Abstract

17-Trifluoromethyl phenyl trinor prostaglandin F2α (17-TPGF2α) is extracted from a Zinc database, but its value for inhibiting breast cancer (BC) through the substance P (SP)/NK1R system remains unknown. This study was designed to investigate the potential antagonist effect of 17-TPGF2α through neurokinin 1 receptor (NK1R). The effect of 17-TPGF2α on the proliferation and apoptosis of BC cell lines was determined through in vitro cell lines. Based on in vitro results we planned to investigate anticancer activity in female Balb/c and used subcutaneous (SC) injection of DMBA for cancer induction. Oral administration of 17-TPGF2α significantly suppresses the tumor volume as compared with an untreated group. The serum parameters like ALP, AST, and ALT and hematological parameters were normalized in test treated group. Histological examination revealed normal histoarchitecture of the mammary gland and focal areas showed minimal inflammatory cell infiltration. There is no necrosis is seen in both test treated and standard treated groups when compared with the DMBA group. All these findings concluded that 17-TPGF2α may have potential as a novel antitumor candidate for BC.

Keywords

Antibreast cancer · Histopathology · Mammary gland · Neurokinin 1 receptor · Tumor volume

Introduction

Neuropeptide-like substance P (SP) is becoming more widely recognized as a powerful cellular growth factor involved in the regulation of normal and pathological cell proliferation (Rozengurt 2002). The expression and secretion of peptides by tumors are gaining popularity, and new avenues for translational research are opening up with the potential to improve tumor diagnostics and treatment (Muñoz and Rosso 2010). Furthermore, research suggests that neuropeptides may have a role in the progression of cancer. SP has been demonstrated to operate as a mitogen on various human cancer cell lines by binding to the neurokinin-1 receptor (NK1R). Antitumor action of NK1R antagonists has been demonstrated in such cell lines, and these antagonists have been proven to promote tumor cell death (Muñoz et al. 2012, 2014). Thus, after specific receptors have been identified, individual neuropeptide antagonists may be utilized to block the paracrine or autocrine loops, implying that they could be employed as a treatment for neuropeptide-secreting malignancies (Orosz et al. 1995). These findings imply that the SP/NK1R system may play a role in cancer development and that NK1R antagonists could be used as a broad-spectrum antitumor drug. In this study, we evaluated the effect of NK1R blocked in Balb/c
mice by using DMBA-induced breast cancer (BC). One in every 8 women in the US is likely to get invasive BC during their lifetime, with 281,550 new invasive BC cases and 49,290 non-invasive BC cases predicted to be identified in women in the US in 2021 (“U.S. Breast Cancer Statistics”). Therefore, it is important the development future therapies targeting this stage of the disease. Research must focus on the drugs with high anticancer activity with minimal adverse effects, and it can be attained, only when the drug is specifically targeted to the tumor cell. In the present study, we evaluated the anticancer activity of 17-trifluorophenyl methyl prostaglandin F2 against BC. The compound was selected based on the in vitro results and molecular docking results.

Materials and Methods

Chemicals

The 7, 12-dimethylbenz (a) anthracene (DMBA) (purity ≥ 95%) was purchased from TCI Chemicals, Tokyo, Japan. Aprepitant was ordered from, 17-trifluoromethyl phenyl trinor prostaglandin F2alpha purchased from Cayman Chemicals, USA.

Animals and Ethical Consideration

Healthy female Balb/c mice, weighing 15–25 g were purchased from TANUVAS Animal Laboratory, Chennai. All the animals were given free access to food and water. The animal experiments were formally approved by the Institutional Animal Ethical Committee of SRM School of Pharmacy (IAEC/243/2021). After a week, the total of 44 Balb/c mice was randomly divided into two experimental groups such as Experiment-I (Acute toxicity) and Experiment-II (Anticancer effect of 17-TPGF2α against DMBA-induced BC).

Experiment-I: Acute Toxicity-423 Guidelines

The acute toxicity study was determined using Organization for Economic Cooperation and Development (OECD)-423 guidelines. In our study, we selected Balb/c female mice that weighed between 18 and 25 g and were 4–5 weeks old. Before the commencement of the study, all of the experimental mice were housed in cages for 7 days to acclimatize to laboratory conditions. The animals were denied food overnight before the commencement of the experiment. We used 20 mice divided into four groups of five mice each. Normal saline was given to animals in Group I, 50 mg/kg of 17-TPGF2α was given to animals in Group II, 300 mg/kg of 17-TPGF2α was given to animals in Group III, and Group IV received 17-TPGF2 α at a dose of 2000 mg/kg of. All animals were observed 30 min, 60 min, and 4 h after the test drug (17-TPGF2α) and vehicle were administered and observation continued for the next 14 days for any mortality and any autonomous or neurological changes (OECD 2002).

Experiment-II (Anticancer Effect of 17-TPGF2α Against DMBA Induced Breast Cancer)

Prepared DMBA solution (20 mg/kg dissolved in flaxseed oil) was subcutaneously administered into the mice’s breast pad based on the body weight. From the first day of induction, animals in the normal and disease group received 0.9% saline water. The current study extended 90 days, and 24 mice were used, divided into four groups of six animals each based on tumour volume. Group-I (normal healthy mice), Group-II (DMBA induced animals), Group-III (Test group) received 50 mg/kg of 17-TPGF2α, and Group-IV (Standard group) received Aprepitant at a dose of 123 mg/kg. The bodyweight of each mouse was measured every week. At the end of the 90th day, blood samples were collected for estimation of biochemical parameters, and the tumors were quickly dissected away from surrounding tissue and weighed, then stored at − 80 °C.

Tumor Volume

Tumor volume (TV) is estimated by measuring the width (W) and length (L) of the tumor using a digital caliper and calculated based on the following formula (Sápi et al. 2015).

\[ TV = \frac{W^2 \times L}{2} \]

Biochemical and Hematological Analysis

The mice serum and blood samples were used to detect the biochemical and hematological parameters. These are aspartate transferase (AST), alanine transferase (ALT), total blood count, white blood cells (WBC), haemoglobin (Hb), mean cell hemoglobin concentration (MCHC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), packed cell volume (PCV).

Histopathological Examination

Mammary glands were dissected and fixed in 10% formalin solution. This slowly allows to penetrate tissue protect against physical changes, then dehydrated using a graded ethanol series. Xylene I and II were used for 20 min for clearing, followed by wax immersion and embedding of the pieces. The pieces were then sliced and stained with hematoxylin and eosin (HE) to look for pathological changes.
Statistical Analysis

All results are shown as mean ± SEM for each experimental group. The statistical analyses were performed by using GraphPad Prism 5.0 and used Way ANOVA followed by Tukey’s method. Statistical significance of difference was considered at a p-value < 0.05.

Results

Experiment-I: Acute Toxicity Study

Balb/c female mice were administered 50, 300, and 2000 mg/kg of 17-TPGF2α in determining the acute toxicity of 17-TPGF2α. Normal group animals received only the vehicle and all treatment groups were then observed for 14 days. Throughout the toxicity study, all behavior and morphological characteristics were found to be normal. Since, on this compound, a toxicity study was studied on the rabbit, where the LD₅₀ was found at the dose of 3742 mg/kg. In the present study, we have selected the dose of 50 mg/kg based on the results of acute toxicity.

Experiment-II

Effect of DMBA on Mammary Glands

Breast tumor was induced in animals that received DMBA (Fig. 1). Experimental animals were weighed weekly and palpated twice a week to check the development of mammary tumors from the first day of acclimatization until the end of the experiment. The tumor appearance was recorded. We began therapy when the average TV reached 100 mm³, with Group-III (Test group) receiving 50 mg/kg of 17-TPGF2α and Group-IV (Standard group) receiving Aprepitant.

Effect of 17-TPGF2α on Mice Body Weight

The bodyweight of animals was changed from 3 weeks of the study as depicted in Fig. 2. A significant (p < 0.05) decrease in the body weight was seen in the disease group as compared with the normal group and a gradual increase in the weight body of test and standard group animals when compared with disease group.

Effect of 17-TPGF2α on Water and Food Intake

The figure represents the alterations in water intake during the treatment. All the animals showed an increase in water intake during the experiment. A significant increase in water intake was observed in animals treated with 17-TPGFα four times per week. Moreover, Aprepitant treated animals also showed a significant rise in water intake when compared to normal animals and the disease control group. A significant rise in food intake was observed in 17-TPGF2α and standard group animals when compared with the DMBA group on day 55 (Figs. 3, 4).

Effect of 17-TPGF2α on Breast Tumors

TV measurement cannot happen right after cancer induction. After 3.5 weeks of DMBA administration, the tumor started to grow out. In our study, TV was measured by using caliper, in that case, tumor diameter (width and length) was measured. The first measurement was done on 25 days and

![Fig. 1 Effect of 17-TPGF2α on tumor growth. Disease group showed increased tumor growth when compared with 17-TPGF2α treated group and also showed decreased tumor growth in standard group. A DMBA induced breast cancer tissue, B 17-TPGF2α treated group’s breast cancer tissue, and C Aprepitant treated group’s breast cancer tissue](image)

![Fig. 2 Effect of 17-TPGF2α on body weight. 17-TPGF2α treated group showed normal body weight as compared with DMBA treated animals. Data are represented as mean ± SEM (n = 6), normal vs. disease (***p < 0.001), disease vs. test (**p < 0.01)](image)
treatment was started after when the TV reached 100 mm³. TVs were measured bi-weekly and estimated by using the above-mentioned formula.

The TV of mice from the disease or DMBA group grew continued to increase until day 90. Where the TV of the DMBA group grew up to 746.2 mm³ and those of test (56.48 mm³) and standard (57.16 mm³, Fig. 5). The animal-received test drug showed a gradual decline in TV from the day of administration to the end of the experiment with an average TV of 56.48 mm³ as compared to the DMBA group. As expected 17-TPGF2α and Aprepitant significantly decreased the incidence of tumor growth and TV.

Effect on Biochemical and Haematological Parameters

The impact of test drug on biochemical and haematological parameters is depicted in Table 1 and Fig. 6. There was normal serum level of ALT (11.45 ± 0.16), AST (25.59 ± 0.199), and ALP (53.93 ± 0.20) in the normal group. While there was increased ALT (19.54 ± 0.19), AST (32.52 ± 0.354), and ALP (60.96 ± 0.42) in the disease or DMBA group. More, interestingly, the test group (17-TPGF2α) had decreased serum levels of ALT (13.04 ± 0.587), AST (26.23 ± 0.70), and ALP (53.93 ± 0.68) than the disease group and further standard group also showed decreased serum levels of ALT (14.0 ± 0.704), AST (24.38 ± 0.54) and ALP (52.58 ± 0.399).

At the dose of 50 mg/kg, 17-TPGF2α regulates normal WBC (1388 ± 108.9), platelet count (6.08 ± 0.02), hemoglobin (15.14 ± 0.014), red blood cells (RBC, 7.193 ± 0.043), PVC (36.42 ± 0.20), MCV (53.52 ± 0.145), MCH (56.21 ± 0.164) and MCHC (41.44 ± 0.09) levels count in test treated group. DMBA administration showed decreased levels of WBC (2647 ± 32.83), platelet count (6.95 ± 0.05), haemoglobin (12.50 ± 0.006), RBC (5.96 ± 0.017), PVC (32.28 ± 0.02), MCV (46.46 ± 0.15), MCH (53.38 ± 0.186) and MCHC (38.69 ± 0.147). Moreover, standard treated group maintained the normal hematological parameters.

Histological Examination

In the present histopathological examination, Fig. 7, the breast tissue section of a normal rat shows normal histoarchitecture of the mammary gland with intervening mature adipose tissue and ducts. Mice received DMBA only shows mammary gland section B, C, and D. The sections shows the presence of mucin, inflammatory cell infiltration, and extensive dedifferentiated neoplastic cells with abnormal nuclei. Adjacent skeletal muscle shows varying sizes and further focal areas showed abundant lymphatic infiltration. The 17-TPGF2α treated and standard treated shows focal areas showed minimal inflammatory cell infiltration and absence of mucin while covered with fibrous connective tissue.
Discussion

Currently, breast tumour is now the largest cause of cancer-related death in both developed and developing countries (Torre et al. 2015). It is critical to understand the underlying molecular pathways that promote BC carcinogenesis to minimize BC incidence and mortality and enhance patient outcomes. Despite advancements in BC diagnosis and treatment, it remains the biggest health concern for women. Existing therapies are confined due to significant side effects; consequently, it is vital to investigate cancer research studies to reach the ultimate objective of developing molecules that selectively target cancer cells while causing minimal side effects (Muñoz and Coveñas 2014).

In the latest days, the involvement of tachykinin neuropeptides in cancer pathogenesis and progression has already been highly drawn interest from researchers. Upregulation of SP and NK1R has been seen in glioblastoma, retinoblastoma, lung, endometrial, and pancreatic cancers, and has been linked to cancer growth and development (Friess et al. 2003; Singh et al. 2000). Several research has recently focused on the anticancer effect of a variety of chemical inhibitors of the SP/NK1R pathway, which have resulted in a considerable reduction in BC carcinogenesis (Munoz and Covenas 2012).

In vivo and in vitro studies have shown that NK1R antagonists like Aprepitant, L-733060, and L-732138 have anticancer properties in hepatoblastoma, small cell and non-small cell lung, pancreatic, and gastrointestinal cancer cells (Berger et al. 2014; Muñoz and Coveñas 2015; Munoz et al. 2015a, b; Muñoz et al. 2017). Accumulating of findings speculated that SP/NK1R complex plays a significant function in tumorigenesis and progression of carcinoma. SP has been linked to cancerous cell growth, neoangiogenesis, and invasiveness, further, NK1Rs being overexpressed in tumoral cells and malignant tissue (Dong et al. 2015; González-Ortega et al. 2014; Munoz et al. 2015a, b).

These findings led us to use molecular docking studies (Schrodinger software) to locate a molecule with a high affinity for NK1R, and we then did in vitro investigations on BC cell lines. In vitro study revealed that 17-TPGF2α inhibited the development of MCF-7 and MDA-MB-468 cells by inducing apoptosis via the Caspase-3 enzyme and increasing expression of Bax and Bad genes. These findings support those of Munoz et al., who found that the NK1R antagonist L-7321 and Aprepitant successfully inhibited proliferation and induced apoptosis in four BC cells, with the suppression of the mitogen activated protein kinase (MAPK) pathway being one probable mechanism for the NK1R antagonist’s apoptotic effects.
Fig. 7 Effect of 17-TPGF2α on microphotographs HandE×800 of mammary glands. A section of mice mammary glands of Group-I group showed normal arrangement of adipocytes (A) and duct (D). Sections B, C and D were from Group-II, where, B section of breast tissue from DMBA induced group showed dedifferentiated cells (DC) and presence of mucin (M) and duct (D). C The section of DMBA induced group showed highly pleomorphic neoplastic cells with pale eosinophilic cytoplasm and some cells in this section showed abnormal shape of nuclei (D)×300. E The tissue from 17-TPGF2α treated animals showed normal histoarchitecture of mammary gland and focal areas showed minimal inflammatory cell infiltration and there is absence of mucin and covered with fibrous tissue (F). There is no necrosis is seen. F Group-IV (Standard: Aprepitant) showed normal histoarchitecture of mammary gland and focal areas showed minimal inflammatory cell infiltration. There is no necrosis is seen.
Our in vivo study made use of subcutaneous injection of DMBA into Balb/c mammary fat pads. The results of a preliminary experiment showed that compared with the normal group, the levels of serum biochemical and haematological parameters were relatively low. Here, it was found that after third week of administration, 70% of animals developed cancer in the DMBA group. The tumor continued to grow until the end of the experiment in DMBA administered group and even some animals died during the experiment. Further, it found that 17-TPGF2α prevents BC growth and tumor incidence by the decrease of the TV and weight when compared to the DMBA group until the last administration on day 90.

In the present study, 17-TPGF2α was found nontoxic to mice when given by oral route. All the administrated animals were safe up to the dose of 50 mg/kg and 17-TPGF2α significantly decreases the haematological parameters when compared to the disease or DMBA group. As far as haematological parameters are concerned, all animals that administered DMBA showed a considerable reduction in RBC levels. Further, animals that received 17-TPGF2α showed normal RBC levels.

DMBA the carcinogen is metabolically converted to the DMBA-DE (DMBA-3,4-diol-1,2-epoxide) by cytochrome p450 enzymes, resulting in the production of BC in mice and the generation of different ROS. The oxidative stress caused by the creation of ROS has a severe impact on other critical organs such as the liver and kidney, leading to disease development. Since the liver and kidneys are such critical organs in our bodies, affecting their normal activities considerably impedes the metabolism of numerous chemotherapy medications, increasing overall body toxicity. The liver is the major organ for xenobiotic substance metabolism, and it is also thought to be damaged by chemical agents (Dakrory et al. 2015; Krishnamoorthy and Sankaran 2016). In the current study, the DMBA-treated group had significantly greater levels of serum ALT, ALP, and AST than the control group. However, after treatment with the 17-TPGF2α, there was a significant reduction in ALT, ALP, and AST in the test group when compared with DMBA treated group.

According to earlier research, activation of the NK1R can cause phosphorylation of the Akt and MAPK pathways, which activates numerous transcription factors that control the expression of target genes. And further simulates inflammatory induced pathogenesis (Akazawa et al. 2009; Christian et al. 1994; Koon et al. 2007; Lieb et al. 1997; Luo et al. 1996). Our study is in agreement with previous research that 17-TPGF2α attenuates inflammation, based on histological examination, observation of cellular morphology showed a significant improvement. The antiproliferative nature of 17-TPGF2α is further confirmed by the histological investigation, as seen by the difference in severity between the 17-TPGF2α treated group and the DMBA treated group. E. Nizam, N. Erin demonstrated macrophage inflammatory protein-2 (MIP-2) an angiogenic and inflammatory chemokine where it would be considered responsible for the metastatic spreading of cancer. It is secreted by aggressive metastatic BC and melanoma cells. MIP-2 secretion is inhibited by an NK1R antagonist, which is likely owing to decreased p38, and MAPK phosphorylation. MIP-2 is implicated in the secretion of both inflammatory and angiogenic cytokines (Nizam and Erin 2018). By our findings, and in vivo anticancer effect for NK1R antagonist has previously been described in malignant glioma cell lines, human BC, brain tumors, human osteosarcoma, and hepatoblastoma cell lines (Molinos-Quintana et al. 2019).

According to Lang et al., SP causes a metastatogenic phenotype in MDA-MB-468 BC cells by enhancing the expression of the adhesive protein b2 integrin. L-733060, an NK1R antagonist, can block this migratory impact. They further suggested that these effects are linked to decreased MAPK pathway activity as well as a lower steady-state of Her2 and epidermal growth factor receptor (EGFR). They also discovered that inhibiting SP lowers cell death in anti-Her2 resistant cells (Lang et al. 2004). According to these findings, our test 17-TPGF2α may exhibit the same effects as those indicated previously. Further research is required to prove the detailed process in our findings. Furthermore, 17-TPGF2α had the same effects as Aprepitant, which we used as a controlled drug in our in vivo trial. Aprepitant has been shown to increase the sensitivity of triple-negative BC cells to doxorubicin (Dox) and reduce the cardiotoxicity caused by Dox. Taken together, the above-mentioned anticholinergic system agent has been shown to exert significant anticancer effects. However, the application in clinical settings has yet to be recognized in further studies.

**Conclusion**

In vivo experiments back up the result that 17-TPGF2α significantly reduces tumor growth in mice. Based on these findings, the Regulation of SP and NK1R expression, as well as suppression of potential signaling pathways linked with SP and NK1R, may be used to initiate novel therapeutic options for BC in upcoming years. The use of an NK1R antagonist in combination with current chemotherapeutics may provide further clinical benefits.

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Author Contributions MM performed the experiments and major contribution in the manuscript writing. TM was responsible for analyzing the data and major contribution in editing the manuscript. TK performed the molecular docking studies and interpreted the results.

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Declarations

Conflict of interest Authors declared that they have no conflicts of interest.

Ethical Approval The study was formally approved by the Institutional Animal Ethical Committee of SRM School of Pharmacy with ID IAEC/243/2021.

Informed Consent Not applicable.

References

Akazawa T, Kwatra SG, Goldsmith LE, Richardson MD, Cox EA, Sampson JH, Kwatra MM (2009) A constitutively active form of neurokinin 1 receptor and neurokinin 1 receptor-mediated apoptosis in glioblastomas. J Neurochem 109(4):1079–1086 (Research Support, NIH, Extramural Research Support, Non-US Gov’t)

Bancroft JD, Layton C (2019) 10—The hematoxylins and eosin. In: Suvarna SK, Layton C, Bancroft JD (eds) Bancroft’s theory and practice of histological techniques, 8th edn. Elsevier, London, pp 126–138

Berger M, Neth O, Ilmer M, Garnier A, Salinas-Martín MV, de Agustín Christian C, Gilbert M, Payan DG (1994) Stimulation of transcriptional regulatory activity by substance P. Neuroimmunomodulation 1(3):159–164 (Research Support, Non-US Gov’t, PHS)

Dakrory AI, Fahmy SR, Soliman AM, Mohamed AS, Amer SA (2015) Protective and curative effects of the sea cucumber Holothuria atra extract against DMBA-induced hepatoparenchymal lesions in rats. Biomed Res Int. https://doi.org/10.1155/2015/563652

Dong J, Feng F, Xu G, Zhang H, Hong L, Yang J (2015) Elevated SP/NK-1R in esophageal carcinoma promotes esophageal carcinoma cell proliferation and migration. Gene 560(2):205–210 (Research Support, Non-US Gov’t)

Fries H, Zhu Z, Liard V, Shi X, Shrikhande SV, Wang L et al (2021) Neurokinin-1 receptor expression and its potential effects on tumor growth in human pancreatic cancer. Lab Invest 83(5):731–742. https://doi.org/10.1038/labinvest.2021.51

González-Ortega A, Sánchez-Vaderrábanos E, Ramiro-Fuentes S, Salinas-Martín MV, Carranza A, Coveñas R, Muñoz M (2014) Uveal melanoma expresses NK-1 receptors and cyclosporin A induces apoptosis in human melanoma cell lines overexpressing the NK-1 receptor. Peptides 55:1–12

Horobin RW (2019) 9 - Theory of histological staining. In: Suvarna SK, Layton C, Bancroft JD (eds) Bancroft’s theory and practice of histological techniques, 8th edn. Elsevier, London, pp 114–125

Koon HW, Zhao D, Zhan Y, Moyer MP, Pothisoulakis C (2007) Substance P mediates antiapoptotic responses in human colonocytes by Akt activation. Proc Natl Acad Sci USA 104(6):2013–2018 (Research Support, NIH, Extramural Research Support, Non-US Gov’t)

Krishnamoorthy D, Sankaran M (2016) Modulatory effect of Pleurotus ostreatus on oxidant/antioxidant status in 7, 12-dimethylbenz (a) anthracene induced mammary carcinoma in experimental rats—a dose–response study. J Cancer Res Ther 12(1):386–394 (Research Support, Non-US Gov’t)

Lang K, Drell TL, Lindecke A, Niggemann B, Kalt Schmidt C, Zaecker KS, Entschladen F (2004) Induction of a metastatogenic tumor cell type by neurotransmitters and its pharmacological inhibition by established drugs. Int J Cancer 112(2):231–238 (Research Support, Non-US Gov’t)

Layton C, Bancroft JD, Suvarna SK (2019) 4—Fixation of tissues. In: Suvarna SK, Layton C, Bancroft JD (eds) Bancroft’s theory and practice of histological techniques, 8th edn. Elsevier, London, pp 40–63

Lieber K, Fiebich BL, Berger M, Bauer J, Schulze-Osthoff K (1997) The neuropeptide substance P activates transcription factor NF-kappa B and kappa B-dependent gene expression in human astrocytoma cells. J Immunol 159(10):4952–4958 (Research Support, Non-US Gov’t)

Luo W, Sharif TR, Sharif M (1996) Substance P-induced mitogenesis in human astrocytoma cells correlates with activation of the mitogen-activated protein kinase signaling pathway. Cancer Res 56(21):4983–4991 (Research Support, Non-US Gov’t Research Support, US Gov’t, PHS)

Molinos-Quintana A, Trujillo-Hacha P, Piruat JI, Bejarano-García JA, García-Guerrero E, Pérez-Simón JA, Muñoz M (2019) Human acute myeloid leukaemia cells express Neurokinin-1 receptor, which is involved in the antileukemic effect of Neurokinin-1 receptor antagonists. Investig N Drugs 37(1):17–26

Munoz M, Covenas R (2012) NK-1 receptor antagonists: a new generation of anticancer drugs. Mini Rev Med Chem 12(7):593–599 (Research Support, Non-US Gov’t Review)

Muñoz M, Coveñas R (2014) Involvement of substance P and the NK-1 receptor in pancreatic cancer. World J Gastroenterol 20(9):2321–2334. https://doi.org/10.3748/wjg.v20.i9.2321

Muñoz M, Coveñas R (2015) Targeting NK-1 receptors to prevent and treat pancreatic cancer: a new therapeutic approach. Cancers 7(3):1215–1232

Muñoz M, Rosso M (2010) The NK-1 receptor antagonist aprepitant as a broad spectrum antitumor drug. Investig N Drugs 28(2):187–193 (Research Support, Non-US Gov’t)

Muñoz M, González-Ortega A, Rosso M, Robles-Frias MJ, Carranza A, Salinas-Martín MV, Coveñas R (2012) The substance P/neurokinin-1 receptor system in lung cancer: focus on the antitumor action of neurokinin-1 receptor antagonists. Peptides 38(2):318–325 (Research Support, Non-US Gov’t)

Muñoz M, González-Ortega A, Salinas-Martín MV, Carranza A, García-Recio S, Almendro V, Coveñas R (2014) The neurokinin-1 receptor antagonist aprepitant is a promising candidate for the treatment of breast cancer. Int J Oncol 45(4):1658–1672. https://doi.org/10.3892/ijo.2014.2565

Muñoz M, Covenas R, Esteban F, Redondo M (2015a) The substance P/NK-1 receptor system: NK-1 receptor antagonists as anti-cancer drugs (Review). J Biosci 40(2):441–463

Muñoz M, Recio S, Rosso M, Redondo M, Covenas R (2015b) The antiproliferative action of [D-Arg (1), D-Phe (5), D-Trp (7, 9)] substance P analogue antagonist against small cell-and non-small-cell lung cancer cells could be due to the pharmacological profile of its tachykinin receptor antagonist. J Physiol Pharmacol 66(3):421–426

Muñoz M, Rosso M, Coveñas R (2017) The neurokinin-1 receptor antagonist L-732138 induces apoptosis in human gastrointestinal cancer cell lines. Pharmacol Rep 69(4):696–701
Nizam E, Erin N (2018) Differential consequences of neurokinin receptor 1 and 2 antagonists in metastatic breast carcinoma cells; effects independent of Substance P. Biomed Pharmacother 108:263–270

OECD (2002) Test No. 423: acute oral toxicity—acute toxic class method. OECD, Paris

Orosz A, Schrett J, Nagy J, Bartha L, Schön I, Nyéki O (1995) New short-chain analogs of a substance-P antagonist inhibit proliferation of human small-cell lung-cancer cells in vitro and in vivo. Int J Cancer 60(1):82–87 (Research Support, Non-US Gov’t)

Rozengurt E (2002) Neuropeptides as growth factors for normal and cancerous cells. Trends Endocrinol Metab 13(3):128–134 (Research Support, US Gov’t, PHS Review)

Sápi J, Kovács L, Drexler DA, Kocsis P, Gajári D, Sápi Z (2015) Tumor volume estimation and quasi-continuous administration for most effective bevacizumab therapy. PLoS ONE 10(11):e0142190. https://doi.org/10.1371/journal.pone.0142190

Singh D, Joshi DD, Hameed M, Qian J, Gascón P, Maloof PB et al (2000) Increased expression of preprotachykinin-I and neurokinin receptors in human breast cancer cells: implications for bone marrow metastasis. Proc Natl Acad Sci USA 97(1):388–393 (Research Support, Non-US Gov’t Research Support, US Gov’t, PHS)

Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A (2015) Global cancer statistics, 2012. CA Cancer J Clin 65:87–108. https://doi.org/10.3322/caac.21262

U.S. breast cancer statistics. https://www.cancer.org/cancer/breast-cancer/about/how-common-is-breast-cancer.html. Accessed 4/2/2021

Wolfe D (2019) 6—Tissue processing. In: Suvarna SK, Layton C, Bancroft JD (eds) Bancroft’s theory and practice of histological techniques, 8th edn. Elsevier, London, pp 73–83

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