaPaC). In this study, we have compared the effect of NaPaC and the parental NaPa molecule on the proliferation of two breast cancer cell lines, MDA-MB-231 and MCF-7, which are representative of two different tumor phenotypes. The MDA-MB-231 cells are invasive and non-hormono-sensitive cells issued from pleural effusion, whereas MCF-7 cells are less invasive and present a hormono-sensitive phenotype. Furthermore, we investigated the cytostatic and apoptotic effects of NaPaC on breast cancer cells as well as on primary endothelial cells

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[human umbilical vein endothelial cells (HUVEC)]. For the intravasation process to occur, tumor cells must first interact with endothelial cells in order to metastasize. Hence, we also studied the paracrine activities of tumor cells on HUVEC endothelial cells in the presence of the NaPa compounds.

Materials and methods

Cell cultures

Breast cancer cell lines, MDA-MB-231 and MCF-7 (ATCC, Rockville, MD), were grown and maintained in Dulbecco's modified Eagle's medium (DMEM; Gibco, Grand Island, NY), supplemented with 10% fetal calf serum (FCS), 15 mM HEPES, 5 μg/ml fungizone, 100 IU/ml penicillin and 100 μg/ml streptomycin, in a humidified atmosphere of 5% CO_2, at 37°C.

The HUVEC were isolated as previously described by Jaffe *et al.* [13] with minor modifications. Briefly, the umbilical vein was washed once with a PBS/glucose buffer (NaH_2PO_4 10 mM, KH_2PO_4 15 mM, KCl 40 mM, NaCl 150 mM, D-glucose 0.1 M), and incubated with 0.2% of collagenase H (Roche, Mannheim, Germany) for 10 min at 37°C. When dissociated, the isolated HUVEC were plated in cell culture dishes and cultured in M199 medium (Gibco) with 20% FCS. Cells were maintained in culture for up to 2 days.

Cell proliferation assay

Cells were plated in 48 well-plates at a density of 1500 (MDA-MB-231), 4000 (MCF-7) and 50 000 cells (HUVEC) per well. After 24 h, cells were treated with increasing concentrations of NaPa (5–40 mM) or NaPaC (0.5–18 mM) for different times (24–96 h) in medium with 2% FCS. Cells were trypsinized and counted on a hemocytometer after Trypan blue staining. The percentage of cells corresponds to a ratio: (total cells–blue cells)/total cells.

Flow cytometry analysis

Cell cycle analysis

Cells (1×10^6) were plated in 10% FCS/DMEM in T_{75} tissue culture flasks. After 24 h, cells were washed with DMEM and incubated with the indicated concentrations of NaPa or NaPaC, diluted in the medium containing 2% FCS. After 96 h, cells were incubated for 4 h with Bromodeoxyuridine (BrdU; PharMingen, San Diego, CA). Then, cells were washed once with PBS, trypsinized and fixed with 70% ethanol. Incorporated BrdU was revealed by using anti-BrdU monoclonal antibody conjugated with FITC (PharMingen). After centrifugation, the cell pellet was resuspended in a staining solution containing 50 μg/ml propidium iodide (PI) (PharMingen) for 10 min. Double-stained cells were analyzed by FACScan (Epics Laser; Beckman Coulter, Fullerton, CA).

Apoptosis analysis

For apoptosis analysis, 1×10^6 cells were plated in 10% FCS/DMEM in T_{75} tissue culture flasks. After 24 h, cells were washed with DMEM and incubated with the indicated concentrations of NaPa or NaPaC, diluted in the medium containing 2% FCS. After 96 h, cells were labeled with Annexin-V and PI as described by the manufacturer (R & D Systems, Lille, France). Double-stained cells were analyzed by FACScan (Epics laser).

Conditioned media (CM) on HUVEC

Tumor cells were grown in T_{25} flasks in media containing 10% FCS (5 ml). At about 80% of confluence, the cell monolayer was washed with DMEM/F12 medium (Gibco) containing 2% FCS and incubated in 5 ml of the same medium. After 24 h, the CM was collected and centrifuged for 5 min at low speed (1500 r.p.m.), filtered (0.45 μm) and stored at –80°C or used immediately. Then, HUVEC were incubated with 5 ml of CM during 96 h.

Statistical analysis

All experiments were performed 3 times. Data are expressed as mean ± SD. Student's *t*-test was used for statistical analyses. $p < 0.01$ compared to control values.

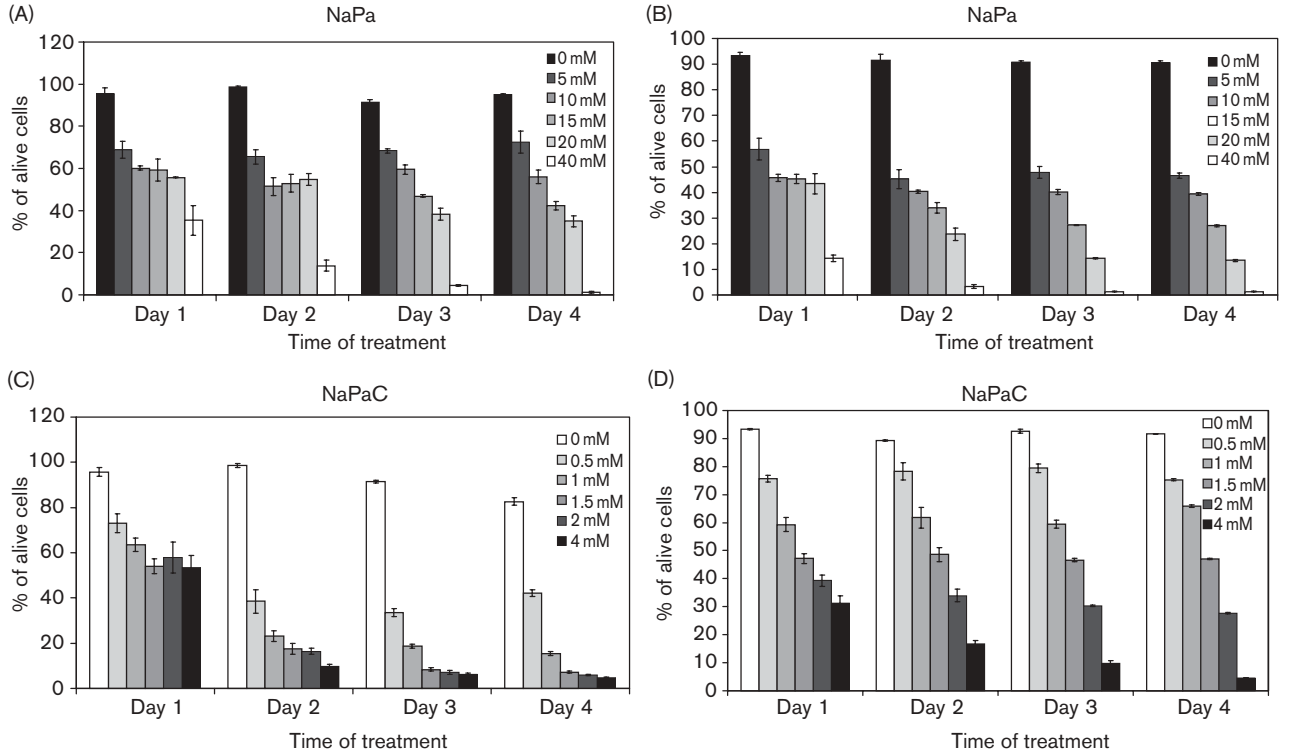
Results

Effects of NaPa or NaPaC on the proliferation of MDA-MB-231 and MCF-7 breast tumor cells

Anti-proliferative effect of NaPa and NaPaC

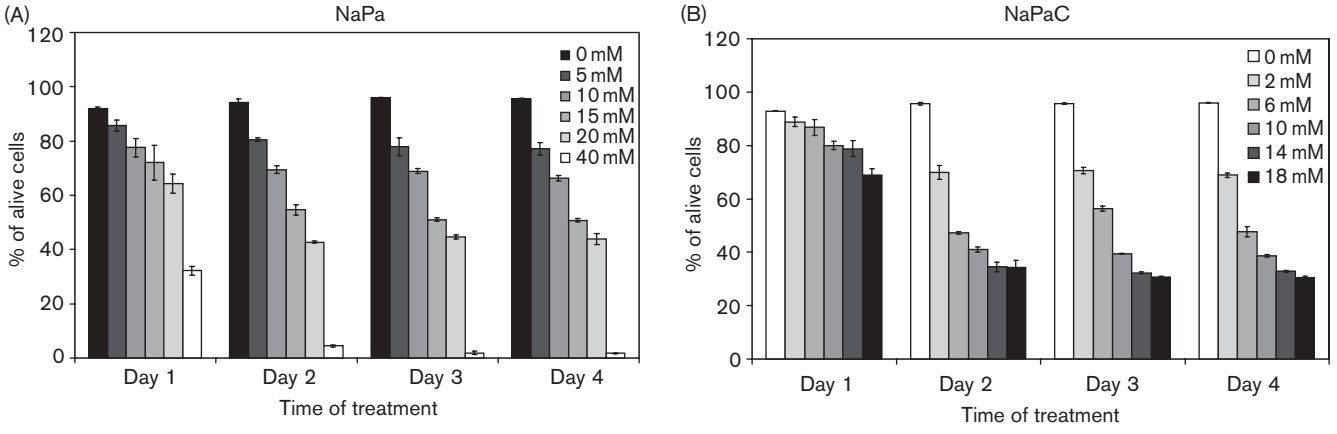
The anti-proliferative effect of NaPa and NaPaC was determined by Trypan blue staining on both human breast cancer cell lines, MDA-MB-231 and MCF-7. Cells were grown in the presence of increasing dose of NaPaC or the parental molecule NaPa for up to 96 h. Our results first showed that NaPaC has a very low cytotoxic activity compared to other well-known anticancer drugs, as previously shown for the parental molecule NaPa [3]. No significant cell death was observed for concentrations of NaPa and NaPaC up to 40 and 4 mM, respectively. From the dose–response curves, we observed that MDA-MB-231 cells were much more sensitive (20-fold) to NaPaC ($IC_{50} = 0.5$ mM) than to the parental molecule NaPa ($IC_{50} = 10$ mM) (Fig. 1A and C). To a lesser extent, we also observed that MCF-7 cells were more sensitive (3-fold) to NaPaC ($IC_{50} = 1.5$ mM) than to NaPa ($IC_{50} = 5$ mM) (Fig. 1B and D). We then studied the effects of NaPaC on HUVEC proliferation, in a time-dependent and dose-dependent manner (Fig. 2). The IC_{50} values indicated that HUVEC were 2.5-fold more sensitive to NaPaC ($IC_{50} = 6$ mM) than to NaPa ($IC_{50} = 15$ mM), but they were 4- and 12-fold less sensitive to NaPaC than MCF-7 and MDA-MB-231 cells, respectively. Thus, our results showed that NaPaC was more active on highly invasive MDA-MB-231 cells than the NaPa parental molecule. Furthermore, NaPaC had

Fig. 1



Growth inhibition of MDA-MB-231 (A and C) and MCF-7 (B and D) cells by NaPa (A and B) or NaPaC (C and D). Cells (10^4/well) were plated in 48-well plates. Twenty-four hours later, NaPa or NaPaC at various concentrations was added to cells in the medium with 2% FCS. Then, 1, 2, 3 or 4 days later, cells were trypsinized and counted. These assays were in triplicate. Results are expressed as mean ± SD.

Fig. 2



Growth inhibition of endothelial cells (HUVEC) by NaPa (A) or NaPaC (B). Cells (5×10^4/well) were plated in 48-well plates. Twenty-four hours later, NaPa or NaPaC at various concentrations was added to cells in the medium with 5% FCS. Then, 1, 2, 3 or 4 days later, cells were trypsinized and counted. These assays were in triplicate. Results are expressed as mean ± SD.

less effect on the proliferation of primary cultured endothelial cells (HUVEC) ($IC_{50} = 6$ mM) than on that of breast cancer cells.

NaPa and NaPaC are G_0/G_1 phase blockers

We studied and compared the effects of NaPa and NaPaC on the cell cycle in MDA-MB-231 and MCF-7 cells using

Table 1 Cytostatic effects of NaPa and NaPaC on MDA-MB-231, MCF-7 cells and HUVEC

	Cells in G_0/G_1 phase (%)			Cells in S phase (%)		
	MDA-MB-231	MCF-7	HUVEC	MDA-MB-231	MCF-7	HUVEC
Control	69.3 ± 0.7	69.9 ± 0.8	74.7 ± 1.3	24.8 ± 0.6	22.5 ± 1.8	13.7 ± 0.8
IC_{50} NaPa	75.7 ± 1.4[a]	76.9 ± 1.1[a]	91.8 ± 0.8[a]	21.0 ± 1.3[a]	17.8 ± 1.0[a]	2.1 ± 1.5[a]
IC_{50} NaPaC	75.3 ± 1.1[a]	73.8 ± 0.6[a]	94.9 ± 1.0[a]	17.2 ± 0.3[a]	19.5 ± 0.3[a]	0.6 ± 1.0[a]

Cells (0.5×10^6/flask of 25 cm^2) were plated in medium with 2% FCS and incubated overnight at 37°C. Twenty-four hours later, NaPa or NaPaC was added to cells at their IC_{50} values for each cell type and incubated for 4 days. Cells were stained with BrdU and PI before analysis with flow cytofluorimetry. These assays were in triplicate. Results are expressed as mean ± SD.
[a] $p < 0.01$.

a flow cytometric approach. The cell cycle profile indicated that 75.3 ± 1.1 and 73.8 ± 0.6% of the cells were arrested in the G_0/G_1 phase after 96 h treatment with NaPaC used at IC_{50} concentration, compared with 69.3 ± 0.7 and 69.9 ± 0.8%, respectively, of the control cells ($p < 0.01$) (Table 1). However, NaPaC acted more efficiently on the invasive MDA-MB-231 than on MCF-7 cells. Thus, the inhibition of proliferation was at least partially explained by a blockage in the G_0/G_1 phase of cell cycle with concomitant reduction of cells in S phase. Similarly to breast cancer cells, we measured the distribution of HUVEC in the different phases of cell cycle after 96 h of treatment with the drugs. Cytofluorimetric analysis of cells showed that NaPaC strongly reduced the number of HUVEC in S phase as also observed with the NaPa molecule (Table 1). This S phase reduction observed with both molecules was associated with cell accumulation in the G_0/G_1 phase of cell cycle. 94.8 ± 1.0% of HUVEC were arrested in the G_0/G_1 phase after 96 h treatment with NaPaC (IC_{50} value) compared to 91.8 ± 0.8% of NaPa (IC_{50} value) treated cells and 74.7 ± 1.3% of the control cells ($p < 0.01$). Our results showed that both molecules had a strong cytostatic effect on HUVEC and NaPaC was even more effective than NaPa. Moreover, the cytostatic effect of NaPa and NaPaC on HUVEC was stronger than on tumor cells. Thus, the inhibition of HUVEC proliferation by NaPa and NaPaC may be mainly explained by a blockage in the G_0/G_1 phase of cell cycle and concomitant reduction of cells in S phase.

Apoptotic effect of NaPaC

Annexin-V and PI labeling was used to measure early and late cell apoptosis, respectively. Breast cancer cell lines exhibited early apoptosis when they were treated during 4 days by NaPaC or NaPa at their respective IC_{50} values (Table 2). Both NaPa and NaPaC were more pro-apoptotic (about 3-fold) on MDA-MB-231 than on MCF-7 cells. Moreover, at IC_{75} values, NaPaC was 3–4 times more pro-apoptotic than NaPa on MDA-MB-231 and MCF-7 cells (Table 2). The anti-proliferative effect of these two molecules may then be explained by a blockage in the G_0/G_1 phase of cell cycle and an important early apoptosis of tumor cells. The late apoptosis also increased when cells were treated with NaPaC or NaPa, with a higher pro-apoptotic effect of NaPaC than NaPa on both MDA-MB-231 and MCF-7 cells (data not shown).

Table 2 Pro-apoptotic effect of NaPa and NaPaC on MDA-MB-231, MCF-7 cells and HUVEC: % of cells in early apoptosis

	MDA-MB-231	MCF-7	HUVEC
Control	9.5 ± 1.2	5.1 ± 1.5	13.6 ± 0.3
IC_{50} NaPa	18.2 ± 0.8[a]	8.5 ± 0.9[a]	25.4 ± 1.1[a]
IC_{50} NaPaC	38.1 ± 0.6[a]	11.6 ± 1.2[a]	30.7 ± 0.9[a]
IC_{75} NaPa	23.6 ± 0.9[a]	9.0 ± 0.8[a]	28.3 ± 1.3[a]
IC_{75} NaPaC	75.1 ± 0.8[a]	21.2 ± 1.3[a]	34.1 ± 1.0[a]

Cells (0.5×10^6 /flask of 25 cm^2) were treated during 4 days with concentrations corresponding to the IC_{50} or IC_{75} of NaPa or NaPaC for each cell type. Percentage of apoptotic cells was determined with flow cytofluorimetry after Annexin-V and PI staining as described in Materials and methods. Each measurement was performed in triplicate. Results are expressed as mean ± SD.
[a] $p < 0.01$.

We also investigated the effect of NaPa or NaPaC on HUVEC apoptosis. As shown for breast cancer cells, both NaPa and NaPaC at their IC_{50} induced HUVEC apoptosis (Table 2), and NaPaC was also more pro-apoptotic than NaPa at IC_{75} (34.1 ± 1.0 versus 28.3 ± 1.3%). However, NaPaC was less pro-apoptotic on HUVEC than on the invasive MDA-MB-231 cells.

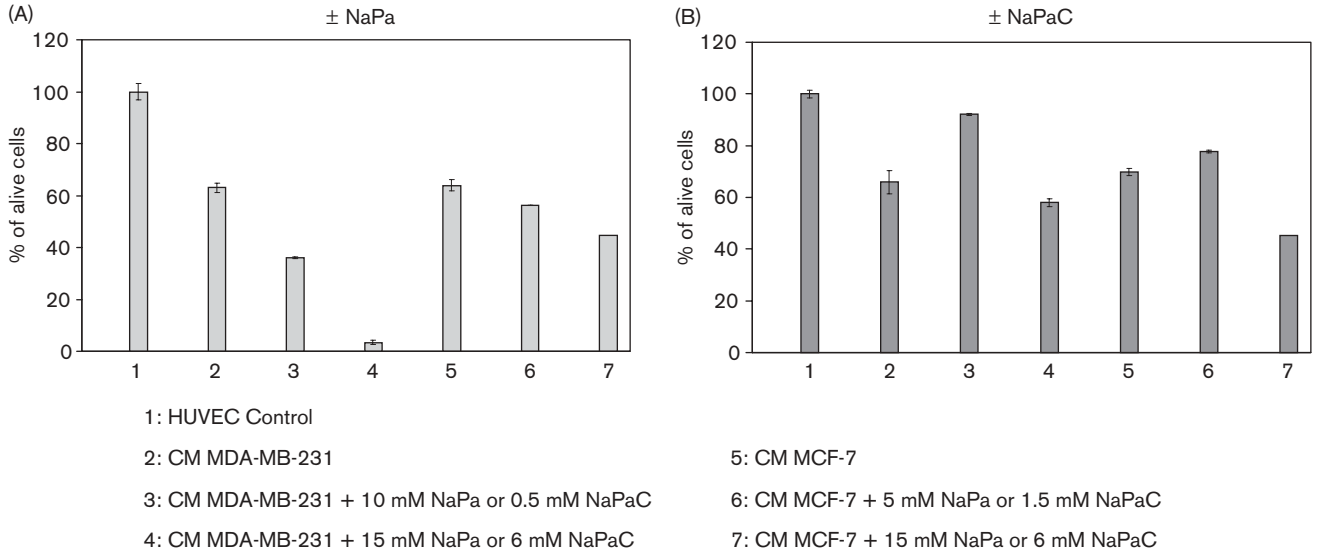
Effects of NaPa or NaPaC on the paracrine activities of tumor cells on HUVEC

Compared effects of NaPa and NaPaC on tumor cell mitogenic activities on HUVEC

To study the cross-talk between tumor cells and endothelial cells, HUVEC were incubated with tumor cell CM for 4 days as described in Materials and methods. Our results showed that MDA-MB-231 or MCF-7 CM inhibited HUVEC proliferation by 40% (Fig. 3). Furthermore, when tumor cell CM were added to HUVEC treated for 4 days with NaPa (Fig. 3A), the HUVEC inhibition was increased; this inhibitory effect was much more pronounced with MDA-MB-231 CM (columns 3 and 4) than with MCF-7 CM (columns 6 and 7), with an inhibition of 90 and 55%, respectively, in the presence of 15 mM NaPa (IC_{50} value of NaPa on HUVEC proliferation).

Different effects were observed when tumor cell CM were added to HUVEC treated with NaPaC (Fig. 3B). When MDA-MB-231 or MCF-7 CM were added to

Fig. 3



Compared effects of NaPa (A) and NaPaC (B) on tumor cell paracrine activities tested on HUVEC. Tumor cells (10^6/flask of 25 cm^2) were plated in DMEM medium and incubated overnight at 37°C. At pre-confluence, DMEM medium was changed by DMEM/F12 medium with 5% FCS. Twenty-four hours later, medium was collected, filtered and incubated with HUVEC in the absence or presence of NaPa or NaPaC. These assays were in triplicate. Results are expressed as mean ± SD.

Table 3 Pro-apoptotic effect of NaPa and NaPaC on MDA-MB-231, MCF-7 cells and HUVEC: % of apoptotic cells

	NaPa				NaPaC			
	0 mM	5 nM	10mM	15 mM	0 mM	0.5 mM	1.5 mM	6 mM
CM MDA-MB-231	20.0 ± 1.5	–	23.2 ± 1.3	25.7 ± 1.2[a]	20.0 ± 1.5	14.9 ± 0.7[a]	–	11.7 ± 1.1[a]
CM MCF-7	17.3 ± 0.9	18.1 ± 1.1	–	21.3 ± 0.8[a]	17.3 ± 0.9	–	15.3 ± 0.8[a]	8.8 ± 0.5[a]

Tumor cell CM were incubated with HUVEC treated or not with NaPa or NaPaC (IC_{50}) during 4 days, and the percentage of apoptotic cells was determined with flow cytofluorimetry after Annexin-V and PI staining as described in Materials and methods. These assays were in triplicate. Results are expressed as mean ± SD.
[a]$p < 0.01$.

HUVEC treated for 4 days with a low concentration of NaPaC (0.5 or 1.5 mM), the inhibitory effect of tumor cell CM on HUVEC proliferation was almost abolished (columns 3 and 6). However, with a higher concentration of NaPaC (6 mM), this inhibition was restored and even increased (columns 4 and 7).

Compared effects of NaPa and NaPaC on breast cancer cell activities on HUVEC apoptosis

To study the mechanism of inhibition of HUVEC proliferation by breast cancer cell CM, early apoptosis marker was measured in cultures where HUVEC were incubated for four days with tumor cell CM. Results in Table 3 showed that breast cancer cell CM induced HUVEC apoptosis (about 20% apoptotic cells). Furthermore, when tumor cell CM were incubated with HUVEC treated by NaPa (15 mM), the number of pro-apoptotic HUVEC was increased by approximately 25%. In contrast, when tumor cell CM were added to HUVEC treated by NaPaC (6 mM), the number of apoptotic HUVEC decreased by about 45%. This differential effect of NaPa and NaPaC on the pro-apoptotic effect of tumor cell CM on HUVEC may explain the differences observed in HUVEC proliferation (Fig. 3).

Discussion

In this study, we compared the anti-proliferative effect of NaPa, an experimental drug under clinical trials [14–17], and NaPaC, a CMDB–NaPa conjugate, on two breast cancer cell lines: MCF-7, a non-aggressive and hormono-dependent cell line, and MDA-MB-231, an aggressive and non hormono-dependent cell line. We have found that the hybrid NaPaC molecule has higher anti-proliferative and pro-apoptotic effects than its parental molecule NaPa. Moreover, we demonstrated that NaPaC is more cytostatic than NaPa on HUVEC cells. Furthermore, the paracrine effect of breast cancer cells was investigated on primary cultured HUVEC. Interestingly, the CM from these tumor cells, which usually stimulates fibroblast cell growth, inhibited HUVEC growth under our experimental conditions.

It was previously shown that NaPa and CMDB, the parental molecules of NaPaC, have anti-proliferative and anti-angiogenic effects in breast tumor models [9–11]. In this study, we have found that NaPaC is, respectively, 20- and 3-fold more efficient than NaPa in inhibiting the proliferation of MDA-MB-231 cells and MCF-7 cells. Interestingly, NaPaC was here shown to act with a higher efficiency on the more invasive MDA-MB-231 breast cancer cells. These results could be explained by the presence of the benzylamide group of CMDB in NaPaC, which preferentially acts on tumor cell proliferation [7]. It was previously shown that CMDB has anti-proliferative and anti-tumoral activities by forming a complex with growth factors such as FGF2 and FGF4, thereby inhibiting their binding to the cell surface [9,10]. This inhibition of proliferation can be enhanced by NaPa [12] which acts by a different mechanism as it forms a complex with glutamine [18,19]. Tumor cells are particularly dependent on glutamine to synthesize DNA. This may explain why NaPa and NaPaC act preferentially on tumor cells compared to normal cells such as HUVEC.

Our present results showed that NaPaC increased the number of breast cancer cells in the G_0/G_1 phase, as also observed for NaPa [5], which could be due to a decreased expression of the cyclin D_1 [5]. However, this partial cytostatic effect could not be totally accountable for the high anti-proliferative effect of NaPa and NaPaC on breast cancer cells, suggesting that other mechanism(s) could be involved. Indeed, further results showed that NaPa and NaPaC induced early apoptosis of breast cancer cells, detected by the appearance of Annexin-V^+/PI^- cells. NaPa's pro-apoptotic effect was similar on both tumor cell lines, whereas NaPaC was 3-fold more pro-apoptotic on MDA-MB-231 than on MCF-7 cells. The NaPaC apoptotic action on MCF-7 cells was mainly due to the NaPa component, since it was observed that CMDB alone, even at high concentrations, did not induce apoptosis of breast tumor cells [12]. However, under our conditions, NaPaC was 2-fold (at IC_{50}) and 4-fold (at IC_{75}) more efficient on MDA-MB-231 cells than the parental NaPa molecule.

Interestingly, in our study we found that NaPa and NaPaC also inhibited HUVEC proliferation, but to a lesser extent than breast cancer cells. This inhibition was mainly attributed to a strong cytostatic effect with almost total disappearance of cells in S phase, although an increase in HUVEC apoptosis was measured. Thus, NaPaC was more cytostatic and less apoptotic on HUVEC cells than on MDA-MB-231 cells. These results are in agreement with our previous results showing that CMDB, the second component of NaPaC, also inhibits HUVEC cell proliferation [11], thus reinforcing the cytostatic effect of NaPa.

Taken together, our results showed that NaPaC, which is more apoptotic and cytostatic than NaPa on the most aggressive MDA-MB-231 tumor cell line, triggers the mechanisms of action of both NaPa and CMDB. Thus, NaPa is believed to modulate the synthesis and/or signal transduction of growth factors [1,20,21], whereas CMDB interacts with growth factors, changing their conformation [22] and preventing their binding to specific receptors [9,10].

Breast tumor cells have been shown to exert paracrine activities on stromal and endothelial cells. CM from breast tumor cells were shown previously to stimulate the proliferation of the endothelial cell line HMEC, an effect attributed to growth factors [9,10]. Surprisingly, our results showed that tumor cell CM inhibited HUVEC proliferation, an effect that was increased in the presence of NaPa. In contrast, the same CM stimulated NIH 3T3 cell growth under our experimental conditions (data not shown). Differently to NaPa, NaPaC at low concentrations partially reversed the inhibitory effect of tumor cell CM on HUVEC proliferation. These results could be related to differential fate of vascular endothelial growth factor (VEGF) present in tumor cell CM, under the various experimental conditions. The VEGF was detected in tumor cell CM incubated for 4 days with HUVEC in the presence of NaPaC, whereas it was not detected in the presence of NaPa (data not shown). A possible mechanism could be that the produced VEGF which binds to the CMDB component in NaPaC is protected from degradation [23], whereas VEGF is unable to react with the NaPa molecule.

In conclusion, we have demonstrated the anti-proliferative activity of NaPaC on two tumor cell lines representative of two breast tumor phenotypes, with a preferential effect on the most aggressive MDA-MB-231 cell line, and also on HUVEC endothelial cells. All together, these results suggest that NaPaC could be a strong anti-tumor and anti-angiogenic molecule for the treatment of breast cancers. Further experiments are in progress to study the anti-tumor and anti-angiogenic effects of NaPaC on MDA-MB-231 tumor xenographs in animals. Further studies should also be conducted to better understand the mechanism of mutual interactions between tumor cells and endothelial cells, especially the inhibition of HUVEC proliferation by tumor cell CM and partial reversion by NaPaC.

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