Substance P/NK1R antagonistic effect of 17-Trifluoromethyl phenyl trinor prostaglandin F2 α in Breast cancer

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Abstract

17-Trifluoromethyl phenyl trinor prostaglandin F2 α (17-TPGF2 α) is extracted from Zinc data base, but its value for inhibiting breast cancer through SP/NK1R system remains unknown. This study was designed to investigate the potential antagonist effect of 17-TPGF2 α through NK1Receptor. The effect of 17-TPGF2 α on the proliferation and apoptosis of breast cancer cell lines were determined through invitro cell lines. Based on invitro results we planned to investigate anticancer activity in Female Balb/c and used SC injection of DMBA for cancer induction. Oral administration of 17-TPGF2 α significantly suppress the tumor volume as compared with an untreated group. The serum parameters like ALP, AST and ALT and haematological parameters were normalized in test treated group. Histological examination revealed normal histoarchitecture of mammary gland and focal areas showed minimal inflammatory cell infiltration. There is no necrosis is seen both test treated and standard treated group when compared with Disease group. All these findings concluded that 17-TPGF2 α may have potential as a novel antitumor candidate for breast cancer.

1. Introduction

Neuropeptide like substance P (SP) is becoming more widely recognized as powerful cellular growth factors involved in the regulation of normal and pathological cell proliferation [1]. The expression and secretion of peptides by tumours is gaining popularity, and new avenues for translational research are opening up with the potential to improve tumour diagnostics and treatment [2]. Furthermore, research suggests that neuropeptides may have a role in the progression of cancer. SP that has been demonstrated to operate as a mitogen on various human cancer cell lines by binding to the neurokinin-1 (NK-1) receptor. Antitumor action of NK-1 receptor antagonists has been demonstrated in such cell lines, and these antagonists have been proven to promote tumour cell death [3, 4]. 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 [5]. These findings imply that the SP/NK-1 receptor system may play a role in cancer development, and that NK-1 receptor antagonists could be used as a broad-spectrum antitumor drug. In this study, we evaluated the effect of NK1 receptor blocked in Balb/c mice by using DMBA induced breast cancer. One in every 8 women in the United States is likely to get invasive breast cancer during their lifetime, with 281,550 new invasive breast cancer cases and 49,290 non-invasive breast cancer cases predicted to be identified in women in the United States in 2021[6]. Therefore, it is an importance for the development of 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 tumour cell. In the present study, we evaluated the anticancer activity of 17-trifluorophenylmethyl prostaglandin F2 against breast cancer. The compound selected based as on the *invitro* results and molecular docking results.

2. Materials And Methods

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

2.2 Animals and Ethical Consideration: Healthy Female Balb/c mice, weighing of 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 Balb/c mice were randomly divided into four groups, comprising normal control, disease control, test group (17-trifluoromethyl phenyl trinor prostaglandin F2 alpha) and Standard group (Aprepitant).

2.3 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 weighted between 18-25g and were 4-5 weeks old. All the animals were divided into 4 groups, 5 animals in each group. Before of 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 commencement of experiment. Normal saline was administered to animal in group1, a normal group while animals in group2, 3 and 4 were given 50mg/kg, 300mg/kg and 2000mg/kg doses of 17-TPGF2a respectively. All animals were observed 30min, 60mins and 4 hrs after test drug and vehicle administered and observation continued for the next 14 days for any mortality and any autonomous or neurological changes [7].

2.4. Breast cancer induction and treatment

Prepared DMBA solution (20mg/kg dissolved in flax seed oil) were subcutaneously administered into the mice breast pad based on the body weight. From the first day of induction, animals in normal and disease group received 0.9% saline water [8]. The test drug was given orally at 50 and 100 mg/kg of body weight on a regular basis. The body weight of each mice was measured every week. At the end of the study, blood samples were collected for an estimation of biochemical parameters and the tumors were quickly dissected away from surrounding tissue and weighed, then stored at -80°C

2.5. Tumor Volume

Tumor volume (TV) is estimated by measuring the width (W) and length (L) of the tumor using digital caliper and calculated based on the following formula [9].

$$\text{TV (mm}^3\text{)} = W^2 \times L \div 2$$

2.6. Biochemical and Hematological analysis

The mice serum and blood samples were used to detect the biochemical and hematological parameters. These are Aspartate transaminase (AST), Alanine transaminase (ALT), Total blood count, White blood

cells (WBC), Haemoglobin (Hb), Mean cell haemoglobin concentration (MCHC), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), Packed cell volume (PCV).

2.7. Histopathological examination

Mammary glands were dissected and fixed in 10% formalin solution. This slowly allows to penetrate into 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 [10–13].

3. Statistical Analysis

All results are shown as mean $\pm$ SEM for each experimental group. The statistical analysis were performed by using Graph pad Prism 5.0 and used one Way ANOVA followed by Tukeys method. Statistical significance of difference was considered at a p value <0.05 .

4. Results

4.1. Acute toxicity study

Balb/c female mice were administered 50, 300 and 2000mg/kg of 17-TPGF2 α in determining the acute toxicity of 17-TPGF2 α . A normal group animals received only the vehicle and all treatment group 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 rabbit, where the LD50 was found at the dose of 3742 mg/kg. In the present study, we have selected the dose of 25.41 mg/Kg based on the results of acute toxicity and dose conversion factor from rabbit dose.

4.1. Effect of DMBA on mammary glands

Breast tumor was induced in animals that received DMBA (Figure.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. When average tumor volume reaches 100mm³, mice were randomized into various group and treated with 17-TPGF2 α orally (dose 25.41 mg/Kg), and Standard (Aprepitant).

4.2. Effect of 17-TPGF2 α on mice body weight

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

4.3. Effect of 17-TPGF2 α on water and Food intake

Figure represents the alterations in water intake during the treatment. All the animals showed an increased in water intake during the course of the experiment. A significant increase in water intake was

observed in animals treated with 17-TPGF2 four times per week. Moreover, Aprepitant treated animals also showed a significant rise of water intake when compared to normal animals and disease control group. A significant rise in food intake was observed in 17-TPGF2 and standard group animals when compared with DMBA group on day 55 (figure 3&4.).

4.4. Effect of 17-TPGF2a on breast tumors

Tumor volume measurement cannot happen right after cancer induction. On 3.5 week of DMBA administration, tumor started to grow out. In our study, tumor volume was measured by using Caliper, in that case tumor diameter (width and length) was measured. First measurement was done on 25 day and treated was started after when the tumor volume was reached 100mm³. tumor volumes were measured bi-weekly and estimated by using the above mentioned formula.

The tumor volume of mice from disease or DMBA group grew continued to increase until day 80. Where, the tumor volume of DMBA group grew up to 746.2 mm³ and those of test (56.48 mm³) and standard (57.16 mm³) Figure 5. The animal received test drug showed gradual decline in tumor volume from the day of administration to the end of the experiment with an average tumor volume 56.48 mm³ as compared to DMBA group. As expected 17-TPGF2a and Aprepitant significantly decreased the incidence of tumor growth and tumor volume.

4.5. Effect on Biochemical and Haematological parameters

The impact of test drug on biochemical and haematological parameters is depicted in Table1. And figure 6. There was a normal serum levels 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 disease or DMBA group. More, interestingly, test group (17-TPGF2a) had decreased serum levels of ALT (13.04±0.587), AST (26.23±0.70) and ALP (53.93±0.68) than 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 25.41mg/kg, 17-TPGF2a regulates normal WBC (1388±108.9), platelet count (6.08±0.02), haemoglobin (15.14±0.014), 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.

Table.1: Biochemical and Haematological parameters

Parameters	Normal Group	Disease(DMBA group)	Test(17-TPGF2a)	Standard (Aprepitant)
ALT	11.45±0.16	19.54±0.19 ^{***}	13.04±0.58 ^{###}	14.00±0.70 ^{aaa}
AST	25.59±0.199	32.52±0.35 ^{***}	26.23±0.70 ^{###}	24.38±0.54 ^{aaa}
ALP	53.93±0.20	60.96±0.42 ^{***}	53.93±0.68 ^{###}	52.58±0.39 ^{aaa}
Total WBC	1350±12.91	2647±32.83 ^{***}	1388±108.9 ^{###}	1363±124.2 ^{aaa}
Platelet count	6.33±0.07	6.95±0.05 ^{***}	6.08±0.02 ^{###}	6.22±0.15 ^{aaa}
Haemoglobin	15.09±0.034	12.50±0.006 ^{***}	15.14±0.04 ^{###}	15.19±0.006 ^{aaa}
RBC	7.17±0039	5.96±0.017 ^{***}	7.19±0.043 ^{###}	7.18±0.08 ^{aaa}
PVC	36.74±0.33	32.28±0.02 ^{***}	36.42±0.20 ^{###}	35.93±0.14 ^{aaa}
MCV	54.30±0.008	46.46±0.15 ^{***}	53.52±0.14 ^{###}	53.84±0.20 ^{aaa}
MCH	56.28±0.074	53.38±0.186 ^{***}	56.21±0.16 ^{###}	56.29±0.09 ^{aaa}
MCHC	41.53±0.14	38.69±0.14 ^{***}	41.44±0.09 ^{###}	41.52±0.13 ^{aaa}

Normal rats given Flax seed oil only, Disease group subcutaneously administered with DMBA and fed with flax seed oil, test group treated with 17-TPGF2a at dose of 25.41mg/kg. Data are represented as mean ± SEM (n=6), normal Vs disease (p<0.05^{*}, p<0.01^{**}, p<0.001^{***}), disease Vs test (p<0.05[#], p<0.01^{##}, p<0.001^{###}), disease Vs standard (p<0.05^a, p<0.01^{aa}, p<0.001^{aaa})

4.6. Histological examination

The mammary glands of mice from normal group showed normal acinic surrounded by a longitudinal sections of skeletal muscle fibers with intervening mature adipose tissue. All showing normal morphology and further, there is no necrosis or inflammation reported. Mice received DMBA only shows an extensive necrotic areas with inflammatory cell infiltration and hyperplasia of mammary glands. Adjacent skeletal muscle shows varying sizes and further focal areas showed abundant lymphatic infiltration. Both test treated and standard treated group showed normal histoarchitecture of mammary gland and focal areas showed minimal inflammatory cell infiltration. There is no necrosis is seen (figure.7).

5. Discussion

Currently, Breast tumour is now the largest cause of cancer-related death in both developed and developing countries [14]. It is critical to understand the underlying molecular pathways that promote BC carcinogenesis in order to minimize BC incidence and mortality and enhance patient outcomes. Despite advancements in breast cancer 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 in order to reach the ultimate objective of developing molecules that selectively target cancer cells while causing minimal side effects [15].

In latest days, the involvement of tachykinin neuropeptides in cancer pathogenesis and progression has already been highly drawn interest from researchers. Up regulation of Substance P and NK1R has been seen in glioblastoma, retinoblastoma, lung, endometrial, and pancreatic cancers, and has been linked to cancer growth and development [16, 17]. Several research has recently focused at the anticancer effect of a variety of chemical inhibitors of the Substance P/NK1R pathway, which have resulted in a considerable reduction in breast cancer carcinogenesis [18].

In vivo and *in vitro* studies have shown that Neurokinin 1 receptor antagonists like Aprepitant, L-733,060, and L-732,138 have anticancer properties in hepatoblastoma, small cell and non-small cell lung, pancreatic, and gastrointestinal cancer cells [19–22]. Accumulating of findings speculated that SP/NK1 receptor complex plays a significant function in tumorigenesis and progression of carcinoma, SP has been linked cancerous cell growth, neoangiogenesis and invasiveness, further, NK1 receptors being overexpressed in tumoral cells and malignant tissue [23–25].

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 invitro investigations on BC cell lines. *In vitro* study revealed that 17-TPGF2 α inhibited the development of MCF-7 and MDA-MB-468 cells through inducing apoptosis via the Caspase-3 enzyme. 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 MAPK pathway being one probable mechanism for the NK1R antagonist's apoptotic effects.

In 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 normal group, the levels of serum biochemical and haematological parameters was relatively low. Here, it was found that after 3rd weeks of administration, 70% of animals developed cancer in DMBA group. The tumor continued to grow until end of the experiment in DMBA administered group and even some animals were died during the experiment. Further, it found that 17-TPGF2 α prevents the breast cancer growth and tumor incidence by the decrease of the tumor volume and weight when compared to DMBA group until the last administration on day 80.

In the present study, 17-TPGF2 α was found nontoxic to mice when given by oral route. All the administrated animals were safe up to the dose 25.41mg/kg and 17-TPGF2 α significantly decreases the

haematological parameters when compared to disease or DMBA group. As far as hematological parameters are concerned, all animals that administered DMBA showed considerable reduction in RBC levels. Further, animals that received 17-TPGF2 α showed normal RBC levels. Moreover, the activities of transaminase like ALT, ALP, and AST were also significantly controlled in test group when compared with disease 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 [26–30]. Our is agreement with previous research that 17-TPGF2 α attenuates inflammation, based on histological examination, observation of cellular morphology showed normal and there is a minimal inflammatory cell infiltration and no necrosis seen when compared to DMBA group. This also suggest a strong believe that the 17-TPGF2 α has potential Antibreast cancer activity.

E.Nizam, N.Erin demonstrated MIP-2 an angiogenic and inflammatory chemokine where it would be considered as responsible for metastatic spreading of cancer. It is secreted by aggressive metastatic breast cancer and melanoma cells. MIP-2 secretion is inhibited by an NK1R antagonist, which is likely owing to decreased p38, MAPK phosphorylation. MIP-2 is implicated in the secretion of both inflammatory and angiogenic cytokines [31]. In accordance with our findings, an *in vivo* anticancer effect for NK1R antagonist has previously been described in malignant glioma cell lines, human breast cancer, brain tumours, human osteosarcoma, and hepatoblastoma cell lines [32].

According to Lang et al., Substance P causes a metastatogenic phenotype in MDA-MB-468BC 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 EGFR. They also discovered that inhibiting SP lowers cell death in anti-Her2 resistant cells [33]. According to these findings, our test 17-PGF2 α may exhibit the same effects as those indicated previously. Further research is required to prove the detailed process in our findings. Furthermore, 17-TPGF2 α had the same effects as Aprepitant, which we used as a control drug in our *in vivo* trial. Aprepitant has been shown to increase the sensitivity of triple negative breast cancer cells to doxorubicin (Dox) and reduce the cardiotoxicity caused by Dox. Taken together, the above mentioned anti-tachykinergic system agent has been shown to exert significant anticancer effects. However, the application in clinical settings has yet to be recognized in further studies.

6. Conclusion

In vivo experiments back up the result that 17-TPGF2 α significantly reduces tumour growth in mice. Based on these findings, Regulation of Substance P and NK1R expression, as well as suppression of potential signalling pathways linked with Substance P and NK1R, may be used to initiate novel therapeutic options for breast cancer in upcoming years. The use of an NK1R antagonist in combination with current chemotherapeutics may provide further clinical benefits.

Declarations

Acknowledgment

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Conflict of interest

Authors declared that they have no conflicts of interest.

Statement and Declaration

All the authors declare that this work has been done by ourselves. We confirmed that the manuscript was read and approved by all authors and there are no other persons who satisfied the criteria for authorship but are not listed.

Author's contributions:

MM performed the experiments and major contribution in the manuscript writing. T.M was responsible for analyzing the data and major contribution in editing the manuscript. T.K was performed the molecular docking studies and interpreted the results

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Figures

Figure 1

Effect of 17-TPGF2a on tumor growth

Disease group showed increased tumor growth when compared with 17-TPGF2a treated group and also showed decreased tumor growth in standard group. (A) DMBA induced breast cancer tissue, (B) 17-TPGF2a treated group's breast cancer tissue. (C) Aprepitant treated group's breast cancer tissue.

Figure 2

Effect of 17-TPGF2a on body weight. 17-TPGF2a treated group showed normal body weight as compared with DMBA treated animals. Data are represented as mean $\pm$ SEM (n=6), normal Vs disease ($p < 0.05^*$, $p < 0.01^{**}$, $p < 0.001^{***}$), disease Vs test ($p < 0.05^{\#}$, $p < 0.01^{\#\#}$, $p < 0.001^{\#\#\#}$)

Figure 3

Effect of 17-TPGF2a on Food intake. Normal animals fed with Flaxen oil; DMBA a negative control treated with flaxen oil; Test group treated with 17-TPGF2a and standard group treated with Aprepitant. The values are significantly represented as mean $\pm$ SEM (n=6), normal Vs disease ($p < 0.05^*$, $p < 0.01^{**}$, $p < 0.001^{***}$), disease Vs test ($p < 0.05^\#$, $p < 0.01^{\#\#}$, $p < 0.001^{\#\#\#}$).

Figure 4

Effect of 17-TPGF2a on Water intake. Normal animals fed with Flaxen oil; DMBA a negative control treated with flaxen oil; Test group treated with 17-TPGF2a and standard group treated with Aprepitant. The values are significantly represented as mean $\pm$ SEM (n=6), normal Vs disease ($p < 0.05^*$, $p < 0.01^{**}$, $p < 0.001^{***}$), disease Vs test ($p < 0.05^\#$, $p < 0.01^{##}$, $p < 0.001^{###}$).

Figure 5

Effect of 17-TPGF2a on Tumor Volume. Normal animals fed with Flaxen oil; DMBA a negative control treated with flaxen oil; Test group treated with 17-TPGF2a and standard group treated with Aprepitant. 17-TPGF2a treated group showed decrease tumor volume from start of the treatment to the end of the experiment (80 day) when compared with diseased group. Data are represented as mean $\pm$ SEM (n=6), normal Vs disease ($p < 0.05^*$, $p < 0.01^{**}$, $p < 0.001^{***}$), disease Vs test ($p < 0.05^\#$, $p < 0.01^{##}$, $p < 0.001^{###}$), disease Vs standard ($p < 0.05^a$, $p < 0.01^{aa}$, $p < 0.001^{aaa}$).

Figure 6

Effect of 17-TPGF2a on serum ALP, AST and ALT parameters. 17-TPGF2a treated group showed normal serum parameters as compared with DMBA treated animals. Data are represented as mean $\pm$ SEM (n=6), normal Vs disease ($p < 0.05^*$, $p < 0.01^{**}$, $p < 0.001^{***}$), disease Vs test ($p < 0.05^{\#}$, $p < 0.01^{\#\#}$, $p < 0.001^{\#\#\#}$).

Figure 7

Histopathological examination

Effect of 17-TPGF2a on microphotographs H& E 800x of mammary glands. (A) Normal group, (B) DMBA administered group, (C) 17-TPGF2a animals, (D) Standard- Aprepitant group. DMBA administered animals showed an extensive necrotic areas with inflammatory cell infiltration and hyperplasia of mammary glands than Normal group. Both test treated and standard treated group showed normal histoarchitecture of mammary gland and focal areas showed minimal inflammatory cell infiltration. There is no necrosis is seen.