Anti-Prolactin and Antigrowth Hormone Effects on Oxidative Stress and Physiological Parameters in MNU Induced Mammary Tumor in Sprague Dawley Rat

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Abstract

Breast cancer is the most common cancer diagnosed in women, accounting for more than 1 in 10 new cancer diagnosed and second most common cause of death among women. This study was intended to assess the effect of cabergoline and octreotide in tumorigenesis and progression prior to and post tumor induction in Sprague Dawley rats. 30 day old inbred SD female rats of body weight (70-80 grams) were taken for this study and N- Methyl-Nitroso-Urea was used for mammary tumor induction. The rats were pre inhibited with cabergoline & octreotide alone and in combination followed by MNU and post treated group (MNU followed by drugs) were studied. The parameters such as tissue glutathione, malondialdehyde and physiological parameters like tumor weight, tumor occurrence, volume were measured.

The Pre- treated combination group showed a significant decrease in mean body weight tumor weight, tumor volume, tumor latency, tumor incidence rate compared to post inhibited group. A near normal levels of tissue GSH, and a decrease levels in MDA was observed in pre-inhibited combination group compared to post hormone inhibited group.

The results suggest that pretreatment with combination of cabergoline and octreotide is a better therapeutic measure than single drug alone for inhibition of tumorgenesis.

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Introduction

Cancer a genetic and the second leading cause of death globally. It remains a significant challenge for societies, healthcare systems, and affected individuals worldwide[1]. As per the estimation about 9.6 million deaths in 2018 globally is due to cancer[2]. Carcinoma of breast is a heterogeneous condition which occurs due to the abnormal growth of the breast epithelial cells[2]. The etiology can be one of these factors, genetic, hormonal, lifestyle, environmental and dietary[3]. Other factors such as genes BRCA1 and BRCA2, also impose greater risk of carcinoma breast[4]. It is known that around two third of all breast cancers diagnosed are as hormone dependent cancer[5]. The steroidal hormones such as estrogen and progesterone have been extensively studied in its involvement on breast cancer. Most recent research suggests the prime importance of prolactin and growth hormone in breast cancer[6].

The hormones and their receptors both, are known to play a key role in diagnosis of breast cancer and management alone or in combination with other hormones. Many experimental and clinical studies on breast cancer patients showed the role of tissue prolactin (locally released) in mammary carcinogenesis[7]. Studies have reported higher prolactin levels were observed despite its pituitary inhibition[8]. Apart from prolactin, growth hormone which is structurally similar to prolactin can easily bind to the prolactin receptors and thus hasten the progression of breast cancer[9].

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Early diagnosis of the cancer remains the keystone for its prevention and cure. Recent research is focused on the diagnostic and pathological relevance of prolactin, growth hormone to breast cancer. Evidences from rat and human breast and prostate tumor models support the fact that locally produced prolactin is a potent stimulator of tumor growth acting via autocrine/paracrine mechanisms[7].

Growth hormone signaling promotes the mesenchymal transition from epithelia by influencing several genes that are involved in the process. It plays a vital role in promoting the free growth of the breast cancer cells[7,10]. Experimental studies on cell lines wherein these hormones were inhibited with specific inhibitors yielded conflicting results[7,9].

Therefore, further studies are to be conducted to evaluate the diagnostic utility of prolactin, growth hormone in the pathobiology of mammary cancer. A lack of extensive information remains, about the effects of hormone inhibitor of prolactin and growth hormone. This forms the basis for the present study which was designed to evaluate the role of cabergoline, prolactin inhibitor and octreotide, growth hormone inhibitor on carcinogenesis and progression.

Materials and methods

In-house bred healthy female Sprague Dawley (SD) rats (40 to 60 days old) of weight 80-100 gmwere selected for the study. The rats were maintained in 12 hours' light and 12 hours' dark cycle and also in temperature and humidity controlled environment. All rats were fed with standard rat food) and water ad libitum. Institutional Animal Ethical Committee (IAEC) approval was obtained before the conduct of the study (IAEC/KMC/28/02/14) and care was taken to handle the rats in humane manner. The study was performed in accordance with the guidelines suggested by Basic and Clinical Pharmacology and Toxicology policy for experimental and clinical studies [12]. The animals were divided into eight groups and each group consists of 6 animals (Table 1)

Experimental design:

Induction of tumor:

Preparation of the carcinogen: Mammary tumor was induced by injecting N-Methyl-N-Nitrosourea dissolved in normal saline with pH- 4 maintained by adding 3% glacial acetic acid. The SD rats were given a single intraperitoneal dose of 50 mg/kgbw of MNU and kept for observation for the mammary tumor development.

Groups (n=6)	Types	Treatment	Duration
I	control	0.9% normal saline	2 months
II	Tumor induced	MNU 50mg/kbw (IP)	Single dose
III	Carbergoline (+ve control)	0.5 mg/kg (OD)	2 months
IV	Carbergoline (c) + MNU(pre treated)	0.5mg (OD) + 50mg/kg	2 months
IVa	MNU+ cabergoline (post treated)	50mg/ 0.5mg	2 months
V	Octreotide (o) (+ve control)	50 µg/kg (OD)	2 months
VI	Octreotide(o) +MNU (pre treated)	50 µg/kg (OD)+ 50mg/kbw	2 months
VIa	MNU+ octreotide	50mg/kg +50 µg/kg	2 months
VII	C+O (positive control)	0.5 mg/kg+50 µg/kg	2 months
VIII	C+O+MNU (pretreated)	0.5 mg/kg +50 µg/kg (OD)+ 50mg/kbw	2 months
VIII A	MNU+ cabergoline + octreotide	50mg/kg+0.5 mg/kg +50 µg/kg	2 months

Table 1. Animal group.

Drugs

Cabergoline (Cabgolin 0.25 mg tablets (Sun Pharma India Limited),were obtained from Pharmacy section, Kasturba Medical College Hospitals, Mangalore. cabergoline (0.5mg/kg body weight) was prepared by dissolving two tablets in 5 ml of distilled water for oral administration to the animals

Octreotide (Neoctide) - 0.05 mg/ml ampoule (Neon Laboratories India Limited) were obtained from Pharmacy section, Kasturba Medical College Hospitals, Mangalore. Standard intradermal dose of octreotide to be given was calculated according to the dosage of 50µgm/kg body weight of the animals. Inbred female SD rats were selected were divided into six groups(n=6).

The animals were divided based on pre and post treatment of drug with induction of MNU (Table 1)

Preparation of Tissue homogenate:

Animals were sacrificed during the morning time (9 am - 11 am) to avoid any variations in the antioxidant levels of the tissue. The mammary tumor was surgically excised and part of the tissue was dissected and weighed

after washing the tissue in an ice cold phosphate buffer saline (pH 7.4) for the tissue homogenate preparation (10% w/v). This homogenate was centrifuged at 8000 rpm for 15 minutes at 4º C and supernatant was used for biochemical estimations. Lipid peroxidation (LPO) assay and tissue reduced Glutathione (GSH) as per standard method [13,14]

Results

Groups (n=6)	Initial weight (g)	Final weight (g)	Weight change ((Δ in g)
Control Group I	71.50±4.08	294.16 ± 12.81	222.66 ± 11.65#
Tumour induced Gr.II (MNU)	72.00±2.44	238.66 ± 9.52	166.66 ± 7.08 *
Cabergoline Gr.III	68.50±1.90	277.83 ± 6.76	209.33 ± 5.75*
Cabergoline + MNU Gr.IV	71.83±1.83	255.33 ± 10.23	183.50 ± 10.76*
MNU+ cabergoline Gr.IVa	73.83±3.97	240.50 ±8.89	166.66 ±5.64*
Octeriotide Gr.V	69.83±2.56	278.00 ± 2.44	208.16 ± 3.65*
Octeriotide+ MNU Gr.VI	74.83±2.92	240.66 ± 4.80	165.83± 3.37*$
MNU +0cteriotide Gr.VIa	76.00±3.40	226.66 ± 8.75	150.66 ± 6.34 *
C+O Gr.VII	70.50±4.37	290.66 ± 3.93	220.16 ± 2.40
C+O+MNU Gr.VIII	75.33±3.72	263.83 ± 10.77	188.50 ± 8.98#
MNU+C+O Gr.VIIIa	76.00±3.40	239.33 ± 4.54	163.33 ± 5.16*

Table 2. Mean Body Weight [Initial, Final and Weight Change (Δ)] in the Groups.
Values expressed as Mean ± SD, (n = 6 animals/ group).
*p<0.001 Vs **Control,** # p<0.001 Vs MNU induced, $ p<0.05 group IV vs group VI.

Groups (n=6)	(TL)Total number of days	TIR	TM
I (MNU induced)	65	100	2$
II Cabergloine + MNU	92 #£	50	1 #$
II A MNU + cabergoline	65	100*	2£
III octeriotide + MNU	80#£	66.66	1#$
IIIA MNU + octeriotide	63	100 *	2£
IV c+O+MNU	110#	16.66	0 #
IV A MNU+C+O	65	83.3$	1#$

Table 3. Comparison of Tumour Latency (TL), Tumour Incidence Rate (TIR) Tumour Multiplicity.
#p<0.01 Vs Tumour induced , $p<0.001 II Vs (III & IV)), £ p<0.001 IV Vs (II & III). * p<0.001IVA vs(IIA, IIIA).

3.1. Change in Mean Body Weight (Final Body Weight – Initial Body Weight)

The results of tumour induced (group II) showed a significant (p<0.001) reduction (final weight and Δ change) in the body weight compared to control (group I) and the pre inhibited drugs group (IV, VI& VII). Pre-inhibition of both GH and PRL in combination (C+O) prior to tumour induction showed significant (p<0.05) increase in body weight (final weight and Δ change) compared to that of octreotide alone (VIa) and tumour induced groups (II). On the other hand, the drug treated animals (positive controls) namely, cabergoline (group III), octreotide (groupV)) had no significant difference in body weight when compared to the normal controls (group I Table 2) Post treated group, IVA& VIA & VIIIA) showed a reduction in the body weight compare to pre inhibited group but did not show significant difference compared to MNU induced group (group II).

3.2 Tumour Latency (TL), Tumour Incidence Rate (TIR) and Tumour Multiplicity (TM) in the Experimental Groups.

The effect of MNU, cabergoline, octreotide, their combination administration on tumour latency, incidence rate and multiplicity are presented in in [Table3]. The results showed that within 45 days of MNU induction (lowest tumour latency) with high tumour multiplicity ($n=_3$) and tumour incidence rate (100%). This was significant (p<0.01) in comparison to all other groups.

Pre-inhibition of hormones namely, PRL and GH together(C+O) lead to a significant increase in tumour latency (p<0.001) as observed by long time taken for the occurrence of tumours in groups compared to that of post hormonal inhibition groups (IVA, VIA& VIIIA).Tumour incidence rate (calculated as percentage) and tumour multiplicity (quantified as the number of tumours present in the rats) were significantly reduced (p<0.01) in pre (,IV , VI) hormone inhibition groups compared to the post-hormone inhibition (IVA,VIA & VIIA). Combined inhibition group showed a significant (p<0.001) reduction in tumour multiplicity when compared to individual inhibition groups (IV & VI).

3.3 Assessment of Extent of Mammary Tumour Progression by Measurement of Tumour Weight and Tumour Volume (Table 4)

The results of the present study showed a significant (p<0.001) difference in the tumour weight and tumour volume in all the groups. Compared to tumour induced group, (II) pre-hormone inhibited groups (IV, VI, VIII) showed significant (p<0.001) reduction in tumour weight and volume and tumour volume alone was significantly (p<0.001)) in post-inhibition groups (IVA, VIA, VIIIA).

Groups (n=6)	Tumor weight	Tumor volume
I (MNU induced group)	2.15±0.22	2.67±0.11
II Cabergloine + MNU	0.33±0.51#*	0.28±0.44#
II A MNU + cabergoline	1.91±0.17$	1.69±0.24#$
III octeriotide + MNU	1.05±0.60#*	0.79±0.39#
IIIA MNU + octeriotide	1.88±0.14$	1.46±0.34 #$
IV c+O+MNU	0.10±0.24#	0.08±0.20#
IV A MNU+C+O	1.56±0.77	0.77±0.39#

Table 4. comparison of (TM) tumour weight & tumour volume.

Values are expressed as Mean ± SD, #p<0.001 Vs MNU, *p<0.05 IV Vs (II &III), $p<0.01 IVa Vs (IIA& IIIA.

Pre inhibition of both prolactin and growth hormone together (VII) significantly (p<0.05) reduced the tumour weight compared to pre inhibition of growth hormone (VI) alone. Whereas in tumour volume in post inhibition of PRL and GH together (VIIIA) showed significant (p<0.01) difference compared to post inhibition of prolactin alone (IVA). On the other hand, the drug treated animals (positive controls) namely, cabergoline (group III), octreotide (group V) and their combination did not show a significant difference in body weight when compared to the normal controls (group I).

3.4 Assessment of Tissue Antioxidant and pro-oxidant status in different groups by the estimation of reduced Glutathione (GSH) and Malondialdehyde (MDA). Table 5.

Groups (n=6)	GSH(µmol/g tissue)	MDA (µmol/g tissue)
Control (group I)	6.10±0.05#	1.01±0.05#
MNU induced (group II)	2.76±0.11*	2.26±0.03 *
Carbergoline Group III	6.56±0.08 NS	1.06±0.07 NS
Cabergoline +MNU (group IV)	3.76±0.11*# €	1.02±0.05# €
MNU+ cabergoline (group IVA)	2.17±0.07*£	2.13±0.05*£
Octreotide (group V)	6.56±0.08 NS	1.06±0.07NS
Octreotide+ MNU (group VI)	2.97±0.14* €	1.16±0.03# €
MNU+ octreotide (group VIA)	2.10±0.07*£	1.66±0.02£
Cabergoline + octreotide (group VII)	6.86±0.07NS	1.05±0.06 NS
Cabergoline+ octreotide +MNU (group VIII)	4.76 ±0.09 *#	0.87±0.05*# €
MNU+ Cabergoline + octreotide (group VIIIa),	2.99 ±0.10*	1.75±0.03*#

Table 5. Estimation of Tissue reduced Glutathione (GSH) and Tissue Malondialdehyde (MDA).

Values are expressed as Mean ± SD
Tissue GSH *p<0.01 Vs Control, # p<0.001 Vs MNU € p<0.001 IVVs (II, III), £ <0.05, IV Vs (II &III),
Tissue MDA *p<0.01 Vs Control, # p<0.001 Vs MNU € p<0.001 IVVs (II, III), £ <0.05, IV Vs (II &III),

The data depicted in (Table3) indicate changes in the tissue GSH and MDA levels.

Tissue GSH levels were significantly (p<0.01) decreased in tumour induced group and in pre -treated drug groups (II, IV, V, VI) compared to control group (I). There was no difference in control (I) and drug treated (positive control) groups (III, V, VII). Pre inhibition of prolactin and growth hormone together showed significant (p<0.05) increase in tissue GSH levels compared pre inhibition of growth hormone alone (VI). The tissue levels of pro-oxidant marker MDA were significantly (p<0.01) increased in tumour induced (II) group compared to control group (I) Pre inhibition of prolactin and growth hormone together resulted in a significant (p<0.001) improvement in oxidative state (tissue MDA levels decreased) compared to post inhibition of prolactin and growth hormone alone except pre-inhibition of prolactin alone.

Discussion

Mammary cancer is one of the most prevalent malignancies affecting women worldwide. The etiology of breast cancer is complex and is an interplay between various risk factors such as age, hormones involved in menstrual cycle namely estrogen and progesterone, genetic predisposition like BRCA 1, BRCA 2, HER2 positivity, unhealthy food habits such as consumption of fat rich and processed diet, alcohol, smoking and sedentary life style leading to obesity[2] In the recent studies on breast cancer the role of prolactin and growth hormone in mammary tumor progression gained maximum focus. [7,9] The effect of prolactin and growth hormone alone or in conjunction with estrogen and progesterone in mammary tumorigenesis and proliferation is an area of research of present times. However, a lack of extensive information remains, about the effects of hormone inhibitor of prolactin and growth hormone. This forms the basis for the present study which was designed to evaluate the role of cabergoline Prolactin inhibitor and octreotide, Growth hormone inhibitor on carcinogenesis and progression.

Experimental rats of our study, demonstrated significant reduction in the body weight compared to control group and increase in body weight in pre inhibited combination group compared to Pre hormone inhibition with octreotide and cabergoline alone, MNU induced and post treated group. (p<0.01). This indicates the pituitary inhibition of GH and PRL release by the drugs was effective in the attenuation of the deleterious effect of carcinogenesis on body weight of the animals. Post hormonal inhibition (after mammary tumor development) with

combination of cabergoline and octreotide, could not reverse the cachexic changes caused due to carcinogenesis. This suggests local production (in vicinity of breast tissue) of these hormones and their role in amplification of mammary tumorigenesis and progression, associated weight deterioration. The results as well indicate that Growth hormone inhibition alone cannot reverse the cachexic changes and will be effective only if both the hormones (PRL and GH) are inhibited together. The pre-hormone inhibition groups showed delayed in the tumor formation with decreased percentage of tumor incidence (%), compared to pre-inhibition done singly again emphasizing the collective inhibitory effect of PRL and GH in limiting the tumour formation. Post hormone inhibition groups had no change as compared to the tumour induced group.

Therefore, the PRL and GH levels need to be assessed for both patients and susceptible cases. In this study we also assessed the tumor weight and volume for the physical analysis of the effect of carcinogen on the rats. The pre hormone inhibited group not only decreased weight but also blocked the formation and progression of cancer cells. This might be due to the fact that the pituitary inhibition resulted in very negligible amount of PRL in serum and therefore the tissue released PRL could not cause much growth of the mammary tumor and progression. Our study propose that if pituitary inhibition of prolactin is done then carcinogenic progression of the cell can be decreased and furthermore this study recommend that combination therapy resulted in delayed tumor growth, non-malignant growth in the mammary tissue, signifying a positive response. This results also showed that pre hormone inhibition groups had normal levels of tissue GSH once again highlighting the oxidative state by decreased or benign growth at mammary tissue. The above beneficial effect of the inhibition of prolactin and growth hormone singly and in combination concludes that carcinogenesis was prevented with improved antioxidant levels of mammary tissue. Lipid peroxidation is considered as a key mechanism for cell damage by producing free radicals. It can change intrinsic membrane properties, which is in turn due to physicochemical changes of oxidized lipids or secondary to cross-linking and polymerization of membrane components affected by malondialdehyde [15]. The pre- hormone inhibition groups showed decreased MDA levels indicating a positive response of the hormonal inhibition at pituitary level by improving the oxidative state and decreasing the oxidative stress of the mammary tissue. These findings are similar to other study where after treatment with specific drugs for breast cancer the MDA levels decreased and attained a near normal levels[16,17].

The post hormone inhibition groups showed higher MDA levels once again indicating the oxidative stress at the mammary tissue level marking the tumorigenesis and progression. However, the groups did not show any improvement by the pituitary inhibition of prolactin and growth hormone alone or in combination after the tumorigenesis, which is in accordance with the earlier study[2]. A significant reduction in MDA levels was observed when pre, inhibition of PRL and GH done together compared to pre, and post inhibition of PRL and GH singly. Therefore, our study suggests that if prolactin and growth hormone are inhibited then it can suppress the tumor progression, decreasing the levels in susceptible cases may have minimum chances of tumor formation.

Conclusions

Pre-treatment in combination of cabergoline and octreotide is a better therapeutic measure in suppression of tumor production and help in the formation of non-malignant growth in the mammary tumor.

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Declaration of Interest

All the authors hereby declare that they have no conflict of interest.

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