UNIVERSIDADE ESTADUAL PAULISTA
"Julio de Mesquita Filho"
INSTITUTO DE BIOCIÊNCIAS DE BOTUCATU

Marcelo Augusto Altieri

REPERCUSSÕES DA EXPOSIÇÃO MATERNA AO ANTIDEPRESSIVO VENLAFAXINA SOBRE O DESENVOLVIMENTO MAMÁRIO E OVARIANO NA PROLE DE FÊMEAS WISTAR

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Resumo:

A depressão materna durante a gravidez e/ou o período pós-parto é um problema crescente de saúde pública em todo o mundo, que pode levar a graves efeitos na saúde da mãe e da criança. A Venlafaxina (VENL), um inibidor da recaptação de serotonina e noradrenalina, tem sido utilizada para tratar um amplo espectro de transtornos de humor, incluindo a depressão materna. Contudo, alguns relatos de caso indicaram efeitos adversos como galactorreia, mamoplasia e ginecomastia associados com o uso crônico de VENL em adultos. Além do mais, concentração elevada deste antidepressivo é encontrada em plasma de crianças, das quais as mães fizeram seu uso, e é uma das mais significativas em comparação com outros antidepressivos comumente utilizados. Levando isso em consideração, estudos mais recentes têm analisado possíveis efeitos colaterais que o uso desse antidepressivo poderia causar nos recém-nascidos, em casos de tratamento da depressão materna. Portanto, este estudo experimental tem como objetivo investigar o efeito da exposição materna a VENL, durante a lactação, sobre: 1) o crescimento da glândula mamária e sua morfologia e do desenvolvimento folicular nos ovários na prole de fêmeas F1 pré-púberes e 2) o risco de desenvolvimento de tumores mamários induzidos quimicamente. Assim, fêmeas de ratos da linhagem Wistar (F0) foram tratadas com VENL por via oral durante a lactação (21 dias) nas doses de 3,85, 7,7 e 15,4 mg/kg nas quais representam as doses humanas de 37,5, 75 e 150 mg. Algumas fêmeas F1 foram eutanasiádas para análise do desenvolvimento da glândula mamária e morfologia, peso e contagem de folículos ovarianos no dia pós-natal (DPN) 22 e 30 (1 filhote/por ninhada/por período). Os níveis séricos de estrógeno e progesterona foram analisados dos diferentes grupos experimentais no DPN 22 e 30. Fêmeas dos diferentes grupos experimentais receberam dose única de N-Metil-N- Nitrosurea (MNU, 50 mg/Kg) no DPN 22 (1 a 2 fêmeas por ninhada) e acompanhadas até o DPN 250 para o desenvolvimento de tumores mamários. Os resultados indicaram que a exposição lactacional a VENL não alterou o desenvolvimento da glândula mamária ou do ovário nas fêmeas F1 nos DPN 22 e 30. Além disso, a exposição lactacional a VENL não influenciou na incidência, latência ou multiplidade de tumores mamários induzidos pela MNU. Assim, em dosagens compatíveis com o uso humano, a exposição materna a VENL foi segura e não resultou em alterações mamárias ou ovarianas na prole de fêmeas nem altera o risco de desenvolvimento de tumores mamários induzidos quimicamente.

Palavras-Chaves: Venlafaxina, Exposição lactacional efeitos adversos, glândula mamária e ovários, tumores mamários, geração F1

Abstract:

The chronic use of selective serotonin reuptake inhibitors or serotonin-norepinephrine reuptake inhibitors (SNRIs) may result in human gynecomastia, mamoplasia, galactorrhea, and elevated breast cancer risk. As antidepressants are frequently used for postpartum depression (PPD) treatment, this study investigated the adverse effects of lactational exposure to venlafaxine (VENL, a selective SNRI) on mammary gland development and carcinogenesis in F1 female offspring. Thus, lactating Wistar rats (F0) received VENL by oral gavage at daily doses of 3.85, 7.7, or 15.4 mg/kg (N = 9, each group) from the lactational day (LD 1) until the weaning of the offspring (LD 21). F1 female offspring were euthanized for mammary gland, and ovary histological analyses on the post-natal day (PND) 22 and 30 (1 pup/litter/period, N = 9, each group). At PND 22, other females (2 pups/litter) received a single dose of carcinogen N-methyl-N-nitrosourea (MNU, 50 mg/kg) intraperitoneally (i.p.) for tumor susceptibility assay until PND 250. Tumor incidence and latency were recorded and representative tumor samples were collected for histopathology. The results indicate that lactational exposure to VENL did not alter the development of the mammary gland (epithelial ductal tree or the mean number of terminal end buds), or the ovary (weight and primary, secondary, tertiary, and Graafian follicles) in prepubertal F1 female offspring. In addition, VENL exposure did not influence tumor incidence or tumor latency in adult female offspring who received MNU. Thus, the findings of this animal study indicated that lactational VENL exposure, a period like human PPD, did not exert an adverse effect on the mammary gland development at the prepubertal phase or on chemically induced mammary tumorigenesis in adult F1 female rats.

Keywords: Venlafaxine; lactational exposure; mammary gland and ovary development, mammary tumors, female rat offspring F1.

LISTA DE ABREVIATURAS E SÍMBOLOS

5-HT	Serotonina
AD	Antidepressivos
ADT	Antidepressivos Tricíclicos
AMH	Hormônio anti-Mülleriano
b.w.	Body weight
BDNF	Fator neurotrófico derivado do cérebro
CONCEA	Brazilian Council on Animal Experimentation Control
CYP2C9	Citocromo P450 2C9
CYP2D6	Citocromo P450 2D6
CYP3A4	Citocromo P450 3A4
CYP3A5	Citocromo P450 3A5
CYP450	Citocromo P450
DAB	3,3'-Diaminobenzidine (Diaminobenzidina)
DG	Dias Gestacionais
DPN	Dia Pós-Natal
ER-α	Estrogen Receptor Alpha (Receptor de Estrogênio Alfa)
F0	Fêmeas de primeira geração (mães)
F1	Fêmeas de segunda geração (filhas)
FDA	Food and Drug Administration (USA)
FSH	hormônio folículo-estimulante
GBD	Global Burden of Diseases, Injuries, and Risk Factors Study
GnRH	hormônio liberador de gonadotrofinas
GSM	Genitourinary Syndrome of Menopause
HRP	Horseradish Peroxidase
i.p.	Intraperitoneally
IMAO	Inibidores da Monoamina Oxidase
LD	Lactation day
LH	Hormônio luteinizante
Lob	Lóbulo
MNU	N-Metil-N-Nitrosurea

NA	Noradrenalina
NIH	National Institutes of Health (Institutos Nacionais de Saúde)
ODV	O-desmetilvenlafaxina
OT	Oxitocina
PBS	Phosphate-buffered saline
PCNA	Proliferating Cell Nuclear Antigen (Antígeno Nuclear de Células Proliferantes)
PPD	Depressão Pós-Parto
PRL	Prolactina
RE	Receptor de Estrogênio
SD	Sprague-Dawley
SERM	Moduladores Seletivos do Receptor de Estrogênio
ISRSN	Inibidores Seletivos de Recaptação de Serotonina e Noradrenalina
ISRS	Inibidores Seletivos da Recaptação de Serotonina
TEBs	Terminal End Buds (Botões Terminais)
VENL	Venlafaxina
VO	Abertura vaginal
WHO	World Health Organization

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1. Revisão de Literatura

1.1. Depressão: Epidemiologia, Fatores de risco, Tratamento e Efeitos colaterais

A depressão é um transtorno emocional caracterizado por alterações significativas no humor e no comportamento, manifestando-se por meio de um conjunto de sinais e sintomas distintos, mas frequentemente observados. Dentre os principais sinais e sintomas clínicos, destacam-se a diminuição do interesse e do prazer nas atividades cotidianas, variações expressivas no peso corporal, pensamentos recorrentes sobre a morte (inclusive ideação suicida), distúrbios no sono (como insônia ou hipersonia) e um forte sentimento de culpa. Para que o diagnóstico de depressão seja estabelecido, esses sinais e sintomas devem persistir por um período superior a duas semanas (Ferrari et al., 2013; SCHULZ e ARORA, 2016; Lopez et al., 2017).

A depressão também é frequentemente associada a um impacto negativo e significativo nas atividades cotidianas e diárias, e no convívio social do indivíduo. Estudos mostram que a persistência dos sintomas depressivos pode comprometer a capacidade de uma pessoa em manter relacionamentos interpessoais e realizar tarefas domésticas, profissionais ou acadêmicas (Kessler et al., 2003). Além disso, a depressão está diretamente ligada a uma série de comorbidades, incluindo transtornos de ansiedade e doenças crônicas, que podem complicar ainda mais o tratamento e sua recuperação (Roy-Byrne et al., 2009). A interação entre esses fatores destaca a importância de uma abordagem integrada e multidisciplinar para o manejo da depressão, visando não apenas aliviar os sintomas principais, mas também melhorar a qualidade de vida geral dos pacientes (Kessler et al., 2003; Roy-Byrne et al., 2009).

Segundo a Organização Mundial de Saúde (OMS), atualmente, mais de 350 milhões de pessoas se encontram-se em quadro depressivo, considerado um problema grave de saúde pública global (Smith, 2014). Em estudo populacional de nível global publicado em 2019, analisou o período entre 1990 e 2017, descreveu que a incidência de depressão aumentou em 49,86% (Liua et al., 2019). O risco de desenvolvimento do quadro depressivo ao longo da vida é de cerca de 15%, e a prevalência do transtorno em mulheres é maior do que em homens. Os principais fatores associados ao desenvolvimento da depressão são: genéticos, hormonais e sociais (Stahl, 2002; Ferrari et al., 2013; Kuehner, 2017).

Nesse contexto, alguns autores descrevem que 23% das mulheres norte-americanas entre 40 e 59 anos relataram uso de AD. Depressão é uma condição médica crônica grave que aumenta o risco do desenvolvimento de câncer da mama por meio da redução da resposta imunitária anti tumoral, caracterizada pela diminuição das células T citotóxicas e de células natural killer, associada a liberação de citocinas pro-inflamatórias no sítio tumoral (Brown et al., 2019; Dorte L. et al., 2016). Em comparação com indivíduos saudáveis, as mulheres com depressão apresentam níveis mais elevados de cortisol, e evidências in vitro sugerem que o cortisol pode aumentar a proliferação de células mamárias tumorais e contribuir para o crescimento do tumor (Brown et al., 2019). Considerando, vale mencionar que o câncer de mama é a neoplasia mais prevalente e a segunda principal causa de mortalidade por câncer entre mulheres nos Estados Unidos (Brown et al., 2015), convém discutir-se o risco do desenvolvimento de câncer de mama em mulheres que fazem uso de antidepressivos.

Inicialmente, o período pós-parto, também conhecido como puerpério apresenta maior risco de desenvolvimento de quadros depressivos devido a alterações hormonais, depressão pré-natal, complicações durante a gravidez, estresse, insatisfação com o parceiro, violência doméstica e questões socioeconómicas em geral. Esse quadro depressivo, conhecido como depressão pós-parto (PPD) e presente nos primeiros seis meses de puerpério pos veses se prolonga por período maiores. (Atif et al., 2015; Hutchens e Kearne, 2020). PPD tem potencial efeitos tanto na saúde da mãe quanto da criança. Para a mae são descritos riscos de futura depressão recorrente, morbidade e mortalidade, Enquanto que para a criança seu desenvolvimento cognitivo e social pode ser influenciado negativamente desenvolvendo introversão e falta de interação com a mãe (Lilja et al., 2011; Atif et al., 2015; Abdollahi et al.,2017).

Como tratamento, além do acompanhamento psicológico, o uso de antidepressivos é o mais recomendado (Berle e Spigset, 2011). Com três principais classes preconizadas: os inibidores da enzima monoamina oxidase (IMAO), os tricíclicos (ADT) e os inibidores seletivos de recaptação (Moreno et al.,1999). Todos têm como objetivo aumentar a quantidade de neurotransmissores monoaminérgicos, noradrenalina (NA) e/ou serotonina (5-HT), na fenda sináptica, melhorando assim a condição sintomática. (Graeff e Guimarães, 1999). A classe dos inibidores seletivos de recaptação envolve a inibição de um transportador específico que remove o neurotransmissor da fenda sináptica e o rearmazena para outro impulso ou o degrada (Moreno et al.,1999).

Entre os diversos medicamentos da classe dos inibidores seletivos de recaptação de serotonina e noradrenalina (ISRSN), destaca-se a Venlafaxina (VENL), indicada para depressão

maior, distúrbio de ansiedade generalizada, síndrome do pânico e fobia social. Esse medicamento também é utilizado para tratar a ocorrência de ondas de calor, conhecidas como fogachos, um dos efeitos adversos da terapia do câncer de mama com o uso de moduladores seletivos do receptor de estrogênio (SERM, do termo em inglês) como o tamoxifeno (Ramaswami et al., 2015; Aiyer et al., 2016; Nassan et al., 2016).

A VENL é um dos dois antidepressivos que constituem a classe dos ISRNS. Clinicamente, VENL tem eficácia semelhante a Imipramina e a Fluoxetina. Em comparação com os tricíclicos e os ISRS, o duplo mecanismo de ação da VENL, ao inibir potentemente a recaptação tanto da serotonina como da noradrenalina, e as diferenças no seu perfil de efeitos secundários lhe conferem ampla utilização no tratamento da depressão, incluindo a que ocorre em mulheres no período pós-natal (Ilett et al., 2006).

Embora os antidepressivos sejam eficazes, o uso prolongado ou em momentos críticos pode acarretar complicações. Efeitos adversos frequentemente observados incluem diminuição da libido, náuseas, constipação, cefaleias e insônia (David e Gourion, 2016; Read e Williams, 2018). Esses efeitos são diretos, isto é, afetam diretamente o indivíduo que está utilizando o medicamento. No entanto, também podem ocorrer efeitos indiretos, como no caso de episódios de depressão pós-parto (DPP). Nessa situação, o uso concomitante de antidepressivos e amamentação pode impactar os recém-nascidos por meio da transferência de substâncias ativas através do leite materno (Newton e Hale, 2015). As diretrizes clínicas para o uso de antidepressivos durante a amamentação recomendam uma avaliação cuidadosa da gravidade do quadro clínico, a consideração de alternativas terapêuticas, como a psicoterapia, e a escolha de antidepressivos que minimizem a exposição ao lactente. Por exemplo, os inibidores seletivos da recaptação de serotonina (ISRS) como a fluoxetina e os inibidores da recaptação de serotonina e norepinefrina (IRSN) como a venlafaxina atingem altas concentrações plasmáticas, sendo recomendados com precaução (Berle e Spigset 2011). Além disso, a exposição intrauterina representa outro meio indireto de impacto, uma vez que sintomas depressivos também podem afetar mulheres grávidas (Ryan et al., 2005; Laurent et al., 2016).

Em um estudo experimental sobre exposição intrauterina detectou modificações na relação entre o peso corporal e o peso da placenta. Observou-se um aumento no peso placentário em ratas que receberam doses de 0, 3, 10, 30 ou 100 mg/kg/dia de VENL durante os dias gestacionais (DG) 8 a 20. Além disso, houve maior incidência de anomalias cardíacas fetais, sugerindo que essas anomalias podem resultar de alterações na sinalização de 5-HT associadas a modificações nas condições placentárias e fetais (Laurent et al., 2016).

Estudos clínicos e experimentais sobre a exposição do lactante a ISRSN de lactentes são

limitados. Mas, pesquisa pré-clínica investigou a exposição lactacional à fluoxetina e constato um atraso na puberdade. Esse atraso foi observado nas fêmeas descendentes de ratas que receberam dose diária de 5 mg/kg. Em outra pesquisa, verificou-se alterações na dinâmica dos folículos e no ciclo reprodutivo das fêmeas descendentes de ratas expostas a doses diárias de 11 e 10 mg/kg de fluoxetina. Alem disso, outros autores descreveram 5-HT influenciando na secreção de gonadotrofinas e induzindo mudanças duradouras nas funções reprodutivas, especialmente por meio do aumento no número de folículos ovarianos no período púbere (Moore et al., 2015; Santos et al., 2016).

1.2. Efeitos adversos dos antidepressivos na glândula mamária

Os inibidores seletivos de recaptação de serotonina e noradrenalina (ISRSN) têm um impacto significativo na prolactina (PRL), um hormônio polipeptídico produzido e secretado pelas células especializadas da hipófise anterior (pars distalis), aumentando sua concentração no sangue. Estudos in vivo demonstraram que a 5-HT possui um efeito estimulador potente na liberação de PRL quando combinada com outros fatores liberadores de prolactina (PRFs), especialmente a oxitocina (OT) e o Peptídeo Intestinal Vasoativo (VIP) (Balsa et al., 1998; Freeman et al., 2000; Emiliano et al., 2004). A PRL desempenha um papel crucial nas funções reprodutivas e, particularmente, na glândula mamária, onde é essencial para o crescimento, desenvolvimento, síntese e manutenção da produção de leite (Freeman et al., 2000). Especificamente, ao se ligar ao seu receptor (PLRr), a PRL promove a transição de um estado inativo para ativo, influenciando a replicação do DNA e consequente a divisão celular no tecido mamário (Porter, 1974; Clevenger et al., 2009).

A glândula mamária é um órgão exclusivo dos mamíferos, cuja principal função é a síntese e secreção de leite para a nutrição dos filhotes. Este processo ocorre principalmente durante a gravidez e a lactação, momentos que representam o ápice funcional e de desenvolvimento do órgão. Além dessas fases, a glândula mamária experimenta alterações estruturais ao longo de toda a vida, abrangendo desde o período embrionário até a pós-menopausa, quando ocorre sua involução (Russo e Russo, 2004; Foteini e Geddes 2012).
Durante a vida embrionária, a estrutura inicial da glândula mamária é formada por ductos que possuem uma ou duas camadas de células epiteliais e uma camada de células mioepiteliais. Com a chegada da puberdade, ocorre a ramificação dos ductos, levando à formação dos terminais end buds (TEBs), que se ramificam novamente para criar estruturas alveolares conhecidas como alveolar buds (ABs). Os ABs sofrem ramificações sucessivas, dando origem

aos lóbulos tipo 1 (Lob 1). Os lóbulos tipo 2 (Lob 2) e tipo 3 (Lob 3) se formam por meio de ramificações sucessivas dos Lob 1. O desenvolvimento e a maturação das glândulas mamárias avançam significativamente durante a gravidez, quando os lóbulos tipo 4 (Lob 4) se formam e o número dos Lob 1 diminui (Cardiff et al., 2000; Russo e Russo, 2004).

Os lóbulos tipo 1 (Lob 1) são considerados a principal origem dos carcinomas ductais, o tipo mais comum de câncer de mama. Isso ocorre porque o Lob 1 é uma estrutura menos diferenciada, propensa ao desenvolvimento de neoplasias mais agressivas (Wellings, 1980; Russo et al., 2004; Javed e Lteif, 2013). Embora os lóbulos mais desenvolvidos também possam ser responsáveis por processos pré-neoplásicos e neoplásicos, estes tendem a ser menos agressivos (Russo e Russo, 2004; Javed e Lteif 2013).

O câncer de mama é atualmente o segundo tipo mais prevalente de câncer e o mais frequente entre as mulheres (Ferlay et al., 2015). Os fatores de risco associados a esta condição incluem idade, uso de anticoncepcionais, terapia hormonal, histórico familiar de câncer, exposição a radiação ionizante e contato com agentes ambientais (Cutuli et al., 2001; Snedeker, 2001; Andrieu et al., 2006; Skol et al., 2016; Inca, 2020).

Portanto, como já discutido as funções da PRL ao longo do desenvolvimento da glândula mamária, com ênfase na gravidez e lactação. No entanto, níveis elevados de PRL, conhecidos como hiperprolactinemia (acima de 25 ng/mL em mulheres e 20 ng/mL em homens), podem resultar em infertilidade tanto em homens quanto em mulheres, além de outras complicações (Peveler et al., 2008).Esses canceres foram correlacionados o aumento na proliferação celular e na resposta ao estrogênio, considerando o papel crucial da PRL na regulação de células-tronco, sejam elas neoplásicas ou não (Clevenger et al., 2003; Chen, 2014).

A hiperprolactinemia pode resultar de fatores fisiológicos ou ser provocada por influências externas (Capozzi et al., 2015). Em um estudo conduzido em 2018, a hiperprolactinemia foi induzida em modelos experimentais através da administração de antipsicóticos, especificamente Risperidona. Este antipsicótico atua como um antagonista monoaminergico, serotoninergica e dopaminergico da dopamina, que normalmente desempenha um papel supressor na produção de prolactina (PRL). Os resultados demonstraram que os animais tratados com Risperidona não apenas apresentaram concentrações plasmáticas elevadas de PRL, como também mostraram um aumento na multiplicidade tumoral e uma menor taxa de sobrevivência livre de tumores (Johnston et al., 2018).

O tamoxifeno reduz em até 50% o risco de recidivade cancer de mama positivo para o receptor de estrógeno em estádios iniciais) (Osborne, 1998). Sua eficácia depende da metabolização por enzimas do citocromo CYP450, incluindo CYP2D6, CYP3A4, CYP3A5 e CYP2C9. A

CYP2D6 é particularmente importante na formação dos dois principais metabólitos ativos do tamoxifeno: o 4-hidroxitamoxifeno e o 4-hidroxi-N-desmetiltamoxifeno, ambos com alta afinidade pelo receptor de estrogênio (Shagufta e Irshad, 2018). Esses metabólitos atuam impedindo ou inibindo o crescimento celular por meio da competição seletiva com o estrógeno.

Antidepressivos, especialmente os ISRSN, são metabolizados pela enzima CYP2D6, a mesma que participa da biotransformação do tamoxifeno. Pesquisas indicam que os ISRSN podem competir com o tamoxifeno ou inibir a atividade da CYP2D6, o que pode diminuir a eficácia do tratamento para o câncer de mama. Essa situação representa um desafio para mulheres com câncer de mama que utilizam ISRSN para tratar depressão ou para mitigar sintomas de ondas de calor durante a pré-menopausa ou a menopausa em conjunto com o tamoxifeno (Stearns, 2003).

1.3. Efeitos adversos dos antidepressivos no ovário

O ovário, órgão reprodutivo feminino, responde a hormônios sexuais, bem como a hormônios do eixo hipotálamo-hipófise-ovário. Isso implica que variações em neurotransmissores ou hormônios hipofisários, ou em qualquer eixo regulado por esses hormônios, podem afetar o desenvolvimento do ovário ou o processo de foliculogênese.

Os folículos são estruturas esféricas localizadas no estroma ovariano que abrigam as células germinativas e podem ser classificados de acordo com seu estágio de desenvolvimento (Gore et al., 2015; Romero-Reyes et al., 2016; Richards, 2018).

A 5-HT desempenha papel na regulação do eixo hipotálamo-hipófise-ovário e possui receptores em todos os três níveis desse eixo (Wada et al., 2006). A serotonina pode promover a liberação dos hormônios folículo-estimulante (FSH) e luteinizante (LH) pela hipófise, através da modulação do hormônio liberador de gonadotrofinas (GnRH) no hipotálamo (Gouveia e Franci, 2004; Wada et al., 2006).

O envolvimento da 5-HT no eixo hipotálamo-hipófise-ovário de ratas fêmeas pré-púberes foi evidenciado em pesquisas anteriores, que mostraram sua influência na modulação do desenvolvimento folicular e no início da puberdade (Moran et al., 2013). Em um estudo com ratas de 30 a 33 dias de idade, a administração de doses agudas e crônicas de 5 mg/kg de fluoxetina causou alterações significativas no desenvolvimento folicular e na ovulação, incluindo aumento da atresia e fragmentação folicular (Romero-Reyes et al., 2016). A fluoxetina apresenta concentrações plasmáticas e no leite materno comparáveis às da venlafaxina (Berle e Spigset 2011). Dada a limitação de estudos sobre os efeitos da exposição

materna a antidepressivos e as possíveis associações biológicas com o desenvolvimento dos ovários e da glândula mamária, é fundamental realizar investigações pré-clínicas para esclarecer os impactos desses medicamentos sobre o desenvolvimento desses órgãos na prole exposta ao antidepressivo VENL durante as fases gestacional e/ou lactacional, bem como o risco subsequente de desenvolvimento de tumores mamários na vida adulta.

2. Objetivo Geral

Portanto, o objetivo principal deste estudo foi avaliar os efeitos da exposição materna ao antidepressivo VENL, na fase lactacional, sobre o desenvolvimento da glândula mamária e dos ovários, assim como sobre o desenvolvimento de tumores mamários induzidos quimicamente.

Neste exame de qualificação serão apresentados resultados sobre a exposição lactacional a VENL e potenciais alterações mamárias e ovarianas.

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RESEARCH PAPER
SAFETY OF LACTATIONAL EXPOSURE TO VENLAFAXINE ON THE RAT MAMMARY GLAND DEVELOPMENT AND CARCINOGENESIS IN F1 FEMALE OFFSPRING

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Abstract:

The chronic use of selective serotonin and norepinephrine reuptake inhibitors may result in gynecomastia, mammoplasia, galactorrhea, and elevated breast cancer risk. As antidepressants are frequently used for postpartum depression (PPD) treatment, this study investigated the adverse effects of lactational exposure to venlafaxine (VENL, a selective SNRI) uterine and ovarian, mammary gland development and carcinogenesis in F1 female offspring. Thus, lactating Wistar rats (F0) received VENL (3.85, 7.7, or 15.4 mg/kg, in which the human doses of 37.5, 75, and 150 mg are represented,n= 9, each group) until weaning. F1 female offspring were euthanized for mammary gland, and ovary histological analyses on the post-natal day (PND) 22 and 30 (1 pup/litter/period, n= 9, each group). At PND 22, other females (2 pups/litter, n=18, each group) received a single dose of N-nitroso-N-methynitrosourea (MNU, 50 mg/kg) for tumor susceptibility assay until PND 250. Tumor incidence and latency were recorded and representative tumor samples were collected for histopathology. The results indicate that lactational exposure to VENL did not alter the development of the mammary gland (epithelial ductal tree or the mean number of terminal end buds), or the ovary (weight and primary, secondary, tertiary, and Graaf follicles) in prepubertal F1 female offspring. In addition, VENL exposure did influence neither tumor incidence nor tumor latency in adult female offspring that received MNU. Thus, the findings of this animal study indicated that lactational VENL exposure, a period similar to human PPD, did not exert an adverse effect on the mammary gland development at the prepubertal phase or on chemically induced mammary tumorigenesis in adult F1 female rats.

Keywords: Venlafaxine; lactational exposure; mammary gland and ovary development, mammary tumors, female rat offspring.

1. Introduction

Although the exact etiology of Major Depressive Disorder (MDD) is not fully understood, it is widely acknowledged that MDD is associated with multiple pathogenic factors. In addition to well-established psychological factors, MDD is also linked to genetic influences, social stress, and even other prevalent chronic conditions. Therefore, the etiology of MDD cannot be explained from the perspective of a single isolated factor [54].

According to the World Health Organization (WHO), an estimated 5% of adults suffer from depressive disorder globally [1], and this situation is considered a serious global public health problem also associated with an increased risk of suicide [2-4]. A global population-level study [i.e., based on the Global Burden of Diseases, Injuries, and Risk Factors Study (GBD) which analyzed the period between 1990 and 2019] indicated that the COVID-19 pandemic has exacerbated both major depressive and anxiety disorders burdens, which are ranked among the top 25 leading causes of burden globally in 2019 [5,6]. The lifetime risk of developing depression is about 10-15%, and the prevalence of the disorder is higher in women than in men [5,6]. The most common types of depression in women include major depression, dysthymia, and postpartum depression. However, there is limited empirical evidence that clarifies the cause of the higher prevalence of depression cases in females, despite genetic/epigenetic, hormonal, and social factors being the primary associated factors [7,8].

Perinatal maternal depression (i.e., during pregnancy and within the first 12 months after delivery) is a growing public health issue worldwide, which can lead to adverse effects on maternal and infant health, including several physical, behavioral, and cognitive disorders [9]. Therefore, several nonpharmacological and pharmacological interventions are available for the treatment of prenatal and postpartum depression (PD) [4,9,10]. Specifically, PD affects up to 15% of new mothers in high-income countries, and the use of serotonin-norepinephrine reuptake inhibitors (SNRIs) has been recommended for the pharmacological treatment of this condition [9,10]. Of those, Venlafaxine (VENL), a selective SNRI, has been considered one of the main therapeutic options for the maternal treatment of depression/anxiety disorders [11-12].

VENL is classified by the U.S. Food and Drug Administration (FDA) as a

category C drug, as it may pose a risk to the embryo/fetus, and can be transferred to the newborn through breast milk in varying amounts depending on the dose ingested by the mother, with the literature indicating maximum doses for this effect ranging from 75 mg/day to a maximum of 225 mg/day [10,13,14]. . In addition, this antidepressant has been recommended to reduce the frequency of vasomotor symptoms (hot flashes) associated with genitourinary syndrome of menopause (GSM), as a nonhormonal medication [15-17], but it was not approved by the FDA for this use [17]. However, some cases of galactorrhea, gynecomastia without galactorrhea, and mammoplasia have been described after chronic use of antipsychotics and antidepressants mainly when related to an increase in serum prolactin (PRL) levels induced during treatment [18-20]. Furthermore, an increased risk for breast cancer occurrence and mortality with antidepressants that elevate serum PRL have been suggested, but few studies corroborate this hypothesis [21-23] since the mechanism of the potential link between antidepressant treatment and hyperprolactinemia (i.e., blocking the dopaminergic inhibition of prolactin release from the anterior pituitary) is still not completely elucidated [23]. However, prospective epidemiologic studies have linked higher PRL exposure to increased risk for breast cancer, particularly for estrogen receptor (ER)-positive tumors in postmenopausal women [24].

The mammary gland is a hormone-dependent tissue whose growth and differentiation (i.e., epithelial ductal tree growth) depend on the orchestrated action of several hormones, cellular interactions, and extracellular cell-matrix signaling cascades [25]. Many human and rodent findings indicate that xenobiotics and hormones can cause transient or persistent effects on mammary gland development, mainly when exposure occurs during critical periods of glandular growth or differentiation [26-27]. PRL is a key factor for mammary gland development contributing to its maturation from a primary ductal system, which grows from terminal end buds, fully mature nonpregnant gland, and pregnant/lactogenic lobuloalveolar differentiation [28,29] Thus, early-in-life exposure to PRL-release-inducing drugs could interfere with specific stages of mammary organogenesis with deleterious effects later in life on mammary development and breast cancer initiation and progression. The ovary, like the mammary gland, is a female reproductive organ and responds to sex hormones as well as hormones from the hypothalamic-pituitary-ovarian axis. This implies that variations in neurotransmitters or pituitary hormones, or in any axis regulated by these hormones, can affect ovarian development or the process of folliculogenesis. Follicles are spherical structures located

in the ovarian stroma that house germ cells and can be classified according to their stage of development (5-HT) plays a role in regulating the hypothalamic-pituitary-ovarian axis and has receptors at all three levels of this axis (Wada et al., 2006). Serotonin can promote the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) by the pituitary, through modulation of gonadotropin-releasing hormone (GnRH) in the hypothalamus. Therefore, this animal study investigated the modifying effects of lactational VENL exposure PD-like on mammary gland development and mammary tumor susceptibility in female offspring of Wistar rats.

2. Materials and Methods

2.1. Animal housing, and experimental design

Pregnant Wistar rats were maintained in individual cages in a room under controlled temperature (23°C ± 2°C) and regulated humidity conditions, with periods of 12 h light/dark cycles and food and drinking water ad libitum. At the end of the gestational phase, the dams were randomly allocated into four groups (n=9 each group): Three groups were treated with venlafaxine (VENL, 1-[2-(dimethylamino)-1-(4 methoxyphenyl) ethyl] cyclohexanol, Cruz Vermelha Pharmacy, 98% purity) at doses of 3.85, 7.7, or 15.4 mg/kg of body weight (b.w.), respectively, from lactation day (LD) 1 until LD 22, by daily oral gavage [30] and a control group, with distilled water and standard chow.. Thus, litters were exposed to VENL through maternal milk in a period where there is a rapid growth of the mammary gland [26,27]. A control group received only the vehicle (distilled water) by daily oral gavage. The VENL doses used in this animal study were based on recommended therapeutic doses for humans, which are 37.5, 75, and 150 mg/day [31], and adjusted based on the animal body surface area [32]. Thus, the dose resulting was as followed: 3.85 mg/kg b.w. (VENL 3.85) equivalent to 37.5 mg/day, 7.70 mg/kg b.w. (VENL 7.7) equivalent to 75 mg/day, and 15.4 mg/kg b.w. (VENL 15.4) equivalent to 150 mg/day. During VENL treatment, lactating dams were weighed on alternate days to adjust VENL administration and evaluate some clinical signs of toxicity. After birth, the number of pups per litter was adjusted to eight pups (four males and four females whenever possible), to allow each animal to receive similar amounts of milk and to prevent litter size effects on the development of the pups. After weaning, F1 female offspring were euthanized at post-natal day (PND) 22 and 30 [(1 pup/litter/period (n=9

each group)] for serum sex hormone determination (control and VENL 15.4) and mammary gland and ovaries analyses (all groups). Other female offspring at PND 22 [2 pup/litter (n= 18 each group)] received a single dose of 50 mg/kg N-methyl-N-nitrosourea (MNU) carcinogen intraperitoneally (i.p.), dissolved in phosphate-buffered saline (PBS) acidified with acetic acid [33] or vehicle (PBS acidified) for tumor mammary assays. All the male pups were used in a published parallel study [30] to evaluate the effects of VENL on the male reproductive system. This animal study was carried out following the Brazilian Council on Animal Experimentation Control (CONCEA) and the protocol was approved by the Ethical Committee on Animal Use of the Institute of Biosciences, São Paulo State University (UNESP) (Protocol number 8262170821 CEUA).

2.2. Mammary gland histological analyses at DPN 22 and 30

At necropsy, the right and left fourth abdominal mammary glands were removed [33]. Briefly, the right mammary glands were air-dried on codified glass slide slides and fixed in 10% buffered formalin (v/v) for 48 hr. The slides were rinsed in 70% ethanol, deionized water, and stained with Carmine (1 g) and aluminum potassium sulfate dodecahydrate (2.5 g) solution (Sigma-Aldrich, USA) for 2 days. Afterward, mammary whole mounts were dehydrated in sequential steps of ethanol (70%, 95%, and 100%), cleared in xylene, mounted with Permount, and cover-slipped. Mammary gland trees were photographed using a magnifying glass at 1× magnification (Leica MZ12 DFC 420, Japan) coupled to a capture system and image analysis. The following morphometric parameters were measured and compared to demonstrate outgrowth [34]: Ductal elongation, perimeter, area of the mammary epithelial trees, the mean number of terminal end buds (TEBs)/mm2 (density) in the external margin of the mammary gland, using a Stemi 2000 stereo zoom microscope (Zeiss, Germany). The images were analyzed using the ImageJ software (NIH, USA; https://imagej.nih.gov/ij). The terminal structures of the mammary gland were identified by size and shape according to the criteria, evaluating the morphology of the ductal and alveolar units, based on their organization and development. However, it is expected that the mammary gland presents different stages of development depending on the female's reproductive cycle and hormonal exposure, which influence the shape and size of the glandular structures, according to Russo and Russo (2004) [35]. .

Left abdominal mammary glands were removed and fixed in 10% phosphate-buffered formalin for 48 hr, embedded in paraffin wax, cut into 5-µm-thick sections, and

submitted to immunohistochemical reactions for proliferating cell nuclear antigen (PCNA), using monoclonal anti-mouse PCNA 1:100 dilution (Dako, Denmark) or estrogen receptor alpha (ER-α) using a mouse monoclonal anti-ER-α, 1:50 dilution (Invitrogen, USA) in a humidified chamber (overnight, 4ºC). Then, the slides were incubated with one-step horseradish peroxidase (HRP)-polymer (EasyPath–Erviegas, Brazil) (30 min). The reaction was visualized with 3-diaminobenzidine (DAB) chromogen (Sigma–Aldrich, USA) and counterstained with Harris' hematoxylin. PCNA and RE-α labeling indexes (%) in the mammary gland sections were evaluated as the number of positively marked epithelial cells divided by the total number of cells scored (400-500 cells/mammary gland).

2.3. Ovary histological analyses at PND 22 and 30

At necropsy, the right and left ovary were removed, weighed, and fixed in 10% buffered formalin for 24 hours, kept in 70% ethanol for further histological processing, and then submitted to hematoxylin and eosin (HE) staining. The ovary was serially sectioned (5 μm thick) and one slide out of every 100 μm (3 sections/per ovary) were obtained [36], mounted on glass slides, stained with HE, and photographed using a magnifying glass at 1× magnification (Leica MZ12 DFC 420, Japan). Ovarian follicles were classified as primordial, primary, secondary or preantral, tertiary or antral, and atretic follicles, according to previously established criteria [36]. The mean number of each ovarian follicle per ovary area (cm2) analyzed was determined. Furthermore, the right and left ovary absolute (mg) and relative (%) weights were calculated considering body weight on the day of euthanasia.

2.4. Serum hormonal analysis at DPN 22 and 30

At PND 22 and 30, blood samples (n=6 each, 1 female per litter) from the control and VENL 15.4 mg/kg groups, since the lower milligram doses did not exhibit significant findings in other similar studies, were obtained through cardiac puncture between 8h30 and 11h30 min, centrifuged (2400× g for 20min) and the serum was stored at -20ºC for further hormonal analysis. Serum estrogen (17β-estradiol, 155 Monobind, 4925-300 CA, USA; sensitivity: 6.5 pg/mL) and progesterone (Monobind, 4825-300, CA, USA; sensitivity: 0.105 ng/mL) were determined by enzyme-linked immunosorbent assay

(ELISA)using manufacturers' instructions.

2.5. Tumor mammary analyses in adult female rats

After MNU administration, F1 female offspring were carefully checked twice a week for the presence of gross mammary tumor development and the number and anatomical site of each palpable mass in the six mammary gland complexes were recorded. Females in which tumor diameter reached approximately 2 cm were euthanized while all other animals were euthanized at post-natal day 250. The animals were euthanized by exsanguination under ketamine/xylazine anesthesia and tumor tissues were carefully removed. Representative samples were fixed for 24 h in 10% neutral buffered formalin, processed, and paraffin-embedded. Paraffin sections (5-µm-thick) were used for conventional HE staining. Mammary tumors were classified histologically according to published criteria [37]. Tumor incidence (percentage of tumor-bearing animals), latency (time between MNU administration and appearance of the first palpable tumor per animal), and histological type were recorded for each group [37].

2.6. Statistical Analyses

The obtained data in this study were expressed as mean ± standard deviation. Groups were compared using one-way ANOVA and Tukey post hoc test (for body weight, ovary weight, and morphometry ovaries parameters), or Duncan's new multiple range test for the mammary glands. Serum hormone analyses and PCNA and ER-α labeling indexes in the mammary gland were analyzed using Student's t-test when the comparison was made only between higher VENL dose and control groups. Incidences of mammary tumors were examined using the Chi-square or Fischer's exact test. The differences between groups were considered significant when $p<0.05$. The statistical analyses were performed using GraphPad Prism 6 Software (GraphPad, USA).

3. Results

3.1. General findings

Analyses of body weight and clinical signs of toxicity were used to assess the

influence of lactational VENL exposure on F1 female offspring after weaning. After the lactation phase, the maternal antidepressant treatment did not induce clinical signs of toxicity or a significant difference in body weight and ovary weights (absolute and relative) in female offspring at DPN 22 e 30, when compared to the control group and regardless of VENL dose (Table 1). In addition, dams treated with VEN did not show either clinical signs of toxicity or alterations in water and food consumption, or weight gain during the lactation phase [30].

3.2. Mammary gland analysis

Analyses of whole mount and tissue sections of the fourth mammary gland were used to assess the influence of maternal antidepressant treatment on F1 female offspring. At PND 22 and 30, VENL exposure did not interfere with mammary development in female offspring as evaluated by ductal elongation (length), area, perimeter, or total number of TEBs in whole-mounts, independently of antidepressant dose (Figure 1). In addition, the cell proliferation (PCNA) and ER-α labeling indexes in epithelial mammary cells in mammary gland tissue sections were similar among the control and high-dose VENL groups in both time points analyzed (Figure 2).

3.3. Ovary and serum hormones analyses

Regarding the analysis of the morphology of the ovary using routine light microscopy, the mean number of ovarian primordial, primary, preantral, antral, and Graaf follicles was evaluated in F1 female offspring at PND 22 and 30. After analysis of each follicular stage, the number of ovarian primary, prentral, antral, and atretic follicles was similar among the groups, independently of VENL exposure (Figure 3). Only at DPN21, a significant increase in the mean number of primary follicles was observed in VENL 3.85 in comparison to the control group. In addition, the serum estrogen levels, but not progesterone levels, were significantly higher in VENL 15.4 group that the control (nontreated) group in F1 female offspring at PND 22 and 30 (Figure 2).

3.4. Mammary tumor analysis

Considering that an increased breast cancer risk and mortality with antidepressant

use have been suggested, the modifying effects of lactational VENL exposure on mammary cancer susceptibility were investigated after a single dose of MNU at PND22. At the end of the study (PND 250), some benign and malignant neoplasms were histologically confirmed in the mammary gland complexes of F1 female rats. Most tumors were classified as adenocarcinomas with either tubular, papillary, or comedo-cribriform patterns while a few adenomas and fibroadenomas were observed (Supplementary Data and Table 2). Lactational exposure to VENL, independently of the dose administered, did not alter the latency period, incidence, or histological patterns (Table 2).

4. Discussion

The findings of the present animal study indicate that lactational (milk) exposure to different doses of VENL does not modify the mammary gland growth/morphology or carcinogenesis induced by a classical carcinogen MNU in female Wistar rats. Thus, maternal VENL exposure, during a critical period of mammary gland development in F1 female offspring rats, did not induce delayed development (atrophy) or tubuloalveolar hyperplasia biomarkers indicative of endocrine-disrupting effects [34,38].

Some studies with rodents suggest that compounds that cause hyperprolactinemia by themselves can produce mammary gland feminization in males and lobuloalveolar hyperplasia in females [38]. In a rat study conducted by Piancetini et al 2003, male Wistar rats that received VENL (20mg/kg) presented an increase in extracellular serotonin and noradrenalin in the hippocampus associated with a significant increase (243% in 60 min) in plasma PRL [39]. Although our study did not measure the amount of serum PRL in lactating dams or female offspring at weaning, the doses used were based on recommended therapeutic doses for humans, resulting in VEN residues detected in milk samples from the dams, as previously published [30].

VENL and its active metabolite, norfluoxetine, readily cross the placenta in humans [40, 41] and are also excreted in human and rat milk [30, 41,42]. Therefore, fetuses and neonates are exposed to these substances during important stages of development of the mammary gland and ovary. In a previous study of our group using the same experimental design and VENL doses, Moreira et al. (2023) [30] showed that dams treated with VENL during lactation did not show significant alterations in water and food consumption, weight gain, or organ weights (liver, kidney, adrenal gland,

spleen, heart, lung, ovary and uterus). In addition, VENL and O-desmethylvenlafaxine (ODV) residues were found in milk samples analyzed from the dams treated in a dose-response manner (1-3 ng/ml) whereas the control dams presented undetectable concentrations [30]. Therefore, F1 female offspring were exposed to this antidepressant in breast milk which may be of concern since that VEN excretion into human milk is relatively higher than the observed for other antidepressants [13].

Human and animal studies have shown that antidepressant use could harm male and female fertility [43-47]. The ovary is a central female reproductive organ that is essential for fertility, sex hormone production, and reproduction, where important perturbations during pre-natal and early post-natal to ovarian development appear to have major effects on post-natal fertility [48]. It is also a known target of environmental and synthetic pharmaceutical compounds that can alter reproductive development and fertility, resulting in reproductive disorders [49,50]. Newly weaned female SD rats were orally treated with paroxetine (3.6 mg/kg) or bupropion (17 mg/kg) until puberty. In special, the chronic treatment with paroxetine significantly reduced serum anti-Müllerian hormone (AMH), luteinizing hormone (LH), and estradiol levels while it significantly induced advanced vaginal opening (VO), vascular dilation and congestion in the ovary and inflammation and vascular dilation in the uterus [51]. In another study, dos Santos et. al. (2016) [43] showed that F1 female Wistar rats, exposed during in-utero and lactational periods to fluoxetine (5 mg/kg), presented delayed puberty onset (VO and first estrous), without significant histological alterations in the uterus and ovaries. In a similar study, Moore et al. (2015) [44] showed that after maternal exposure during 2 weeks before mating until weaning to fluoxetine (10 mg/kg), F1 female Wistar rats presented prolonged estrus phase and more number of secondary follicles and total follicles and increased apoptotic ovarian cells, without alter serum levels of estradiol, FSH and LF levels. Although some adverse effects of antidepressants on ovary development and pubertal maturation of the reproductive system have been described in female rats, our findings finding did not demonstrate that VENL induces ovary toxicity.

Some clinical evidence has demonstrated the efficacy of hormonal therapeutics including progestins and estradiol, and gamma-aminobutyric acidergic neuroactive steroids in the treatment of PPD [51]. In counterpart, some studies have demonstrated that antidepressant administration may increase serum estradiol levels in female rats and postmenopausal women [45,52]. In a study conducted by Ulker et al. (2020), paroxetine oral treatment (3.6 mg/kg/day, during the PND 21 to PND 90−93), significantly increased

serum estradiol levels in female Sprague-Dawley rats [45]. In addition, ovariectomized Wistar rats treated with VENL (10 mg/kg/day, over 4 weeks) presented an increase in hippocampal brain-derived neurotrophic factor (BDNF) and serum estradiol levels [53]. In our study, a significant increase in serum estrogen levels was detected in VENL 15.4 group compared to the control group at PDN 22 and 30. Thus, further studies are necessary to investigate on the adverse effects of lactational VENL on estradiol levels and puberty onset (vaginal opening and estrous cycle), and fertility capacity in the F1 female offspring rats.

5. Conclusion

This animal study provides the first evidence that lactational exposure to the antidepressant VENL can be safe since it did not modify mammary gland development or carcinogenesis susceptibility in F1 female Wistar rats, nor presented potential ovarian follicle development. However, the impact of lactational VENL exposure on ovary morphology/function requires further examination in a more comprehensive set of female reproductive endpoints, including studies on reproductive development and performance (puberty onset, fertility, and sexual behavior).

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships with funders that could have appeared to influence the writing and interpretation of findings reported in this research.

Data Availability

Relevant data is presented in the manuscript. Raw data will be provided upon reasonable request.

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Legend for figures:

Figure 1- A) Representative photomicrographs of the mammary gland whole-mounts of F1 female offspring at post-natal day (PND) 22 and 30 from non-treated (control) or venlafaxine (VENL)-treated groups. B e C) Morphometric parameters measured to analyze mammary gland development. Scale bar = 200 μm.

Figure 2- A) Representative photomicrographs of the mammary gland tissue sections immunostaining for PCNA and estrogen receptor alpha (ER-α) and progesterone receptor (PR) of F1 female offspring at post-natal day (PND) 22 and 30 from non-treated (control) or venlafaxine (VENL 15.4 mg/kg/day)-treated groups. B) PCNA. C) ER-α labeling indexes and serum estradiol and progesterone levels of F1 female offspring at post-natal day (PND) 22 and 30 from non-treated (control) or venlafaxine (VENL 15.4 mg/kg/day). Scale bar = 50 μm.

Figure 3- A) Representative photomicrographs of the ovary tissue sections staining with hematoxylin-eosin (HE) of F1 female offspring at post-natal day (PND) 22 and 30 from non-treated (control) or venlafaxine-treated groups. B e C) Mean number of ovarian follicles per ovary area (cm2) of F1 female offspring at post-natal day (PND) 22 and 30 from non-treated (control) or venlafaxine-treated groups. Scale bar= 500 μm.

Supplementary Data

Representative photomicrographs from tumors histological sections stained by hematoxylin–eosin (scale bar = 50 μm). A) Fibroadenoma (Control group), B) Adenoma (VENL 15.4 group) C) Adenocarcinoma (VENL 3.85 group), and D) Invasive adenocarcinoma (VENL 7.7group), respectively. Arrows: Indicate skeletal muscle fibers from abdominal muscle.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Education Personnel (CAPES-PROAP).

Table 1- Effects of lactational venlafaxine (VENL) exposure on body weight and ovary weights (absolute and relative) in F1 female offspring groups at post-natal day (PND) 22 and 301.

Parameters	Groups			
	Control	VENL 3.85	VENL 7.7	VENL15.4
Body weight (g)	N=9	N=9	N=9	N=9
PND 22	45.34 ± 6.39	46.31 ± 6.54	45.49 ± 5.40	45.14 ± 5.06
PND 30	91.00 ± 8.88	92.18 ± 8.93	93.27 ± 8.15	85.02 ± 9.78
Right ovary weights	N=9	N=9	N=9	N=9
Absolute (mg)				
PND 22	11.00 ± 2.39	13.38 ± 3.42	12.05 ± 2.56	12.00 ± 2.78
PND 30	15.16 ± 2.48	14.33 ± 3.39	13.83 ± 2.93	12.50 ± 2.04
Relative (%)				
PND 22	24.59 ±4.15	29.56 ± 5.91	26.74 ± 3.77	27.62 ± 3.70
PND 30	16.63 ± 2.55	15.59 ± 3.52	14.98 ± 3.55	14.88± 2.64
Left ovary weights	N=9	N=9	N=9	N=9
Absolute (mg)				
PND 22	12.00 ± 2.20	14.03 ± 2.86	12.62 ± 2.61	12.12 ± 1.95
PND 30	15.17 ± 2.86	14.67 ± 2.50	16.17 ± 1.52	14.17 ± 2.13
Relative (%)				
PND 22	26.97 ± 4.01	30.14 ± 4.67	26.94 ± 3.78	27.62 ± 3.70
PND 30	16.62 ± 2.54	15.98 ± 3.39	17.44 ± 1.58	16.93 ± 3.48

1 Values are indicated as median ± standard deviation; N= number of animal/group (1 female/litter). There are no differences among the groups using ANOVA test.

Table 2 - Effects of lactational venlafaxine (VENL) exposure on tumor mammary development in F1 female offspring groups initiated with N-Methyl-N-Nitrosourea (MNU).

Parameters	Group/Treatment1			
	Control	VENL 3.85	VENL 7.7	VENL 15.4
Number of females1	18	18	18	18
Body weight (g)2	260. 17 ± 16.52	266.00 ± 20.02	261.00 ± 19.39	258.50 ± 13.60
Incidence of tumor3	6 (33%)	8 (44%)	7 (38%)	7 (38%)
Time to first tumor4	16.3 ± 9.25	18.6 ± 7.4	17.9 ± 9.7	12.7 ± 8.8
Number of tumors	6	6	7	8
Malignant				
Adenocarcinoma	5 (83%)	4 (67%)	6 (86%)	7 (88%)
Benign				

Adenoma	-	-	-	1 (12%)
Fibroadenoma	1 (17%)	2 (33%)	1 (14%)	-

1 Number of animal/group (2 female/litter); 2Values are indicated as median ± standard deviation; 3 Include the animals euthanized before post-natal day 250; 3 Incidences are expressed as the number of animals bearing tumors. 4 Time (weeks) between MNU administration and the appearance of the first palpable tumor.

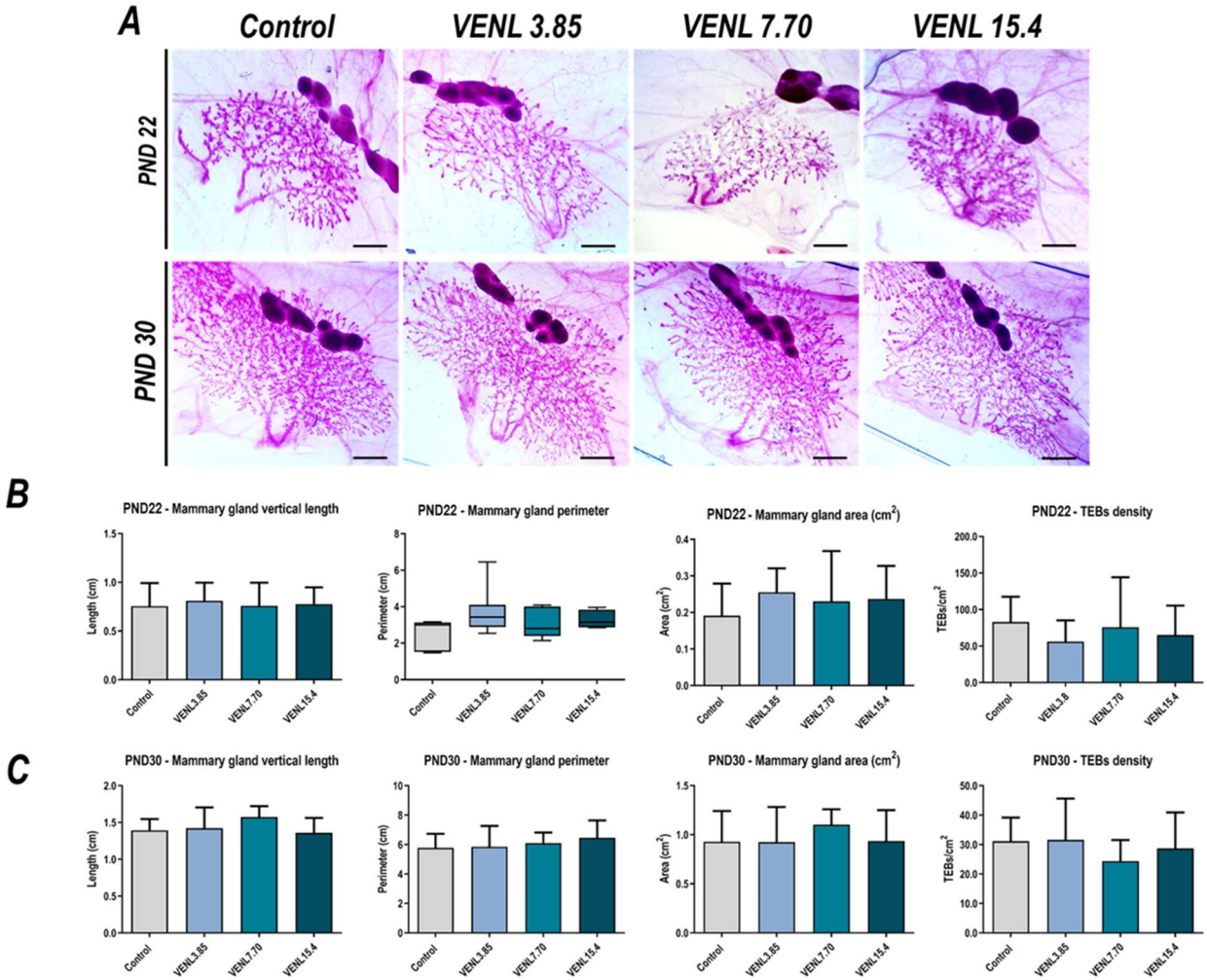


Figure 1

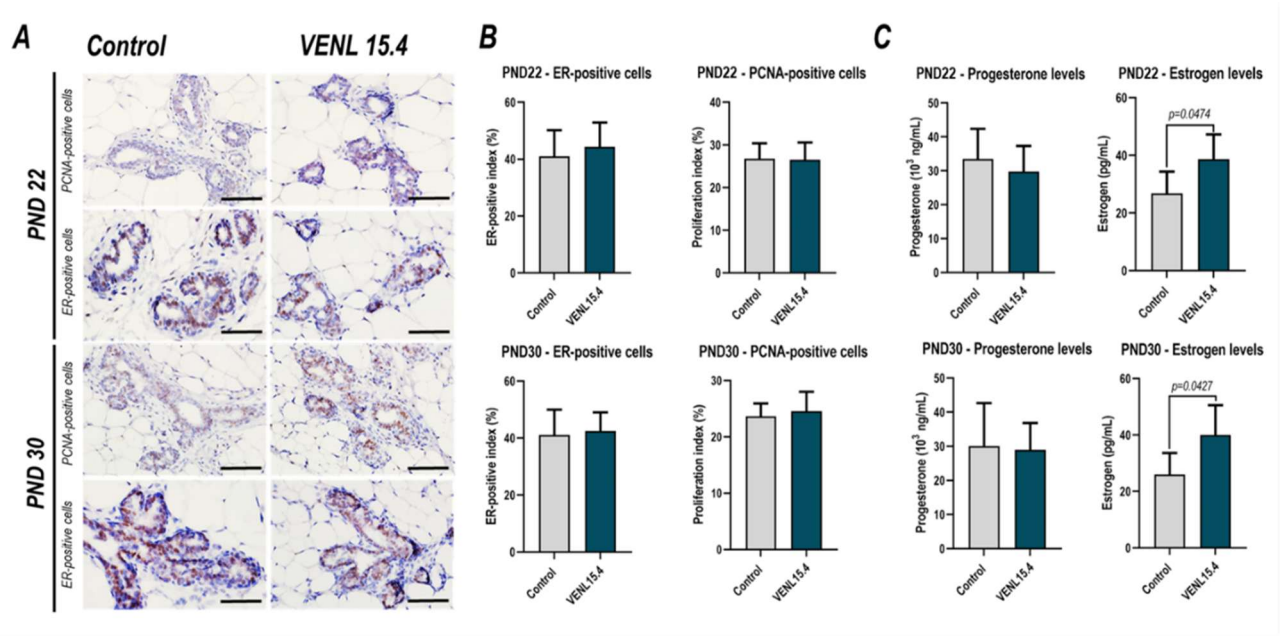


Figure 2

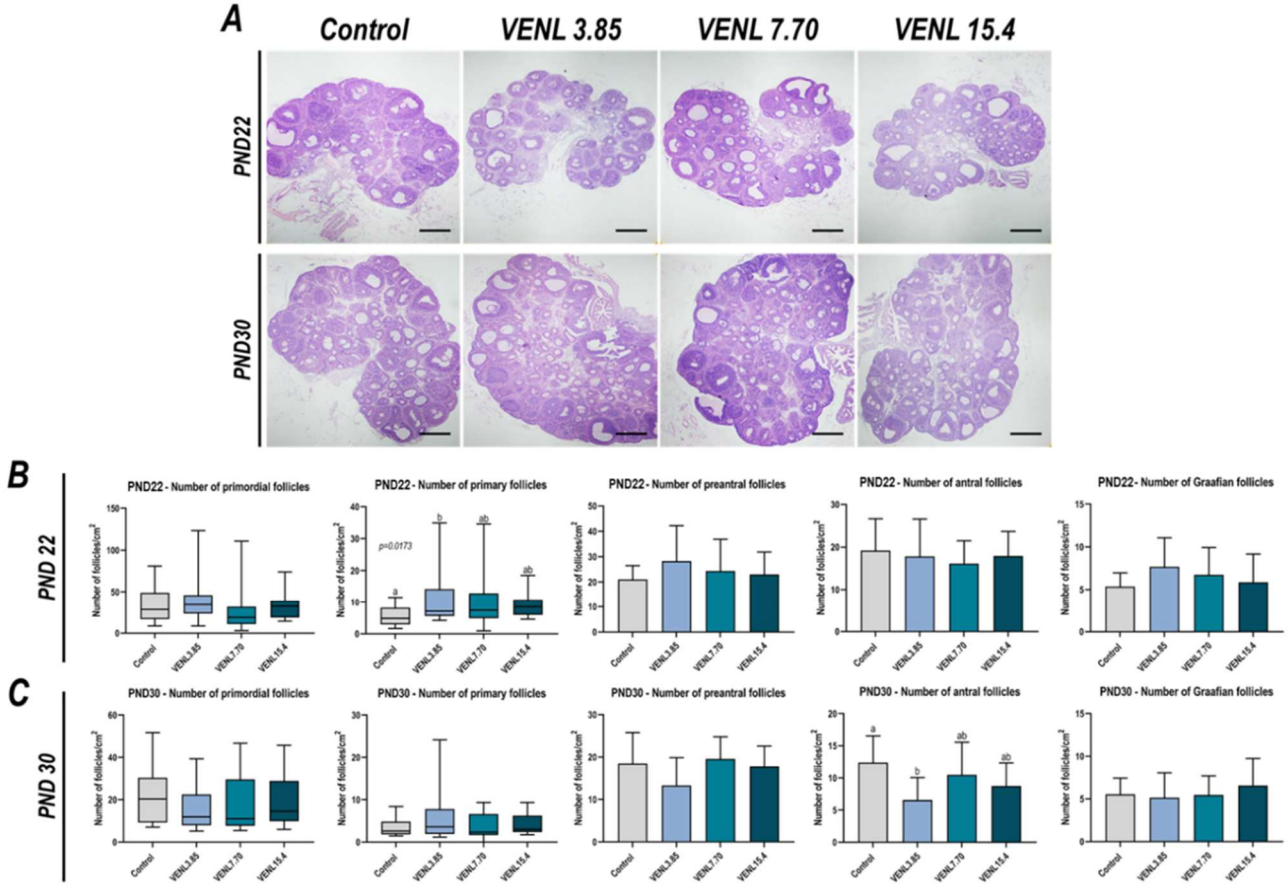
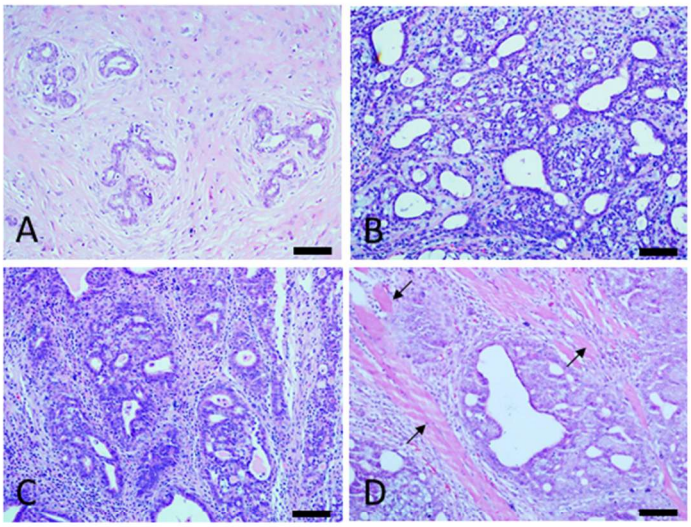


Figure 3



Supplementary Data

Representative photomicrographs from tumors histological sections stained by hematoxylin–eosin (scale bar = 50 µm). A) Fibroadenoma (Control group), B) Adenoma (VENL 15.4 group) C) Adenocarcinoma (VENL 3.85 group), and D) Invasive adenocarcinoma (VENL 7.7group), respectively. Arrows: Indicate skeletal muscle fibers from abdominal muscle.