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# Decreased analgesic effect of morphine, but not buprenorphine, in patients with advanced P-glycoprotein$^+$ cancers

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**Abstract:**
**Background:** P-glycoprotein (P-gp) is expressed on the blood-brain barrier (BBB) and acts as a transporter regulating the analgesic effect of morphine. The P-gp is also expressed by different types of tumors. The aim of this study was to determine the potential association of the P-gp expression in malignant tumors with analgesic effects in patients.
**Methods:** The P-gp expression in 120 malignant tumors was examined by immunohistochemistry. The analgesic responses of individual patients to morphine and buprenorphine (BNP) were evaluated by visual analog scale (VAS). The levels of plasma morphine and BNP were determined by HPLC.
**Results:** We found that there was no significant difference in the values of VAS between patients with P-gp$^+$ and P-gp$^-$ malignant tumors in responses to 0.000025 g × kg$^{-2}$ of BNP administered by patient-controlled intravenous analgesia (PCIA), accompanied by similar levels of plasma BNP in those patients. In contrast, the values of VAS in response to 0.00075 g × kg$^{-2}$ of morphine in patients with P-gp$^+$ tumors were significantly greater than those in the patients with P-gp$^-$ tumors, although similar levels of plasma morphine were detected in both groups of patients. Furthermore, treatment with a higher dose **(**0.0011 g × kg$^{-2}$**)** of morphine effectively controlled pain in those with P-gp$^+$ tumors.
**Conclusion:** Our data indicated that patients with P-gp$^+$ tumors required a higher dose of morphine to achieve an analgesic effect and that the P-gp expression in tumors may be valuable for predicting the analgesic responses of patients with severe pain to morphine.

**Key words:**
analgesia, buprenorphine, morphine, pain, P-glycoprotein

**Abbreviations:** BBB – blood-brain barrier, BNP – buprenorphine, PCIA – patient-controlled intravenous analgesia, P-gp – P-glycoprotein, VAS – visual analog scale

## Introduction

Approximately, 75% of patients with advanced cancer suffer with an ever-troublesome symptom of pain. Failure to relieve pain can lead to unnecessary consequences, decreased activity, anorexia, and sleep deprivation [6]. Opioid analgesics are generally used for the treatment of acute and chronic pain, but the efficacies of analgesics are widely variable in the clinic [16, 17, 19]. However, little is known on what factors contribute to the variable responses of individual patients to analgesic medicines.

A recent study has shown that inherited differences in the expression levels of drug-metabolizing enzy-

mes, drug-transporters, and/or opioid receptors (μ, δ, and κ-opioid) in the tumor tissues may affect the effectiveness of opioid analgesics in individual patients [12]. Previous studies have indicated that transport of morphine across the blood-brain barrier (BBB) seems to be the most important step for its analgesic effect [22, 30]. Notably, the permeability glycoprotein (P-gp) is an ATP-dependent efflux transporter and the P-gp is expressed in a variety of tissues, including capillary epithelial cells on the luminal membrane of the BBB as well as different types of tumors. The P-gp is a critical regulator of the pharmacokinetics of opioid medicines because the P-gp can mediate the efflux of opioid medicines, reducing the accumulation and analgesic effect of opioids in the brain [3, 8, 9, 11, 23, 26, 28].

Morphine, a potent natural opiate, has been commonly used for the treatment of patients with tumor-related severe pain in the clinic. Although morphine can permeate through the BBB, the efficacy in the penetration and accumulation of morphine in the brain is rather limited. A previous study has shown that morphine and other pharmacologically active agents, such as cyclosporin A, digoxin and terfenadine, are substrates of the drug efflux pump P-gp [2]. Evidentially, increased analgesic effect of morphine was observed in P-gp$^{-/-}$ mice [28]. Furthermore, treatment with a P-gp inhibitor altered the BBB permeability of morphine, and the P-gp may participate in the transport of morphine across the BBB [18, 32]. Morphine and fentanyl can activate the P-gp ATPase on the membranes of brain capillary endothelial cells, which in turn regulate the antinociceptive potencies of these opioids [7]. Interestingly, Wakako et al. [31] have shown that the analgesic effects of morphine are negatively correlated with the levels of P-gp expression and basal P-gp ATPase activities in the mouse brain. Unfortunately, it is very difficult to detect the P-gp expression on the BBB of humans. Notably, the P-gp is expressed in many types of malignant tumors. However, whether the P-gp expression in the tumor tissues could be associated with the analgesic effect of morphine in patients with advanced malignant tumors has not been explored.

Buprenorphine (BNP), an opioid agonist/antagonistic analgesic, is widely used for the control of either acute postoperative pain or the moderate to severe terminal cancer pain in the clinic [10, 14, 24]. BNP can readily pass through the BBB of rats [20, 21]. Although the P-gp can transport lipohilic and cationic compounds [25], whether the expression of P-gp in malignant tumors could be associated with the responses of patients to analgesic BNP is unknown.

In this study, we examined the analgesic effects and the concentrations of plasma morphine and BNP in patients with advanced P-gp$^+$ or P-gp$^-$ malignant tumors following treatment with morphine or BNP. We found significantly reduced analgesic effects of morphine, but not BNP, in patients with advanced P-gp$^+$ malignant tumors. We discussed the implications of our findings.

## Materials and Methods

### Patients

A total of 163 cancer patients at a terminal stage were referred consecutively to the Palliative Care Unit, the People's Hospital of Wuxi for pain relief and cancer-related symptoms from January 1, 2005 to December 1, 2010. Written informed consent was obtained from individual patients or their close relatives, and the study was approved by the Ethics Committee of Nanjing Medical University. Patients with malignant tumors were diagnosed by histological examination, and all of the patients received chemotherapy and/or surgical treatment. These patients suffered with malignant tumors, predominantly including esophageal cancer, lung cancer, gastric cardia cancer, breast cancer, colon cancer and rectum cancer. Individual patients with histologically confirmed malignant tumors at stage IV, able to communicate effectively with the healthcare providers, regardless of previous chemotherapy and surgical treatment, were included. On the other hand, individual tumor patients with opioid intolerance, previous usage of strong opioids, severe renal or hepatic dysfunction, predominantly neuropathic pain, or breakthrough pain; or individuals who needed neural block or neuroablative treatment for pain relief, with impaired sensory or cognitive function, with coma or other mental disorders were excluded.

### Immunohistochemistry

Cancerous specimens were obtained from individual patients following chemotherapy either through biopsy or surgery and fixed in 10% neutralized formalin overnight, followed by paraffin-embedded. The tissue

sections at 5-μm sections were deparaffinized and rehydrated, and the expression of P-gp in the tumor tissue sections were characterized by immunohistochemistry by the avidin-biotin complex (ABC) technique using a specific kit, according to the manufacturer's instructions (Labvision, Kalamazoo, USA). Briefly, individual tissue sections were probed with mouse anti-human P-pg monoclonal antibody (Maxim Biomedical, Rockville, USA) or control mouse IgG at 4°C overnight, and after washing, the sections were incubated with biotinylated rabbit-anti-mouse IgG at room temperature for 2 h. Subsequently, the bound antibodies were detected with horseradish peroxidase (HRP)-conjugated ABC and visualized by 3,3'-diaminobenzidine (DAB), followed by imaging under a light microscope. Two known positive and negative sections were used as controls to ensure the quality of experiments. The intensity of anti-P-gp staining was analyzed semi-quantitatively, and the percentage of positively staining cells in a given area selected randomly from individual sections was determined in a blinded manner. Specimens with fewer than 10% of positively staining cells were classified as (–), and those with 10% and more than 10% were classified as (+).

### Analgesia

Individual patients were randomized into different groups of $M_1$, $M_2$, $B_1$ and $B_2$, according to the status of P-gp expression in their tumor tissues and their demographic characteristics. The $M_1$ and $B_1$ groups of patients had P-gp$^+$ tumors, while the $M_2$ and $B_2$ groups of patients had P-gp$^-$ tumors. All patients with pain due to surgery, tumor progression, or metastases were initially treated orally with 0.05 g of diclofenac sodium suppositories (Jiangsu Yuan Heng Pharmaceutical Company, Nanjing, China) every 8–12 h, 0.1 g of sustained-releasing tramadol hydrochloride (Beijing Adorable Pedicle Pharmaceutical Company, Beijing, China), or 0.03 g of sustained-releasing morphine hydrochloride (Southwest Pharmaceutical Company, Chongqing, China) every 12 h. Individual patients, who still suffered with unsustainable pain, received a patient-controlled intravenous analgesia (PCIA) pump (Dragon Medical Device, Zhangjiagang, China). Individual patients were educated about the pain management strategies and assessment as well as the medicinal pump operation. After the patients were demonstrated to control the medicinal pump effectively, they were provided with medicines for analgesia.

The $M_1$ and $M_2$ groups of patients received a load dose of 0.0025 g morphine (Shenyang Pharmacological Company, Shenyang, China), while the $B_1$ and $B_2$ groups of patients were treated with a load dose of 0.00015 g BNP (Tianjing Pharmacological Company, China). Subsequently, the patients in the $M_1$ and $M_2$ groups were provided with the PCIA solution containing 0.00075 g × kg$^{-2}$ morphine and 0.01 g azasetron in 100 ml of saline with consistent transfusions of 2 ml per h, self-adjusted with 0.5 ml of PCA solution and a lock time of 20 min. The $B_1$ and $B_2$ groups of patients were treated with 0.000025 g × kg$^{-2}$ BNP and 0.01 g azasetron in 100 ml of saline with the same condition as the $M_1$ and $M_2$ groups of patients. One week later, due to the poor responses, the $M_1$ group of patients received 0.0011 g × kg$^{-2}$ morphine and 0.01 mg azasetron using the same treatment condition.

### Evaluation of analgesic

Before assessment, individual patients were educated about the procedure of visual analog scale (VAS) test. Patients from different groups were tested for the analgesic effect before treatment ($T_0$) and 4 h ($T_1$), 12 h ($T_2$), 24 h ($T_3$), and 36 h ($T_4$) post initial analgesia in a double blinded manner using VAS: 0 (no pain feeling and highly satisfactory); 1–2 (satisfactory), 3–5 (primary satisfactory), 6–7 (primary unsatisfactory), 8–9 (unsatisfactory), and 10 (the utmost pain and highly unsatisfactory). During the measurement, their blood pressure, heart rate and pulse oxygen saturation were monitored and recorded. Individual patients were kept awake, alert, and breathing spontaneously with a ventilatory frequency of 12–20 breaths per min and a pulse oximetry reading ($SpO_2$) of at least 90% (with or without supplemental oxygen). All patients were able to be interviewed and to address the questions of examiners.

### Detection of the concentrations of morphine and BNP

Blood samples were obtained from individual patients at 4 h ($T_1$), 12 h ($T_2$), 24 h ($T_3$), and 36 h ($T_4$) post initial analgesia, and their plasmas were prepared by centrifuging at 2,700 × g for 10 min. The concentrations of plasma morphine or BNP in individual patients at each time point after treatment were deter-

mined by HPLC/MS (Agilent, Santa Clara, USA). Individual plasma samples (200 µl each) were mixed with 1.0 ml of diethyl ether and vortexed for 2 min, followed by centrifuging at 2,700 × g for 10 min. After carefully extracting the organic layer, the organic layer was evaporated to dryness. Subsequently, the residues were re-dissolved in 100 µl of acetonitrile-water, analyzed in duplicate by chromatography using an acetonitrile-water (70:30 v/v) solvent system at a flow rate of 0.6 ml/min, and then characterized by an MS analyzer. A known concentration of morphine or BNP plasma sample was used for the validation of specificity, linearity, precision, accuracy, extraction recovery and stability of this assay.

### Statistical analysis

Data are expressed as the mean ± SD, median (range), or real values. Qualitative data were analyzed by Fisher exact test, and continual data were analyzed by analysis of variance (ANOVA) among the different time points in the same analgesia group and paired-Student's *t*-test between groups. A p value of $< 0.05$ was considered statistically significant.

## Results

To determine the analgesic effects of morphine and BNP on patients with advanced malignant tumors, a total of 163 patients with different types of malignant tumors at advanced stages were screened, and 43 patients did not meet the criteria (14 subjects declined participation, 11 received adjuvant chemotherapy, 9 with elevated levels of serum transaminase and jaundice, and 9 with respiratory tract infection). The remaining 120 patients were recruited, and their tumor tissues were characterized for the P-gp expression by immunohistochemistry (Fig. 1). There were 60 tumor samples that were positive for anti-P-gp staining and other 60 tumor samples were negative. These patients were randomly divided into the M1, M2, B1 and B2 groups, and further stratified, according to the tumor type, the status of P-gp expression, and their demographic characteristics. As shown in Table 1, there was no significant difference in the distribution of patients in terms of their demographic and clinical characteristics, as well as tumor types and the pathological stages among these groups of patients.

Furthermore, there was no significant difference in the basal VAS score (Tab. 2), Analysis of analgesic responses of different groups of patients revealed that following treatment with BNP, the values of VAS in both the $B_1$ and $B_2$ groups of patients decreased, and were undistinguishable between these two groups of patients. Interestingly, patients with P-gp$^+$ malignant tumors responded poorly to morphine. Evidentially, although the values of VAS decreased in the $M_1$ group of patients who had received 0.00075 g × kg$^{-2}$ of morphine, but were significantly higher than those in the $M_2$ group of patients who had P-gp$^-$ malignant tumors and had received the same dose of morphine. More importantly, following treatment with a higher dose of morphine (0.0011 g × kg$^{-2}$), the values of VAS in the $M_1$ group of patients decreased and were not significantly different from those in the $M_2$ group of patients who received a lower dose of morphine. These data indicated that patients with P-gp$^+$ malignant tumors required a higher dose of morphine to achieve an analgesic effect.

Further analysis of the concentrations of plasma morphine and BNP indicated that there were similar levels of plasma BNP in both the $B_1$ and $B_2$ groups of patients, regardless of the expression of P-gp and

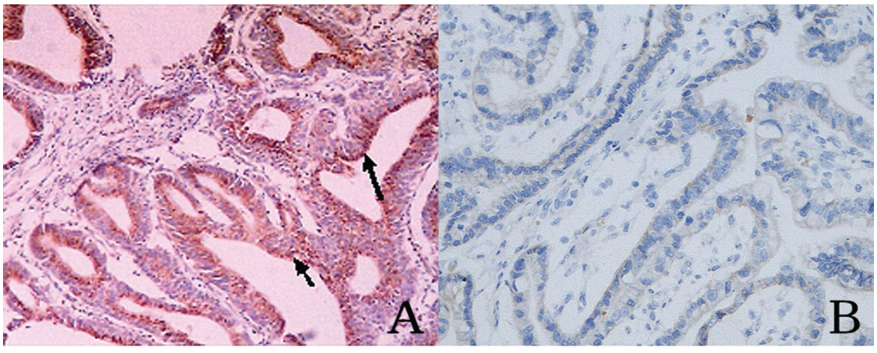


**Fig. 1.** Immunohistochemistry analysis of P-gp expression in tumor tissues. The tumor tissue sections (5 µm) were deparaffinized, rehydrated, and stained with monoclonal anti-human P-gp, followed by visualization using the avidin-biotin complex (ABC) and DAB. Data shown are representative images (magnification 200×) from the P-gp$^+$ and P-gp$^-$ tumors (n = 60 per group). (**A**) The P-gp$^+$ tumors; (**B**) the P-gp$^-$ tumors. The arrows indicate anti-P-gp staining in tumor tissues

**Tab. 1.** The demographic and clinical characteristics of patients

| Characteristics | Group $M_1$ | Group $M_2$ | Group $B_1$ | Group $B_2$ | p value |
|---|---|---|---|---|---|
| Number of subjects | 30 | 30 | 30 | 30 | 0.05 |
| Expression of P-gp | + | – | + | – | |
| Age (years) | 57.5 ± 10.4 | 57.2 ± 11.1 | 57.4 ± 10.2 | 57.5 ± 9.7 | > 0.05 |
| (range) | (51 ~ 64) | (52 ~ 63) | (54 ~ 64) | (53 ~ 65) | |
| Weight (kg) | 59.1 ± 13.8 | 58.7 ± 14.1 | 58.9 ± 13.0 | 58.7 ± 12.7 | > 0.05 |
| (range) | (56 ~ 63 ) | (55 ~ 64 ) | (54 ~ 64) | (55 ~ 64 ) | |
| Sex (male/female) | 21/9 | 20/10 | 22/8 | 21/9 | > 0.05 |
| Type of cancer | | | | | |
| Esophageal cancer | 6 | 7 | 8 | 8 | > 0.05 |
| Cardia cancer | 5 | 4 | 3 | 3 | > 0.05 |
| Breast cancer | 4 | 5 | 5 | 4 | > 0.05 |
| Lung cancer | 7 | 7 | 7 | 7 | > 0.05 |
| Colon cancer | 4 | 3 | 3 | 4 | > 0.05 |
| Rectum cancer | 4 | 4 | 4 | 4 | > 0.05 |
| Education | | | | | |
| None | 0 | 0 | 0 | 0 | > 0.05 |
| Primary | 1 | 0 | 1 | 1 | > 0.05 |
| Junior high | 3 | 4 | 4 | 3 | > 0.05 |
| High school | 18 | 17 | 16 | 18 | > 0.05 |
| College or university | 8 | 9 | 9 | 8 | > 0.05 |

indistinguishable levels of morphine in both the $M_1$ and $M_2$ groups of patients following transfusion with a lower dose of morphine (Tab. 3). Following treatment with a higher dose of morphine, the concentrations of morphine in the $M_1$ group of patients, as expected, were obviously higher than those in the same group of patients with a lower dose of morphine. These data further indicated that patients with P-gp$^+$ malignant tumors need a higher dose of morphine for the control of their severe pain in the clinic.

**Tab. 2.** The VAS scores of different groups of patients

| Groups | $T_0$ | $T_1$ | $T_2$ | $T_3$ | $T_4$ |
|---|---|---|---|---|---|
| $M_1$ (0.75 mg/kg) | 8.0 ± 1.5 | 4.1 ± 2.4* | 4.4 ± 1.9* | 4.3 ± 1.6* | 4.3 ± 2.3* |
| $M_2$ | 8.1 ± 1.7 | 1.7 ± 1.1*$^{\Delta}$ | 1.8 ± 1.6*$^{\Delta}$ | 1.9 ± 1.4*$^{\Delta}$ | 1.8 ± 1.4*$^{\Delta}$ |
| $M_1$ (1.1 mg/kg) | 8.0 ± 1.7 | 1.8 ± 1.4*$^{\Delta}$ | 1.8 ± 1.9*$^{\Delta}$ | 1.7 ± 1.6*$^{\Delta}$ | 1.9 ± 1.8*$^{\Delta}$ |
| $B_1$ | 7.8 ± 1.6 | 1.5 ± 0.9* | 1.6 ± 0.7* | 1.4 ± 0.7* | 1.5 ± 1.0* |
| $B_2$ | 7.9 ± 1.2 | 1.6 ± 0.8* | 1.5 ± 1.0* | 1.4 ± 0.9* | 1.4 ± 1.1* |

Data are expressed as the mean ± SD of each time from each group of patients (n = 30 per group); * $p < 0.05$ *vs.* the values of $T_0$; $^{\Delta}$ $p < 0.05$ *vs.* the values of other groups at the same time point

**Tab. 3.** The concentrations of plasma morphine and BNP

| Groups | $T_1$ | $T_2$ | $T_3$ | $T_4$ |
|---|---|---|---|---|
| $M_1$ (0.75 mg/kg) | 82.3 ± 5.1 | 81.6 ± 5.7 | 84.0 ± 5.6 | 83.9 ± 5.5 |
| $M_2$ | 81.0 ± 4.7 | 78.9 ± 5.1 | 81.6 ± 5.1 | 82.8 ± 6.2 |
| $M_1$ (1.1 mg/kg) | 135.6 ± 6.2$^\Delta$ | 143.9 ± 7.7$^\Delta$ | 137.2 ± 9.1$^\Delta$ | 141.1 ± 7.9$^\Delta$ |
| $B_1$ | 0.18 ± 0.07 | 0.18 ± 0.07 | 0.19 ± 0.06 | 0.18 ± 0.06 |
| $B_2$ | 0.17 ± 0.06 | 0.17 ± 0.05 | 0.18 ± 0.06 | 0.18 ± 0.05 |

Data are expressed as the mean ± SD of ng/ml of plasma opioid from 30 subjects in each group at each time point; $^\Delta$ $p < 0.05$ *vs.* the values of other groups at the same time point

## Discussion

The P-gp acts as an energy-dependent efflux pump, which transports a wide array of structurally divergent compounds, including opioid analgesics, from the intracellular to the extracellular compartment [1]. There is clear evidence that the levels of P-gp expression in animal brains are related to the analgesic effects of some opioids [3, 8, 9, 11, 23, 26, 28]. However, due to the technical difficulty to detect P-gp expression in human brains, the influence of P-gp expression in the brain on the pharmacokinetics and central pharmacodynamics of opioids in humans is unknown. Previous studies have shown that the P-gp is expressed on different types of malignant tumors [13, 15, 29]. In this study, we tested the efficacy of two analgesics in patients with P-gp$^+$ or P-gp$^-$ malignant tumors. We found that patients with P-gp$^+$ or P-gp$^-$ malignant tumors had similar analgesic responses to BNP, but those patients displayed significantly different analgesic responses to morphine. Evidentially, the values of VAS in patients with P-gp$^+$ malignant tumors were significantly greater than those with P-gp$^-$ malignant tumors following treatment with a lower dose of morphine. Furthermore, treatment of the patients, who had poor responses to lower doses of morphine, with a higher dose of morphine achieved analgesic effect, which was similar to that in the low dose of morphine-treated patients with P-gp$^-$ malignant tumors. The poor responses to low dose of morphine in the patients with P-gp$^+$ malignant tumors unlikely came from the rapid metabolism of morphine because the concentrations of plasma morphine in those patients were similar to that in the P-gp$^-$ malignant tumor patients who achieved analgesic effect. Rather, these data indicated that patients with P-gp$^+$ malignant tumors required a higher dose of morphine to achieve analgesic responses. These novel findings suggest that the P-gp expression in tumors may be valuable for predicting the responses of individual patients to analgesics in the clinic.

Pharmacokinetic studies have shown that intramuscularly injected morphine predominantly distributes to visceral parenchymal organs and muscle tissues in humans, but only a little of the morphine can pass through the BBB. Given that morphine has very high affinity to its opioid receptors, the small amount of morphine can have potent analgesic effect [4, 5, 27]. It is possible that high levels of P-gp are also expressed in the lumenal borders of several normal tissues, including human brain capillary endothelium, which is a vital component of the BBB of patients with P-gp$^+$ malignant tumors. The P-gp would act as a transporter, leading to the efflux of morphine and reducing its analgesic effect in those patients, particularly when they received a low dose of morphine. Indeed, treatment with a higher dose of morphine to reach a significantly higher concentration of plasma morphine effectively relieved the pain in the patients with P-gp$^+$ malignant tumors, suggesting that following treatment with a higher dose of morphine, sufficient amount of morphine reached the brain and achieved analgesic effect in those patients. Because there was no significant difference in the responses to BNP between patients with P-gp$^+$ tumors and those with P-gp$^-$ tumors, the P-gp may be inefficient in the efflux of BNP in those patients. Apparently, our findings may provide a new base for the design of analgesic therapy for advanced tumor patients with severe pain in the clinic.

In summary, our data indicated that there was significant difference in analgesic responses to morphine, but not BNP, between patients with P-gp$^+$ and P-gp$^-$ malignant tumors and that patients with P-gp$^+$ tumors required a higher dose of morphine to achieve analgesic effect. Hence, the P-gp expression in tumors may be valuable for predicting the analgesic responses of individual patients with advanced malignant tumors. Our findings may aid in the design of analgesic treatment of patients with P-gp$^+$ malignant tumors.

**Conflicts of interest:**
The authors declare no conflicts of interest.

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