NK-1 Receptor Antagonists: A New Generation of Anticancer Drugs

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Abstract: After binding to the neurokinin-1 (NK-1) receptor, substance P (SP) induces tumor cell proliferation, angiogenesis, and the migration of tumor cells for invasion and metastasis. After binding to NK-1 receptors, NK-1 receptor antagonists inhibit tumor cell proliferation, angiogenesis and the migration of tumor cells. These antagonists are broad-spectrum antitumor drugs. In addition, in the host they display beneficial effects: anxiolytic, antiemetic, neuroprotector, nephroprotector, hepatoprotector, antiinflammatory and analgesic. In combination therapy with classic cytostatics, NK-1 receptor antagonists have synergic effects and minimize the side-effects of these classic drugs. Thus, NK-1 receptor antagonists could offer a new and promising generation of anticancer drugs.

Keywords: Substance P, neoangiogenesis, metastasis, tumor cells, apoptosis.

INTRODUCTION

There is a considerable body of data suggesting that the substance P (SP)/neurokinin (NK)-1 receptor system plays an important role in cancer: the presence of SP and NK-1 receptors has been reported in a wide variety of tumors; SP , through the NK-1 receptor, induces tumor proliferation; NK-1 receptors are involved in the viability of tumor cells (Fig. **1**), and the tachykinin 1 (*TAC1R*) gene is overexpressed in tumor cells [1-23]. It is not only SP that is associated with cancer; other peptidergic systems are also involved. Receptors for several peptides (e.g., galanin, somatostatin) have been described in many types of tumor [24, 25]. However, it should be remarked that glioblastoma and astrocytoma tumors expressing the most malignant phenotypes show an increased percentage of NK-1 receptor expression and that normal human cells express a lower number of NK-1 receptors than tumoral human cells (e.g., mRNA NK-1 receptor expression is increased in malignant tissues in comparison with benign tissues) [13, 26-28]. Thus, advanced tumor stages could exhibit significantly higher NK-1 receptor levels and the expression of NK-1 receptors could be correlated with the degree of malignancy. This means that the SP/NK-1 receptor system is a good candidate to test the action of NK-1 receptor antagonists as antitumoral agents and that the NK-1 receptor could be a new target in cancer treatment aimed at blocking tumor growth [3, 7, 22]. The overexpression of NK-1 receptors by tumor cells also suggests a specific treatment against these cells using NK-1 receptor antagonists, in this case considerably decreasing the possible side-effects of the treatment. This is quite important, since one of the goals of cancer therapy should be to cure the disease with none or fewer side-effects than the side-effects exerted by cytostatic drugs. These drugs used in chemotherapy show a very low safety profile and induce very severe clinical side-effects because they are not specific against tumor cells. The overall contribution of curative and adjuvant cytotoxic chemotherapy to 5-year survival in adults was respectively estimated to be 2.3% and 2.1% in Australia and in the USA [29]. That is, the short-medium-term perspectives are not very promising for oncologic patients and hence new pathways must be opened up since the discoveries in certain fields of enquiry (e.g., stem cell research, the search for new cytostatic agents) have not been fully successful. Thus, NK-1 receptor antagonists represent an important opportunity for exploitation as a new, promising generation of broad-spectrum anticancer drugs [1-9, 15-17, 19, 30] (Fig. (**1**); Table **1**).

SUBSTANCE P AND THE NEUROKININ-1 RECEPTOR PROMOTE CANCER

SP is an undecapeptide belonging to the tachykinin family of peptides. SP and NK-1 receptors have a widespread distribution throughout the body; SP is derived from the preprotachykinin A gene and the biological actions of SP are preferentially mediated by the NK-1 receptor [31-36]. SP has been implicated in a wide range of biological functions, such as the regulation of the cardiovascular system, gastric motility, inflammation, immunological action, stress, emesis, pain and depression [35, 37-45].

In breast cancer, an increased expression of preprotachykinin A in comparison with normal mammary epithelial cells has been observed [26]. The expression of SP has been reported in metastatic neuroblastoma cells, malignant glioma/retinoblastoma, metastatic melanomas, primary invasive malignant melanomas, *in situ* melanomas, keratocystic odontogenic tumors, and in oral squamous cell and larynx carcinomas [2, 10-12, 30, 46, 47] (Fig. **1**). In the three latter tumors, SP-immunoreactivity was found in both the cytoplasm and in the nucleus of tumor cells (the involvement of SP as a transcription factor should be

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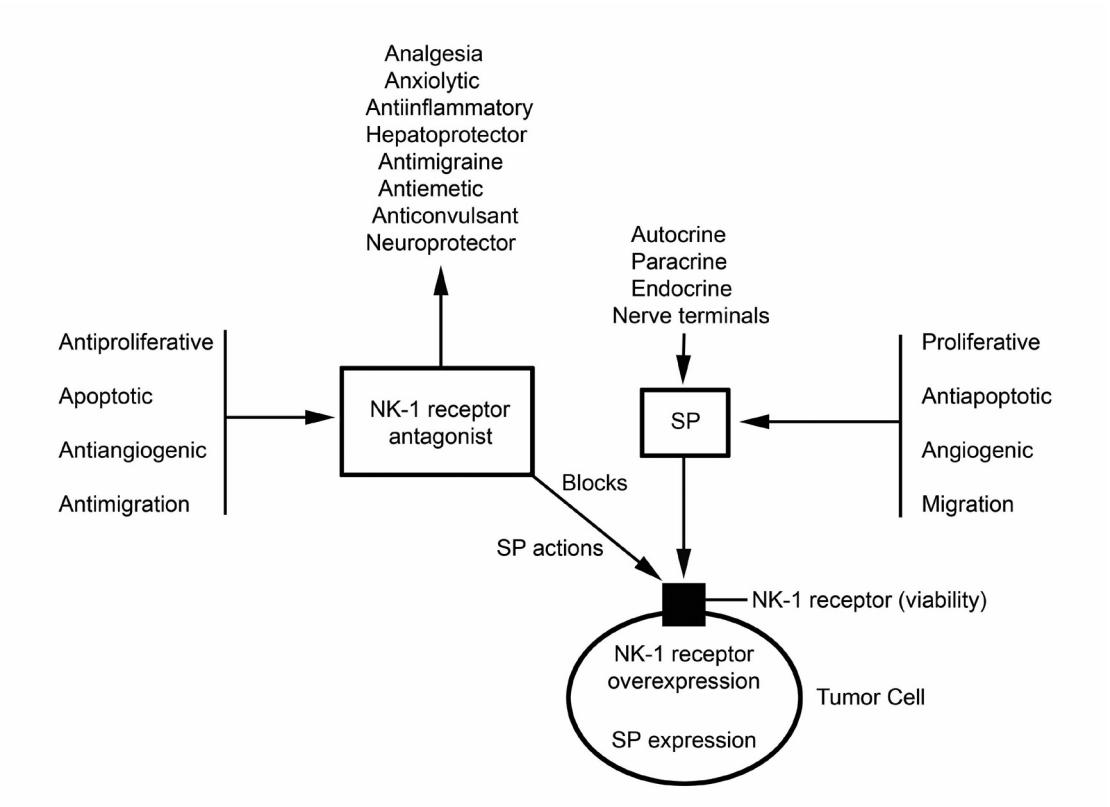

Fig. (1). Involvement of the SP/NK-1 receptor system in human cancer.

addressed in the future) [10-12]. SP has been observed in both the peritumoral tissues and the tumor mass, and the peptide exerts a mitogenic action on endothelial cells, stimulating vessel growth [27, 30, 48]. It has also been reported that SP-containing nerve fibers in human gastric cancer are related to gastric cancer differentiation; that SP could promote the proliferation, migration and invasion of MKN45 cells *in vitro*, and that after SP stimulation the intracellular calcium level of MKN45 cells is increased [49].

NK-1 receptors have been demonstrated in human primary tumors and neoplastic cells, in human cancer cell lines and in intra- and peritumoral blood vessels (e.g., smooth muscle cells, endothelial cells) of most of the tumors investigated [2, 6, 7, 9, 14-17, 27, 50]. In oral squamous cell and larynx carcinomas, primary retinoblastoma, gastric and colon adenocarcinomas, primary invasive malignant melanoma, and in metastatic melanoma tissues, NK-1 receptors were located in the cytoplasm of the tumor cells [2, 9-12]. Moreover, immunoreactive NK-1 receptors were confined to the plasma membrane of tumor cells [9, 17]. The presence of NK-1 receptors in the cytoplasm of tumor cells can be explained in terms of the notion that after the binding of SP to the NK-1 receptor, both are internalized into early endosomes; then, SP is degraded and the NK-1 receptor is recycled to the cell surface [51, 52]. It should be remarked that keratinocytes of the thorny and granular strata of the supratumoral epidermis (melanoma) show evident nuclear immunoreactivity, suggesting that NK-1 receptors are present in the nuclei of such cells [9]. Thus by using immunohistochemical methods, NK-1 receptor visualization should facilitate the identification of tumors overexpressing NK-1 receptors for diagnostic and therapeutic intervention (e.g., the administration of NK-1 receptor antagonists). Four isoforms (75, 54-58, 46, 33-38 kDa) of the NK-1 receptor have been reported in human tumor cell lines (e.g. melanoma) [2, 6, 9, 13, 16, 17], but their functional roles are currently unknown. It is possible that these isoforms may be of different functional significance [see 35].

After binding to NK-1 receptors, SP stimulates mitogenesis in normal and tumor cells [1-7, 9, 14-18, 53, 54]. It seems that the increase in DNA synthesis and mitogenesis by SP is regulated by the mitogen-activated protein kinase (MAPK) pathway, including extracellular signal-regulated kinases 1 and 2 (ERK1/2) [18]. Once activated, ERK1/2 is translocated into the nucleus, inducing proliferation and protecting the cell from apoptosis [see 7] (Fig. **1**).

SP can be secreted from tumor cells exerting an autocrine and paracrine (e.g., acting as a mitogen on endothelial cells and promoting angiogenesis) mechanism; the peptide can be released from nerve terminals (a direct interaction between the nervous system and tumor cells occurs) and/or it can arrive from the blood stream (an endocrine mechanism related to emotional behavior) [see 7]. Through these four pathways, SP can induce not only the growth of the tumor (Fig. **1**), but also the peptide could induce the migration and spreading of tumor cells (metastasis development and cancer progression) [49, 55]. This is of huge importance since the prevention of the development of metastases is one of the major goals in the treatment of cancer. It is known that SP

Table 1. Cytostatic Actions Versus NK-1 Receptor Antagonist Actions

Organ/Site	Classic Cytostatics	NK-1 Receptor Antagonists
Cell specificity	- Non-specific cytotoxicity	- Specific cytotoxicity against tumor cells, through the NK-1 receptor
Antitumor action	- Mitogenesis inhibition - Cell death by necrosis	- Mitogenesis inhibition - Cell death by apoptosis - Angiogenesis inhibition - Inhibition of the migration of tumor cells
Central nervous system	- Emesis - Anxiety - Seizure - Encephalopathy	- Antiemetic - Anxiolytic - Anticonvulsant - Neuroprotector
Peripheral nervous system	- Polyneuropathy	- Neuroprotector
Liver	- Hepatotoxicity	- Hepatoprotector
Kidney	- Nephrotoxicity	- Nephroprotector
Systemic	- Pain - Inflammatory	- Analgesic - Antiinflammatory

induces changes in cellular shape (including blebbing) and that membrane blebbing is an important event in cell movement and spreading, cancer cell invasion and metastasis [56, 57]. SP and NK-1 receptors have been located in both intra- and peri-tumoral blood vessels and it is known that SP, by increasing endothelial cell proliferation (inside and around the tumor), stimulates vessel growth (angiogenesis) and hence tumor development by increasing tumoral blood flow and by fostering stromal development [13, 26, 27, 48] (Fig. **1**).

NEUROKININ-1 RECEPTOR ANTAGONISTS: ANTI-TUMOR ACTION

All the above-mentioned data suggest that NK-1 receptor antagonists could exert antiproliferative, antiangiogenic and antimigration actions, and could also exert a selective action (contrary to classic cytostatics) against tumor cells, since they overexpress the NK-1 receptor (Fig. (**1**); Table **1**) [3, 7, 9, 13, 22, 26, 27, 48]. NK-1 receptor antagonists can be divided in two groups: peptide NK-1 receptor antagonists and non-peptide NK-1 receptor antagonists (L-733,060 (benzylether piperidines); RP-67,580 (perhydroiso-indolones); WIN- 51,708 (steroid); L-732,138 (tryptophan-based); CP-99,994 (benzylamino piperidines); CP-96,345 (benzylamino and benzylether quinuclidine)) [34, 45, 58-65]. The binding sites for non-peptide NK-1 receptor antagonists, SP and peptide NK-1 receptor antagonists are different. SP and peptide NK-1 receptor antagonists bind to the extracellular ends of the transmembrane helices and especially to the extracellular loops of the receptor, whereas non-peptide NK-1 receptor antagonists bind more deeply between the transmembrane segments. After binding to NK-1 receptor, NK-1 receptor antagonists could block the effect of SP (e.g., mitogenesis, angigenesis, and the migration of tumor cells). NK-1 receptor antagonists attenuate pain, with reduced tolerance, pelvic pain and mechanical allodynia and they also produce anxiolytic-like, antiinflammatory, antimigraine, neuroprotective, anticonvulsant and hepatoprotective effects [40, 65-72] (Fig. (**1**); Table **1**)**.** In an animal model of colitis-associated colon cancer, the NK-1 receptor antagonist SR-140,333 significantly decreased the incidence of inflammatory bowel disease [73]. It is known that cancer-related visceral pain originates from post-synaptic dorsal column neurons placed in the spinal cord and that these neurons start to express NK-1 receptors after visceral stimulation [74]. These data suggest a new target for the development of pharmacological strategies (e.g., NK-1 receptor antagonists) to control cancer-related visceral pain.

It should be noted that although many NK-1 receptor antagonists have been tested in clinical trials they showed a lack of effectiveness [60]. Currently, aprepitant (EMEND) and fosaprepitant are the only NK-1 receptor antagonists available for clinical use. These antagonists are indicated for the treatment of acute and delayed chemotherapy-induced nausea and vomiting [60, 75, 76]. Chemotherapy induces the release of SP and aprepitant blocks the unwanted actions exerted by SP on the central nervous system [75]. The water-soluble injectable form of aprepitant, called fosaprepitant dimeglumine, is also used in clinical practice for the prevention of postoperative nausea and vomiting and for inhibiting chemotherapy-induced nausea and vomiting [58]. It has been reported that aprepitant exerts antipruritic effects (SP is a major mediator of pruritus), but these results must be confirmed in future randomized, controlled clinical trials [77].

Antiproliferation and Apoptotic Effects on Tumor Cells

Many works have demonstrated that NK-1 receptor antagonists (e.g., L-733,060 (piperidine), L-732,138 (L-tryptophan), aprepitant (morpholine)…) exert a dose-

dependent antitumor action against many human tumor cell lines expressing NK-1 receptors and that such antitumor action is caused by apoptosis [1-6, 9, 13, 15-17, 19, 20, 22, 26, 30, 78, 79, 80-84] (Fig. **1**). The antitumoral action of L-733,060 against human cancer cell lines is more potent than that of aprepitant, and the antitumoral action of aprepitant is more potent than that of L-732,138 [5, 9, 21, 23]. The induction of apoptosis by NK-1 receptor antagonists could represent one of the most appropriate methods for cancer treatment, although this should be tested in future clinical trials. Currently, the mechanisms responsible for inducing apoptosis in tumor cells are unknown. It has been reported that NK-1 receptor antagonists could inhibit both DNA synthesis and cell proliferation through the MAPK pathway; that they could prevent the nuclear translocation of ERK1y2, inhibiting proliferation and inducing apoptosis, and that they decrease the basal phosphorylation of Akt and cause the proteolysis of poly-(ADP-ribose) polymerase and the cleavage of caspase-3 [18, 22, 83, 85]. It seems that the overexpression of NK-1 receptors by tumor cells renders these cells highly dependent upon the potent mitotic signal mediated by SP, which counteracts the death signal pathways activated in tumor cells by their own genetic damage, suppressor gene losses, oncogene activation, etc. Thus, by increasing the phenotypic expression of NK-1 receptors tumor cells neutralize the pathways leading to cell death, since the different death signals are overridden by the SP-mediated mitotic stimulus. Accordingly, when the NK-1 receptor is blocked by the NK-1 receptor antagonist the cell dies, because the balance inside the cell could be favorable to apoptotic/death signals [see 20, 22].

The above data suggest that NK-1 receptor antagonists could be candidates for broad-spectrum antineoplastic drugs [86]. However, as previously mentioned, aprepitant and fosaprepitant are the only NK-1 receptor antagonists available for clinical use. The safety of aprepitant has been reported, as well as its safety against human fibroblast cells [75, 78, 87]. Thus, aprepitant is the main NK-1 receptor antagonist candidate for use in the treatment of cancer, since its possible evaluation as an antitumor agent could be easier or faster than for less investigated compounds, because many of the required safety and characterization studies have already been completed [60, 75].

Antiangiogenic and Antimigration Actions

It has been reported that NK-1 receptor antagonists block the mitogenic action mediated by SP on endothelial cells, decreasing tumor-associated angiogenesis; that they block rapid SP-induced changes in cellular shape (including blebbing), and that L-733,060 inhibits the SP-mediated metastatic progression of cancer cells [56, 88, 89] (Fig. (**1**); Table **1**). In patients with breast cancer the risk of recurrence or metastasis is reduced four-fold over a 2.5-4 year follow-up period when surgery is associated with paravertebral anesthesia [90]. The SP/NK-1 receptor system could be involved in this process, since it has been suggested that paravertebral anesthesia blocks the SP-induced migration, invasion and metastasis of tumor cells [see 8]. This hypothesis is quite interesting, because the use of paravertebral anesthesia prior to cancer surgical intervention in cancer could reduce the number of recurrences and metastases in the surgical treatment of tumors and hence pretreatment with NK-1 receptor antagonists prior to surgical intervention could have synergic effects [see 8].

In sum, since tumor-cell migration is mediated by SP and is a prerequisite for invasion and metastasis and since the peptide is involved in angiogenesis, it seems that NK-1 receptor antagonists could be used to inhibit the above processes (Fig. (**1**); Table **1**). However, it should be remarked that most of the findings concerning the involvement of the SP/NK-1 receptor system in cancer were obtained from preclinical studies, and then most of them from *in vitro* studies. This means that *in vivo* studies must be carried out in experimental animal models in order to fully demonstrate the involvement of SP/NK-1 receptor/NK-1 receptor antagonist in tumor growth, angiogenesis and the migration of tumor cells before developing clinical trials in humans (e.g., using aprepitant). It will be very important to investigate the cellular signaling pathways downstream from the NK-1 receptor, after the binding of SP or the NK-1 receptor antagonist.

CYTOSTATIC DRUGS AND NEUROKININ-1 RECEPTOR ANTAGONISTS

In an *in vitro* study, it has been demonstrated that co-administration of L-733,060/ vinblastine or microtubule-destabilizing agents was synergistic for the inhibition of the growth of cancer cells expressing NK-1 receptors, but not for non-tumoral cells. It seems that L-733,060 reduces antiapoptotic NK-1 receptor signaling and hence enhances vinblastine-induced cell death. This strategy might be clinically useful for cancer chemotherapy [91]. Moreover, it has been reported that cyclophosphamide (an antineoplastic agent) and X-radiation induce neurogenic inflammation, this process being mediated by SP; that placlitaxel (a cytostatic) exerts adverse pulmonary actions, this also being mediated by SP, and that cisplatin impairs renal function. In all three cases, an improvement was observed when NK-1 receptor antagonists were administered [92-94]. These data suggest that these antagonists, in addition to exerting an antitumor action, should decrease the side-effects produced by chemotherapy and radiation treatments to a considerable extent [91-94].

THE SUBSTANCE P/NEUROKININ-1 RECEPTOR SYSTEM IN CANCER

This mini-review highlights the data published about the SP/NK-1 receptor system in cancer and the possible use of NK-1 receptor antagonists as new antitumoral agents, since it seems that these antagonists exert an antitumoral action (inducing apoptosis in tumor cells), an antiangiogenic action (by blocking endothelial cell proliferation) and the antimigration activity of tumor cells. NK-1 receptor antagonists counteract the physiological actions mediated by SP in cancer: tumor cell proliferation (SP seems to be a universal mitogen for tumor cells expressing/overexpressing NK-1 receptors), angiogenesis (SP stimulates the growth of endothelial cells) and the migration of tumor cells (SP activates the migration of tumor cells) (Fig. **1**). Thus, summing up the data currently available concerning the involvement of the SP/NK-1 receptor system in cancer, the

following key points emerge (Fig. **1**): 1) SP is expressed in cancer cells; 2) SP has been located in the nucleus and in the cytoplasm of tumor cells; 3) NK-1 receptors are overexpressed in cancer cells; 4) NK-1 receptors have been located in the cytoplasm, in the plasma membrane, and in the nucleus of the tumor cells; 5) Cancer cells have different NK-1 receptor complex isoforms (75, 54-58, 46, 33-38 kDa); 6) The tachykinin 1 (*TAC1R*) gene is overexpressed in tumor cells; 7) The NK-1 receptor is involved in the viability of tumor cells; 8) SP induces tumor cell proliferation, after binding to the NK-1 receptor; 9) SP stimulates angiogenesis; 10) SP and NK-1 receptors are located in intra- and peritumoral blood vessels; 11) SP induces the migration of tumor cells; 12) NK-1 receptor antagonists block the SP-induced mitogen stimulation of tumor cells; 13) NK-1 receptor antagonists inhibit SP-induced rapid changes in cellular shape; 14) NK-1 receptor antagonists inhibit SP-mediated increased migratory activity of tumor cells; 15) NK-1 receptor antagonists exert an antiangiogenic action; 16) NK-1 receptor antagonists inhibit tumor cell growth in a dose-dependent manner. They act as a broad-spectrum antitumor agent; 17) The specific antitumor action of the NK-1 receptor antagonists occurs through the NK-1 receptor; and 18) Tumor cell death is due to apoptosis.

CONCLUSIONS

All the data reported above suggest that the SP/NK-1 receptor system could play an important role in cancer (NK-1 receptor antagonists could be selective against tumor cells, since these cells overexpress NK-1 receptors); that the NK-1 receptor could be a new target in cancer treatment, and that NK-1 receptor antagonists could offer a new and promising new generation of anticancer drugs. In coming years, the preclinical data mentioned in this review should be confirmed in experimental animal models and, later, in human trials.

CONFLICT OF INTEREST

MM: USPTO Application no. 20090012086 “Use of non-peptidic NK-1 receptor antagonists for the production of apoptosis in tumor cells”.

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REFERENCES

[1] Muñoz, M.; Rosso, M.; Pérez, A.; Coveñas, R.; Rosso, R.; Zamarriego, C.; Soult, J.A.; Montero, I. Antitumoural action of the neurokinin-1-receptor antagonist L- 733,060 and mitogenic action of substance P on human retinoblastoma cell lines. *Invest. Ophthalmol. Vis. Sci.,* **2005**, *46*, 2567-70.

[2] Muñoz, M.; Rosso, M.; Coveñas, R.; Montero, I.; González-Moles, M.A.; Robles, M.J. Neurokinin-1 receptors located in human retinoblastoma cell lines: antitumor action of its antagonist, L-732,138. *Invest. Ophthalmol. Vis. Sci.,* **2007**, *48,* 2775-81.

[3] Muñoz, M.; Coveñas, R. Neurokinin-1 receptor: a new promising target in the treatment of cancer. *Discov. Med.,* **2010**, *10*, 305-13.

[4] Muñoz, M.; Pérez, A.; Coveñas, R.; Rosso, M.; Castro, E. Antitumoural action of L- 733,060 on neuroblastoma and glioma cell lines. *Arch. Ital. Biol.,* **2004**, *142*, 105- 12.

[5] Muñoz, M.; Pérez, A.; Rosso, M.; Zamarriego, C.; Rosso, R. Antitumoural action of NK1 receptor antagonist L-733,060 on human melanoma cell lines. *Melanoma Res.,* **2004**, *14*, 183-8.

[6] Muñoz, M.; Rosso, M.; Pérez, A.; Coveñas, R.; Rosso, R.; Zamarriego, C.; Piruat, J.I. The NK1 receptor is involved in the antitumoural action of L-733,060 and in the mitogenic action of substance P on neuroblastoma and glioma cell lines. *Neuropeptides,* **2005**, *39*, 427-32.

[7] Muñoz, M.; Rosso, M.; Coveñas, R. A new frontier in the treatment of cancer: NK-1 receptor antagonists. *Curr. Med. Chem.,* **2010**, *17*, 504-16.

[8] Muñoz, M.; Rosso, M.; Casinello, F.; Coveñas, R. Paravertebral anesthesia: how substance P and the NK-1 receptor could be involved in regional block and breast cancer recurrence. *Breast Cancer Res. Treat.,* **2010**, *122*, 601-3.

[9] Muñoz, M.; Rosso, M.; Robles-Frías, M.J.; Salinas-Martín, M.V.; Rosso, R.; Gonzalez-Ortega, A.; Coveñas, R. The NK-1 receptor is expressed in human melanoma and is involved in the antitumor action of the NK-1 receptor antagonist aprepitant on melanoma cell lines. *Lab. Invest.,* **2010**, *90*, 1259-69.

[10] González-Moles, M.A.; Mosqueda-Taylor, A.; Esteban, F.; Gil-Montoya, J.A.; Díaz-Franco, M.A.; Delgado, M.; Muñoz, M. Cell proliferation associated with actions of the substance P/NK-1 receptor complex in keratocystic odontogenic tumours. *Oral. Oncol.,* **2008**, *44*, 1127-33.

[11] Brener, S.; González-Moles, M.A,; Tostes, D.; Esteban, F.; Gil-Montoya, J.A.; Ruiz-Avila, I.; Bravo, M.; Muñoz, M. A role for the substance P/NK-1 receptor complex in cell proliferation in oral squamous cell carcinoma. *Anticancer. Res.,* **2009**, *29*, 2323-9.

[12] Esteban, F.; González-Moles, M.A.; Castro, D.; Martín-Jaén, M.M.; Redondo, M.; Ruiz-Avila, I.; Rosso, M.; Muñoz, M. Expression of substance P and neurokinin-1- receptor in laryngeal cancer: linking chronic inflammation to cancer promotion and progression. *Histopathology,* **2009**, *54*, 258-60.

[13] Friess, H.; Zhu, Z.; Liard, V.; Shi, X.; Shrikhande, S.V.; Wang, L.; Lieb, K.; Korc, M.; Palma, C.; Zimmermann, A.; Reubi, J.C.; Buchler, M.W. Neurokinin-1 receptor expression and its potential effects on tumor growth in human pancreatic cancer. *Lab. Invest.,* **2003**, *83*, 731-42.

[14] Palma, C., Nardelli, F.; Manzini, S.; Maggi, C.A. Substance P activates responses correlated with tumour growth in human glioma cells line bearing tachykinin NK1 receptors. *Br. J. Cancer,* **1999**, *79*, 236-43.

[15] Muñoz, M.; Rosso, M.; Coveñas, R. The NK-1 receptor is involved in the antitumoural action of L-733,060 and in the mitogenic action of substance P on human pancreatic cancer cell lines. *Lett. Drug Des. Discovery,* **2006**, *3*, 323-9.

[16] Muñoz, M.; Rosso, M.; Aguilar, F.J.; González-Moles, M.A.; Redondo, M.; Esteban, F. NK-1 receptor antagonists induce apoptosis and counteract substance P- related mitogenesis in human laryngeal cancer cell line HEp-2. *Investig. New Drugs,* **2008**, *26*, 111-8.

[17] Rosso, M.; Robles-Frías, M.J.; Coveñas, R.; Salinas-Martín, M.V.; Muñoz, M. The NK-1 receptor is expressed in human primary gastric and colon adenocarcinomas and is involved in the antitumor action of L-733,060 and the mitogenic action of substance P on human gastrointestinal cancer cell lines. *Tumor Biol.,* **2008**, *29*, 245-54.

[18] Luo, W.; Sharif, T.R.; Sharif, M. Substance P-induced mitogenesis in human astrocytoma cells correlates with activation of the mitogen-activated protein kinase signaling pathway. *Cancer Res.,* **1996**, *56*, 4983-91.

[19] Bigioni, M.; Benzo, A.; Irrisuto, C.; Maggi, CA.; Goso, C. Role of NK-1 and NK-2 tachykinin receptor antagonism on the growth of human breast carcinoma cell line MDA-MB-231. *Anticancer Drugs,* **2005**, *16,* 1083-9.

[20] Esteban, F.; Muñoz, M.; González-Moles, M.A.; Rosso, M. A role for substance P in cancer promotion and progression: a mechanism to counteract intracellular death signals following oncogene activation or DNA damage. *Cancer Metast. Rev.,* **2006**, *25,* 137-45.

[21] Muñoz, M.; Rosso, M.; González-Ortega, A.; Coveñas, R. The NK-1 receptor antagonist L-732,13 8 induces apoptosis and counteracts substance P-related mitogenesis in human melanoma cell lines. *Cancers,* **2010**, *2*, 611-23.

[22] Muñoz, M.; Rosso, M.; Coveñas, R. The Nk-1 receptor: a new target in cancer therapy. *Curr. Drug Targets.,* **2011**, *12*, 909-21.

[23] Muñoz, M.; González-Ortega, A.; Coveñas, R. The NK-1 receptor is expressed in human leukemia and is involved in the antitumor action of aprepitant and other NK- 1 receptor antagonists on acute lymphoblastic leukemia cell lines. *Invest. New Drugs,* **2011**, DOI 10.1007/s10637-010-9594-0.

[24] Reubi, J.C. Peptide receptors as molecular targets for cancer diagnosis and therapy. *Endocr. Rev.,* **2003**, *24*, 389-427.

[25] Berger, A.; Santic, R.; Hauser-Kronberger, C.; Schilling, F.H.; Kogner, P.; Ratschek, M.; Gamper, A.; Jones, N.; Sperl, W.; Kofler, B. Galanin and galanin receptors in human cancers. *Neuropeptides,* **2005**, *39*, 353-9.

[26] Singh, D.; Joshi, D.D.; Hameed, M.; Qian, J.; Gascon, P.; Maloof, P.B.; Mosenthal, A.; Rameshwar P. Increased expression of preprotachykinin-I and neurokinin receptors in human breast cancer cells: implications for bone marrow metastasis. *Proc. Natl. Acad. Sci. USA.,* **2000**, *97,* 388-93.

[27] Hennig, I.M.; Laissue.; J.A.; Horisberger, U.; Reubi, J.C. Substance-P receptors in human primary neoplasms: tumoral and vascular localization. *Int. J. Cancer,* **1995**, *61*, 786-92.

[28] Fowler, C.J.; Brannstrom, G. Substance P enhances forskolin-stimulated cyclic AMP production in human UC11MG astrocytoma cells. *Methods. Find. Exp. Clin. Pharmacol.,* **1994**, *16*, 21-8.

[29] Morgan, G.; Ward, R.; Barton, M. The contribution of cytotoxic chemotherapy to 5-year survival in adult malignancies. *Clin. Oncol. (R. Coll. Radiol.),* **2004**, *16*, 549- 60.

[30] Palma, C.; Bigioni, M.; Irrissuto, C.; Nardelli, F.; Maggi, C.A.; Manzini, S. Anti-tumour activity of tachykinin NK1 receptor antagonists on human glioma U373 MG xenograft. *Br. J. Cancer,* **2000**, *82*, 480-7.

[31] Mai, J.K.; Stephens, P.H.; Hopf, A.; Cuello, A.C. Substance P in the human brain. *Neuroscience,* **1986**, *17*, 709-39.

[32] Furuya, S.; Furuya, K.; Shigemoto, R.; Sokabe, M. Localization of NK-1 receptors and roles of substance P in subepithelial fibroblasts of rat intestinal villi. *Cell Tissue Res.,* **2010**, *342*, 243-59.

[33] Haneda, E.; Higuchi, M.; Maeda, J.; Inaji, M.; Okauchi, T.; Ando, K.; Obayashi, S.; Nagai, Y.; Narazaki, M.; Ikehira, H.; Nakao, R.; Zhang, MR.; Suzuki, K.; Suzuki, H.; Suhara, T. *In vivo* mapping of substance P receptors in brains of laboratory animals by high-resolution imaging system. *Synapse,* **2007**, *61*, 205-15.

[34] Quartara, L.; Maggi, C.A. The tachykinin NK1 receptor. Part I: ligands and mechanisms of cellular activation. *Neuropeptides,* **1997**, *31*, 537-63.

[35] Douglas, S.D.; Leeman, S.E. Neurokinin-1 receptor: functional significance in the immune system in reference to selected infections and inflammation. *Ann. NY Acad. Sci.,* **2011**, *1217*, 83-95.

[36] Hökfelt, T.; Pernow, B.; Wahren, J. Substance P: a pioneer amongst neuropeptides. *J. Intern. Med.,* **2001**, *249*, 27-40.

[37] Miyano, K.; Morioka, N.; Sugimoto, T.; Shiraishi, S.; Uezono, Y.; Nakata, Y. Activation of the neurokinin-1 receptor in rat spinal astrocytes induces Ca^{2+} release from IP-3-sensitive Ca^{2+} stores and extracellular Ca^{2+} influx through TRPC3. *Neurochem. Int.,* **2010**, *57*, 923-34.

[38] Quartara, L.; Maggi, C.A. The tachykinin NK1 receptor. Part II: distribution and pathophysiological roles. *Neuropeptides,* **1998**, *32*, 1-49.

[39] Varty, G.B.; Cohen-Williams, M.E.; Hunter, J.C. The antidepressant-like effects of neurokinin NK1 receptor antagonists in a gerbil tail suspension test. *Behav. Pharmacol.,* **2003**, *14*, 87-95.

[40] Bang, R.; Sass, G.; Kiemer, A.K.; Vollmar, A.M.; Neuhuber, W.L.; Tiegs, G. Neurokinin-1 receptor antagonists CP-96,345 and L-733,060 protect mice from cytokine-mediated liver injury. *J. Pharmacol. Exp. Ther.,* **2003**, *305*, 31-9.

[41] Koon, H.W., Shih, D.; Karagiannides, I.; Zhao, D.; Fazelbhoy, Z.; Hing, T.; Xu, H.; Lu, B.; Gerard, N.; Pothoulakis, C. Substance P modulates colitis-associated fibrosis. *Am. J. Pathol.,* **2010**, *177*, 2300-9.

[42] Castellani, M.L.; Galzio, R.J.; Felaco, P.; Tripodi, D.; Toniato, E.; De Lutiis, M.A.; Conti, F.; Fulcheri, M.; Conti, C.; Theoharides, T.C.; Caraffa, A.; Antinolfi, P.; Felaco, M.; Tete, S.; Pandolfi, F.; Shaik-Dasthagirisaheb, Y.B. VEGF, substance P and stress, new aspects: a revisited study. *J. Biol. Regul. Homeost. Agents,* **2010**, *24*, 229-37.

[43] Vinet-Oliphant, H.; Alvarez, X.; Buza, E.; Borda, J.T.; Mohan, M.; Aye, P.P.; Tuluc, F.; Douglas, S.D.; Lackner, A.A. Neurokinin-1 receptor (NK1-R) expression in the brains of SIV-infected rhesus macaques: implications for substance P in NK1-R immune cell. *Am. L. Pathol.,* **2010**, *177*, 1286-97.

[44] Frisch, P.; Bilkei-Gorzó, A.; Rácz, I.; Zimmer, A. Modulation of the CRH system by substance P/NKA in an animal model of depression. *Behav. Brain Res.,* **2010**, *213*, 103-8.

[45] Hegde, A.; Koh, Y.H.; Moochhala, S.M.; Bhatia, M. Neurokinin-1 receptor antagonist treatment in polymicrobial sepsis: molecular insights. *Int. J. Inflam.,* **2010**, *2010*, 601098.

[46] Khare, V.K.; Albino, A.P.; Reed, J.A. The neuropeptide/mast cell secretagogue substance P is expressed in cutaneus melanocytic lesions. *J. Cutan. Pathol.,* **1998**, *25*, 2-10.

[47] Tarkkanen, A.; Tervo, T.; Tervo, K.; Eranko, L.; Eranko, O.; Cuello, A.C. Substance P immunoreactivity in normal human retina and in retinoblastoma. *Ophthalmic. Res.,* **1983**, *15*, 300-6.

[48] Ziche, M.; Morbidelli, L.; Pacini, M.; Gepetti, P.; Alessandri, G.; Maggi, C.A. Substance P stimulates neovascularization *in vivo* and proliferation of cultured endothelial cells. *Microvasc. Res.,* **1990**, *40*, 264-78.

[49] Feng, F.; Yang, J.; Tong, L.; Yuan, S.; Hong, L.; Wang, W.; Zhang, H. Substance P immunoreactive nerve fibers are related to gastric cancer differentiation status and could promote proliferation and migration of gastric cancer cells. *Cell Biol. Int.,* **2011**, DOI: 10.1042/CBI20100229.

[50] Johnson, C.L.; Johnson, C.G.; Stauderman, K.A.; Buck, S.H. Characterization of substance receptors in human astrocytoma cells. *Ann. NY. Acad. Sci.,* **1991**, *632*, 410-2.

[51] Grady, E.F.; Garland, A.M.; Gamp, P.D.; Lovett, M.; Payan, D.G.; Bunnett, N.W. Delineation of the endocytic pathway of substance P and its seven transmembrane domains NK1 receptor. *Mol. Biol. Cell.,* **1995**, *6*, 509-24.

[52] Mantyh, P.W.; Allen, C.J.; Ghilardi, J.R.; Rogers, S.D.; Mantyh, C.R.; Liu, H.; Basbaum, A.I.; Vigna, S.R.; Maggio, J.E. Rapid endocytosis of a G protein-coupled receptor: substance P evoked internalization of its receptor in the rat striatum *in vivo*. *Proc. Natl. Acad. Sci, USA.,* **1995**, *92*, 2622-6.

[53] Payan, D.G.; Brewster, D.R.; Goetzl, E.J. Specific stimulation of human T lymphocytes by substance P. *J. Immunol.,* **1983**, *131*, 1613-5.

[54] Ziche, M.; Morbidelli, L.; Pacini, M.; Dolara P.; Maggi, C.A. NK1-receptors mediate the proliferative response of human fibroblasts to tachykinins. *Br. J. Pharmacol.,* **1990**, *100*, 11-4.

[55] Entschladen, F.; Lang, K.; Drell, T.L.; Joseph, J.; Zaenker, K.S. Neurotransmitters are regulators for the migration of tumor cells and leukocytes. *Cancer Immunol. Immunother.,* **2002**, *51*, 467-82.

[56] Meshki, J.; Douglas, S.D.; Lai, J.P.; Schwartz, L.; Kilpatrick, L.E.; Tuluc, F. Neurokinin 1 receptor mediates membrane blebbing in HEK293 cells through a Rho/Rho-associated coiled-coil kinase-dependent mechanism. *J. Biol. Chem.,* **2009**, *284*, 9280-9.

[57] Fackler, O.T.; Grosse, R. Cell motility through plasma membrane blebbing. *J. Cell. Biol.,* **2008**, *181*, 879-84.

[58] Huang, S.C.; Korlipara, V.L. Neurokinin-1 receptor antagonists: a comprehensive patent survey. *Expert. Opin. Ther. Pat.,* **2010**, *20*, 1019-45.

[59] Millan, M.J.; Dekeyne, A.; Gobert, A.; Mannoury la Cour, C.; Brocco, M.; Rivet, J.M.; Di Cara, B.; Lejeune, F.; Cremers, T.I.; Flik, G.; de Jong, T.R.; Olivier, B.; de Nanteuil, G. S-41,744, a dual neurokinin (NK) 1 receptor antagonist and serotonin (5-HT) reuptake inhibitor with potential antidepressant properties: a comparison to aprepitant (MK869) and paroxetine. *Eur. Neuropsychopharmacol.,* **2010**, *20*, 599-621.

[60] Rost, K.; Fleischer, F.; Nieber, K. Neurokinin-1 receptor antagonists: between hope and disappointment. *Med. Monatsschr. Pharm.,* **2006**, *29*, 200-5.

[61] Giuliani, G.; Cappelli, A.; Matarrese, M; Masiello, V.; Turolla, E.A.; Monterisi, C.; Fazio, F.; Anzini, M.; Pericot Mohr, G.L.; Riitano, D.; Finetti, F.; Morbidelli, L.; Ziche, M.; Giorgi,G.;

Vomero, S. Non-peptide NK(1) receptor ligands based on the 4-phenylpyridine moiety. *Bioorg. Med. Chem.,* **2011**, *19*, 2242-51.

[62] Obach, R.S.; Margolis, J.M.; Logman, M.J. *In vitro* metabolism of CP-122,721((2S,3S)-2-phenyl-3-[(5-trifluoromethoxy-2-methoxy)benzylamino]piperidine), a non-peptide antagonist of the substance P receptor. *Drug Metab. Pharmacokinet.,* **2007**, *22*, 336-49.

[63] Sindrup, S.H.; Graf, A.; Sfikas, N. The NK1-receptor antagonist TKA731 in painful diabetic neuropathy: a randomized, controlled trial. *Eur. J. Pain,* **2006**, *10*, 567-71.64.

[64] Kart, E.; Jocham, G.; Müller, C.P.; Schlömer, C.; Brandão, M.L.; Huston, J.P.; de Souza Silva, M.A. Neurokinin-1 receptor antagonism by SR140,333: enhanced *in vivo* ACh in the hippocampus and promnestic post-trial effects. *Peptides,* **2004**, *25*, 1959-69.

[65] Muñoz, M.; Coveñas, R. NK-1 receptor antagonists: a new paradigm in pharmacological therapy. *Curr. Med. Chem.,* **2011**, *18*, 1820-31.

[66] Largent-Milnes, T.M.; Yamamoto, T.; Nair, P.; Moulton, J.W.; Hruby, V.J.; Lai, J.; Porreca, F.; Vanderah, T.W. Spinal or systemic TY005, a peptidic opioid agonist/neurokinin 1 antagonist, attenuates pain with reduced tolerance. *Br. J. Pharmacol.,* **2010**, *161*, 986-1001.

[67] Rudick, C.N.; Schaeffer, A.J.; Klumpp, D.J. Pharmacologic attenuation of pelvic pain in a murine model of interstitial cystitis. *BMC Urol.,* **2009**, *9*, 16.

[68] Sahbaie, P.; Shi, X.; Guo, T.Z.; Qiao, Y.; Yeomans, D.C.; Kingery, W.S.; Clark, J.D. Role of substance P signaling in enhanced nociceptive sensitization and local cytokine production after incision. *Pain,* **2009**, *145*, 341-9.

[69] Rupniak, N.M.; Carlson, E.C.; Harrison, T.; Oates, B.; Seward, E.; Owen, S.; de Felipe, C.; Hunt, S.; Wheeldon, A. Pharmacological blockade or genetic deletion of substance P (NK(1)) receptors attenuates neonatal vocalization in guinea-pigs and mice. *Neuropharmacology,* **2000**, *39*, 1413-21.

[70] Bolay, H.; Reuter, U.; Dunn, A.K.; Huang, Z.; Boas, D.A.; Moskowitz, M.A. Intrinsic brain activity triggers trigeminal meningeal afferents in a migraine model. *Nat Med.,* **2002**, *8*, 136-42.

[71] Zachrisson, O.; Lindefors, N.; Brené, S. A tachykinin NK1 receptor antagonist, CP-122,721-1, attenuates kainic acid-induced seizure activity. Brain Res. Mol. *Brain Res.,* **1998**, *60*, 291-5.

[72] Yu, J.; Cadet, J.L.; Angulo, J.A. Neurokinin-1 (NK-1) receptor antagonists abrogate methamphetamine-induced striatal dopaminergic neurotoxicity in the murine brain. *J. Neurochem.,* **2002**, *83*, 613-22.

[73] Pagán, B.; Isidro, A.A.; Coppola, D.; Chen, Z.; Ren, Y.; Wu, J.; Appleyard, C.B. Effect of a neurokinin-1 receptor antagonist in a rat model of colitis-associated colon cancer. *Anticancer Res.,* **2010**, *30*, 3345-53.

[74] Wang, Y.; Mu, X.; Liu, Y.; Zhang, X.; Wu, A:, Yue, Y. NK-1-receptor-mediated lesion of spinal post-synaptic dorsal column neurons might improve intractable visceral pain of cancer origin. *Med. Hypotheses,* **2011**, *76*, 102-4.

[75] Hargreaves, R.; Ferreira, J.C.; Hughes, D.; Brands, J.; Hale, J.; Mattson, B.; Mills S. Development of aprepitant, the first neurokinin-1 receptor antagonist for the prevention of chemotherapy-induced nausea and vomiting. *Ann. NY Acad. Sci.,* **2011**, *1222*, 40-8.

[76] Diemunsch, P.; Grelot, L. Potential of substance P antagonists as antiemetics. *Drugs,* **2000**, *60*, 533-46.

[77] Ständer, S.; Siepmann, D.; Herrgott, I.; Sunderkötter, C:, Luger, T.A. Targeting the neurokinin receptor 1 with aprepitant: a novel antipruritic strategy. *PLoS One,* **2010**, *5*, e10968.

[78] Muñoz, M.; Rosso, M. The NK-1 receptor antagonist aprepitant as a broad spectrum antitumor drug. *Invest. New Drugs,* **2010**, *28*, 187-93.

[79] Rosati, R.; Adil, M.R.; Ali, M.A.; Eliason, J.; Orosz, A.; Sebestye, N.F.; Kalemkerian, G.P. Induction of apoptosis by a short-chain neuropeptide analog in small cell lung cancer-stimulation of multiple neuropeptides and inhibition by broad spectrum antagonists *in vitro* and *in vivo*. *Peptides,* **1998**, *19*, 1519-23.

[80] Woll, P.J.; Rozengurt, E. [D-Arg^{1}-D-Phe^{5}, D-Trp$^{7,\ 9}$, Leu^{11}] Substance P, a potent bombesin antagonist in murine Swiss 3T3 cells, inhibits the growth of human small cell lung cancer cells *in vitro*. *Proc. Natl. Acad. Sci. USA.,* **1988**, *35*, 1859-63.

[81] Reeve, J.G.; Bleehen, N.M. [D-Arg^{1}-D-Phe^{5}, D-Trp$^{7,\ 9}$, Leu^{11}] Substance P induces apoptosis in lung cancer cell lines *in vitro*. *Biochem. Bioph. Res. Com.,* **1994**, *199*, 1313-9.

[82] El-Salhy, M.; Starefeldt, A. Direct effects of octreotide, galanin and serotonin on human colon cancer cells. *Oncol. Rep.,* **2003**, *10*, 1723-8.

[83] Akazawa, T.; Kwatra, S.G.; Goldsmith, L.E.; Richardson, M.D.; Cox, E.A.; Sampson, J.H.; Kwatra, M.M. A constitutively active form of neurokinin 1 receptor and neurokinin 1 receptor-mediated apoptosis in glioblastomas. *J. Neurochem.,* **2009**, *109*, 1079-86.

[84] Huang, W.Q.; Wang, J.G.; Chen, L.; Wei, H.J.; Chen, H. SR140333 counteracts NK-1 mediated cell proliferation in human breast cancer cell line T47D. *J. Exp. Clin. Cancer Res.,* **2010**, *24*, 29-55.

[85] DeFea, K.A.; Vaughn, Z.D.; O'Bryan, E.M.; Nishijima, D.; Dery, O.; Bunnett, N.W. The proliferative and antiapoptotic effects of substance P are facilitated by formation of a beta-arrestin-dependent scaffolding complex. *Proc. Natl. Acad. Sci. USA.,* **2000**, *97*, 11086-91.

[86] Giardina, G.A.M.; Gagliardi, S.; Martinelli, M. Antagonists at the neurokinin receptors-Recent patent literature. *Drugs,* **2003**, *6*, 758-72.

[87] Kramer, M.S.; Cutler, N.; Feighner, J.; Shrivastava, R.; Carman, J.; Sramek, J.J.; Reines, S.A.; Liu, G.; Snavely, D.; Wyatt-Knowles, E.; Halle, J.J.; Mills, S.G,.; MacCoss, M.; Swain, C.J.; Harrison, T.; Hill, R.G.; Hefti, F.; Scolnick, E.M.; Cascieri, M.A.; Chicchi, G.G.; Sadowski, S.; Williams, A.R.; Hewson, L.; Smith, D.; Carlsson, E.J.; Hargreaves, R.J.; Rupniak, N.M. Distinct mechanism for antidepressant activity by blockade of central substance P receptors. *Science,* **1998**, *281*, 1640-5.

[88] Lang, K.; Drell, T.L.; Lindecke, A.; Niggemann, B.; Kaltschmidt, C.; Zaenker, K.S.; Entschladen, F. Induction of a metastatogenic tumor cell type by neurotransmitters and its pharmacological inhibition by established drugs. *Int. J. Cancer,* **2004**, *112*, 231-8.

[89] Guha, S.; Eibl, G.; Kisfalvi, K.; Fan.; R.S.; Burdick, M.; Reber, H.; Hines, O.J.; Strieter, R.; Rozengurt, E. Broad-spectrum G protein–coupled receptor antagonist, [D-Arg1,DTrp5,7,9,Leu11]SP: a dual inhibitor of growth and angiogenesis in pancreatic cancer. *Cancer Res.,* **2005**, *65*, 2738-45.

[90] Exadaktylos, A.K.; Buggy, D.J.; Moriarty, D.C.; Mascha, E.; Sessler, D.I. Can anesthetic technique for primary breast cancer surgery affect recurrence or metastasis? *Anesthesiology,* **2006**, *105*, 660-4.

[91] Kitchens, C.A.; McDonald, P.R.; Pollack, I.F.; Wipf, P.; Lazo, J.S. Synergy between microtubule destabilizing agents and neurokinin 1 receptor antagonists identified by an siRNA synthetic lethal screen. *FASEB. J.,* **2009**, *23*, 756.13.

[92] Alfieri, A.B.; Cubeddu, L.X. Role of NK1 receptors on cisplatin-induced nephrotoxicity in the rat. *Naunyn. Schmiebergs. Arch. Pharmacol.,* **2000**, *361*, 334- 8.

[93] Alfieri, A.B.; Cubeddu, L.X. Efectos de los antagonistas de los receptores NK1 y de la dexametasona sobre la inflamación neurogénica inducida por ciclofosfamida y por radiación X, en la rata. *AVFT.,* **2004**, *23*, 61-6.

[94] Sendo, T.; Itoh, Y.; Goromaru, T.; Hirakawa, T.; Ishida, M.; Nakano, H.; Oishi, R. Role of substance P in hypersensitivity reactions induced by paclitaxel, an anticancer agent. *Peptides,* **2004**, *25*, 1205-8.

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