Anti-cancer effects of morphine through inhibition of tumour necrosis factor-α release and mRNA expression

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Morphine is mainly used to relieve pain in the terminal stage of cancer patients. We found that morphine has inhibitory effects on growth of various human cancer cell lines, with IC_{50} from 2.7 to 8.8 mM, and BALB/3T3 cells, with IC_{50} of 1.5 mM. Although the IC_{50} values were relatively high, we decided to study the mechanisms of anti-carcinogenic effects of morphine. Morphine inhibited activation of protein kinase C induced by teleocidin, one of the 12-O-tetradecanoylphorbol-13-acetate (TPA)-type tumour promoters (IC_{50}, 1 mM). Based on our previous evidence that tumour necrosis factor-α (TNF-α) acts as an endogenous tumour promoter on BALB/3T3 cells initiated with 3-methylcholanthrene, we found that morphine dose-dependently inhibited TNF-α release from KATO III cells (IC_{50}, 5.6 mM) and also from BALB/3T3 cells (IC_{50}, 1.3 mM) induced by okadaic acid, one of the non-TPA type tumour promoters. Moreover, morphine inhibited expression of TNF-α mRNA in BALB/3T3 cells (IC_{50}, 1.6 mM), but not expression of early response genes. Morphine may improve condition of cancer patients by suppression of tumour growth and reduction of amounts of an endogenous tumor promoter, TNF-α, in tissues. The high dosage of morphine required to induce anti-carcinogenic effects is also discussed.

Introduction

Cancer pain is one of the most excruciating problems for patients at the far-advanced or terminal stage (1). Medical use of morphine in Japan increased 25 times between 1979 and 1991 in accordance with development of adequate palliative care (2). At present, morphine is most frequently used to relieve severe cancer pain. Recently, there have been claims that the unexpectedly extended life spans of advanced cancer patients were often achieved by administration of high dosage of morphine in palliative care (F. Takeda, personal communication). Based on this evidence, we studied the possibility that morphine has theoretically inhibitory effects on cancer development through inhibition of tumour promotion or progression.

We previously reported that tumour necrosis factor-α (TNF-α*) stimulated cell transformation of BALB/3T3 cells treated with 3-methylcholanthrene and induced clonal growth of Bhas 42 cells, BALB/3T3 cells containing v-H-ras gene, indicating that TNF-α acts as an endogenous tumour promoter (3). Moreover, TNF-α is reported to be implicated in tumour invasion and metastasis (4). Like TNF-α, other cytokines, such as leukaemia inhibitory factor (LIF), interleukin-6 (IL-6) and interferon-γ (IFN-γ) were reported to play a significant role as humoral mediators of cachexia, which is a state of body weight loss, weakness and anemia in cancer patients (5). Some of these cytokines suppress lipoprotein lipase activity in adipocytes (6,7). In animals bearing cancer cell lines producing these cytokines, cachexia develops (8,9).

It is now possible to analyse additional anticarcinogenic effects of morphine, aside from analgesic, in relation to cytokine networks. We first studied growth inhibition of various human cancer cell lines and BALB/3T3 cells by morphine, because opioids were reported to inhibit growth of neuroblastoma in mice (10) and human lung cancer cell line (11). In addition, we studied whether morphine inhibits activation of protein kinase C (PKC) by teleocidin, one of the 12-O-tetradecanoylphorbol-13-acetate (TPA)-type tumor promoters. This will partly give answers to the mechanisms of anticarcinogenic effects.

Next we studied inhibition of TNF-α release from human cancer cell lines, such as KATO III cells, human stomach cancer cell line and HL-60 cells; human promyelocytic leukemia cell line, along with BALB/3T3 cells. The mechanism of TNF-α release inhibition by morphine was investigated through inhibition of TNF-α mRNA expression. This paper presents the first evidence that morphine may inhibit growth of cancer cell lines mediated through inhibition of TNF-α release and TNF-α mRNA expression, and probably resulting in improvement of cachexic state in cancer patients.

Materials and methods

Growth inhibition of cell lines by morphine

Morphine was subjected to cultures of seven human cancer cell lines and BALB/3T3 cells. Human cancer cell lines were: PC-9 lung cancer cell line provided by Dr Nagahide Saigo at the National Cancer Center Research Institute, Tokyo; HL-60 cells, a promyelocytic leukemia cell line; neuroblastoma cell line established from neuroblastoma of an infant and provided by Dr Yasuhiko Kaneko at Saitama Cancer Center (12); KATO III cells, stomach cancer cell line and SEKI cells, malignant melanoma cell line, provided by the Japanese Cancer Research Resources Bank, Tokyo; SKNO-1 cells, acute myeloblastic leukemia cell line, provided by Dr Masayoshi Tsuusumi at the Center for Molecular Biology and Cytogenetics, Tokyo (13); U 251 cell, glioma cell line, purchased from RIKEN Cell Bank. All cell lines were maintained in a humidified incubator with a 5% CO_{2} atmosphere in air at 37°C. For the PC-9 cells, HL-60 cells, KATO III cells, SEKI cells, U 251 cells and SKNO-1 cells, exponentially growing cells (2 x 10^5 cells/ml) were seeded in growth medium containing various concentrations of morphine HCl (Sankyo Co., Ltd). With each usage, morphine HCl was dissolved into sterile water. Neuroblastoma cells and BALB/3T3 cells (1 x 10^5 cells/ml) were maintained in 0.1 ml medium for 24 h, and the cells were treated with various concentrations of morphine. Both morphine and medium were replaced after 3 days of incubation. After incubation for the indicated period, the number of cells was determined by trypan blue staining or 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The optical density of formazan crystals dissolved in 200 μl DMSO was measured at 550 and
Fig. 1. Inhibition of growth of human cancer cell lines by morphine. (A) PC-9, (B) HL-60 and (C) neuroblastoma cell line. Control (O), 1 mM (●), 2 mM (△), 4 mM (▲) and 8 mM (□) of morphine concentration. The experiments were conducted three times.

![Graph showing growth inhibition of cell lines by morphine](image)

Fig. 2. Inhibition of PKC activation by morphine. Various concentrations of morphine were added to the assay mixture including 2.2 μM teleocidin, phosphatidylserine and Ca\(^{2+}\). PKC activity was expressed as percentage of \(^{32}\)P-incorporation into histone H1.

![Graph showing PKC activation inhibition](image)

Table I. Growth inhibition of cell lines by morphine

<table>
<thead>
<tr>
<th>Cell line</th>
<th>IC(_{50}) (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC-9</td>
<td>4.8</td>
</tr>
<tr>
<td>HL-60</td>
<td>2.7</td>
</tr>
<tr>
<td>Neuroblastoma</td>
<td>3.3</td>
</tr>
<tr>
<td>KATO III</td>
<td>8.8</td>
</tr>
<tr>
<td>SEKI</td>
<td>4.2</td>
</tr>
<tr>
<td>U 251</td>
<td>3.4</td>
</tr>
<tr>
<td>SKNO-1</td>
<td>3.8</td>
</tr>
<tr>
<td>BALB/3T3</td>
<td>1.5</td>
</tr>
</tbody>
</table>

\(^{4}\text{IC}_{50}\), concentration for 50% cell growth inhibition.

Inhibitory effects of morphine on TNF-α mRNA expression

BALB/3T3 cells in semi-confluent condition were treated with various concentrations of morphine dissolved in 4.5 ml medium. After 1 h, 200 nM okadaic acid was added and made 5.0 ml medium. KATO III cells (4 \(\times 10^5\) cells/ml) were first treated with various concentrations of morphine. One hour later, cells were treated with 50 nM okadaic acid. Eight hours after treatment with okadaic acid, total RNA was isolated from BALB/3T3 cells and KATO III cells. Samples of 1 μg total RNA were subjected to reverse transcription with the reverse transcriptase of murine leukaemia virus at 37°C for 1 h. cDNA was amplified using an RNA polymerase chain reaction (PCR) kit (Roche Molecular Systems Inc., USA) as described previously (15). The amount of TNF-α mRNA was analysed by BAS 2000 image analyser and normalised by glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA as control. TNF-α mRNA was expressed in relative terms compared with that of non-treated control.

Effects of morphine on jun B gene expression in BALB/3T3 cells

BALB/3T3 cells were treated with morphine and okadaic acid as described above. Total RNA was isolated from BALB/3T3 cells, and 20 μg total RNA for each sample was electrophoresed and blotted onto a nylon membrane. Northern blot analysis was performed by using α-\(^{32}\)P labelled cDNA probes. cDNAs of jun B and GAPDH were obtained as reported previously (16).

Results

Growth inhibition of cell lines by morphine

Morphine inhibited growth of all seven human cancer cell lines. Figure 1 shows growth inhibition of PC-9, HL-60 and
Anti-cancer effects of morphine

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Concentration of morphine ( mM

Fig. 3. Inhibition of TNF-α release from BALB/3T3 cells (A) and KATO III cells (B) by morphine. BALB/3T3 cells were treated with 200 nM okadaic acid. KATO III cells were treated with 50 nM okadaic acid. After incubation for 24 h, TNF-α released into the medium was determined with an enzyme linked immunosorbent assay kit. TNF-α levels are expressed as a percentage relative to morphine untreated control. Values are means of triplicate assays.

Inhibition of PKC activation by morphine

Figure 2 shows that morphine dose-dependently inhibited PKC activation induced by 2.2 μM teleocidin. The concentration for 50% inhibition was 1 mM. In this experiment, we found that morphine directly inhibited PKC activation induced by a tumour promoter in vitro. At higher concentrations of morphine than shown here, PKC activation was inhibited to the basal level (data not shown). This suggested that the inhibitory effect of morphine was induced by a direct interaction of morphine with PKC as well as with enzyme complex including phosphatidylserine and a tumour promoter.

Inhibition of TNF-α release from BALB/3T3 cells and KATO III cells induced by okadaic acid

Figure 3 shows morphine dose-dependently inhibited TNF-α release from BALB/3T3 cells and KATO III cells induced by okadaic acid. Their IC<sub>50</sub> values were 1.3 and 5.6 mM, respectively. Morphine also dose-dependently inhibited TNF-α release from HL-60 cells induced by 10 nM TPA (data not shown).

Inhibition of TNF-α mRNA expression

To determine the mechanism of TNF-α release inhibition by morphine, we studied whether TNF-α mRNA expression is inhibited by morphine. In the case of BALB/3T3 cells, morphine dose-dependently inhibited TNF-α mRNA expression induced by 200 nM okadaic acid (Figure 4), but it did not inhibit jun B mRNA expression (Figure 5). The IC<sub>50</sub> value was 1.6 mM, similar to that of TNF-α release inhibition. Thus, inhibition of TNF-α release by morphine appears to be caused by inhibition of mRNA transcription.

Discussion

Morphine has various actions at the transcription and translation level depending on the cells. For example, morphine inhibited TNF-α mRNA expression, but did not inhibit expression of early response genes, such as jun B gene in BALB/3T3 cells treated with okadaic acid. In KATO III cells treated with okadaic acid, morphine inhibited TNF-α release from the cells, but did not inhibit TNF-α mRNA expression. It has been reported that a variety of human and animal tumors have specific opioid receptors (17). The effective concentration of our dosage is relatively high, although we did not examine the number of opioid receptors of these cell lines. It is suggested that these effects of morphine may not be mediated through opioid receptors, but direct effects. Although TNF-α was originally found to cause haemorrhagic necrosis in transplanted solid tumours (18), TNF-α is now known to have pleiotropic effects (19), such as cell proliferating activity (20), tumour invasion and metastasis (4). Interestingly, the dose-response curves of morphine for inhibition of TNF-α release from BALB/3T3 cells was almost the same as that of inhibition of PKC activation. We think that morphine contributes to improvement of patient condition mediated through inhibition of TNF-α release and inhibition of signal transduction.

We also found that TNF-α released a cytokine, LIF from KATO III cells and this LIF release was inhibited by morphine (data not shown). LIF is known as a mediator of cachexia, just as TNF-α is. It has been reported that anti-TNF-α antibody attenuates development of cachexia (21), but it is still difficult to use anti-TNF-α antibody for cachexia patients. In other words, morphine is a more suitable compound than anti-cytokine antibodies for easing cachectic state in cancer patients by inhibiting the amounts of TNF-α and LIF in the tissues. This would make it possible to unexpectedly extend the lifespan of patients.

As for the dosage of morphine, growth inhibition of cancer cells was obtained by high concentrations in in vitro experiments. For our preliminary in vivo experiments, morphine was...
Fig. 4. Effects of morphine on TNF-α mRNA expression in BALB/3T3 cells (left) and KATO III cells (right). BALB/3T3 cells and KATO III cells were first treated with morphine. One hour later, 200 nM and 50 nM okadaic acid were added in BALB/3T3 cells and KATO III cells, respectively; morphine alone (○) and morphine plus okadaic acid (●). After 8 h, total RNA was isolated from cells and TNF-α mRNA expression was determined by RT-PCR.

Fig. 5. Effects of morphine on jun B gene expression in BALB/3T3 cells. First, BALB/3T3 cells were treated with morphine. After incubation for 1 h, 200 nM okadaic acid was added in BALB/3T3 cells. Total RNA was isolated from cells after 8 h and jun B gene expression was detected by Northern blot analysis.

Injected into mouse at a concentration of 20 mg/kg/day, resulting in a reduction of tumour volume in mouse subcutaneously inoculated using colon 26 cells (data not shown). The plasma concentration of morphine in such cases is estimated at 8 μM according to available literature (22). For cancer patients, morphine is administered in concentrations of from 60 to 200 mg/day (1). The morphine concentration in plasma of these patients has been reported to be as high as 3 μM (23).

The metabolites of morphine, such as morphine-6-glucuronide and morphine-3-glucuronide, are also active analgesics (24). It is reported that morphine-6-glucuronide, in particular, is 20 times as potent as ordinary morphine, and the amounts of morphine-6-glucuronide in plasma are sometimes 20-fold concentrations than those of morphine (23). It is possible that these metabolites of morphine can strongly inhibit cytokines release in vivo and may contribute to inhibition of cachexia in patients. In addition to the standard use of morphine as an analgesic, we think potential contribution of morphine to palliative care warrants further study.

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References


