A Pilot "Window of Opportunity" Neoadjuvant Study of Propranolol in Breast Cancer
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1. Background and Rationale:

Despite great advances in the treatment of breast cancer over the past 2 decades, breast cancer is still a deadly disease claiming too many precious lives. According to the American Cancer Society (www.Cancer.org) in the U.S., around 40,000 deaths occur each year secondary to breast cancer. There is a continuous need for more research and advances in this field.

Much of the research efforts over the past decade have focused on new molecular diagnostics and genomic profiling. This has promoted the development of multiple novel targeted agents, however despite these advances, several limitations persist: 1) Those agents are effective but also associated with significant toxicity and costs, and additional efforts are needed to better predict the benefit of new targeted agents; 2) Single-therapy options have been less effective than combinations of hormonal, chemotherapeutic, and new molecular drugs, demonstrating that it may be more important to hit an oncologic signaling pathway, rather than a single gene mutation; 3) In many patients molecular diagnostics do not reveal a discrete signaling pathway driving tumor growth, leading patients to depend on a general attack via chemotherapy. Therefore, identifying additional pathways and novel, more affordable treatment approaches in breast cancer remain desirable.

Sympathetic nervous system regulation of cancer cell biology and the tumor microenvironment has clarified the molecular basis for long-suspected relationships between stress and cancer progression. Several preclinical studies in a number of diverse cancer types have demonstrated that stimulation of beta adrenergic receptor (ADRB) signaling by the catecholamines epinephrine and norepinephrine regulate fundamental biological processes underlying the progression and metastasis of tumors, including promotion of inflammation, angiogenesis, migration, invasion, and resistance to programmed cell death, as well as inhibition of DNA damage repair and cellular immune response [1].

We recently reported that inhibition of catecholamine signaling using ADRB antagonists including the beta blockers propranolol, timolol, and atenolol reduces proliferation and promotes apoptosis in a large panel of sarcoma cell lines (angiosarcoma, osteosarcoma, and Ewing's

sarcoma), and inhibits sarcoma growth in a xenograft tumor model [2]. Moreover, we have demonstrated that propranolol behaves synergistically when combined with standard chemotherapy agents to enhance sarcoma cell death, suggesting that beta blockade sensitizes tumors to the cytoxicity of these drugs [2]. Retrospective analyses in human carcinomas have shown incredible responses to beta blockade including a 57% reduction in breast cancer metastatic development [3] and a 17% reduction in patient mortality across all major cancer types [4]. Propranolol co-administered with other therapies has shown a 4.6 month increase in progression free survival and a 5.4 month increase in patient survival for pancreatic cancer (ASCO 2015, suppl 3, abstr 302); improved progression free survival in early and metastatic breast cancer (ASCO 2013, suppl; abstr e12564); a reduced risk of recurrence in melanoma patients (ASCO 2011, suppl; abstr 8524); and a sustained complete response in a patient with angiosarcoma [5].

2- Preliminary Observations

Dr. Bryan's lab has demonstrated that ADRB proteins are strongly unregulated in breast cancers relative to normal breast tissue **(Figure 1)**. Given this key finding and the published retrospective analyses showing efficacy of beta blockade in reducing metastasis and significantly improving patient survival rates [3, 4], we propose a Phase II "Window of Opportunity" trial to prospectively test the efficacy of the beta blocker propranolol in breast cancer patients at the Texas Tech Health Science Center and University Medical Center in El Paso, Texas. By ascertaining safety and potential benefits of the beta blocker propranolol in breast cancer, we would generate a novel and cost–effective strategy that would advance treatment options for cancer patients, especially those living in low resource setting.

3. Goals and Objectives:

This trial is registered at www.clinicaltrials.gov and planned as a Phase II study using the "window-of-opportunity" (WOO) design in which the treatment-free window between breast cancer diagnosis and surgical tumor resection is used to study the biological effects of the beta blocker propranolol (Figure 2). The proliferative index of the tumor after treatment will serve as the study's primary endpoint. We have strategically chosen the WOO design as it allows us to

cost effectively evaluate molecular endpoints that support the efficacy of propranolol against breast cancer, and can be used to obtain subsequent larger grant funding from outside agencies for further clinical studies. WOO pre-surgical studies will expedite the process of testing propranolol in cancer patients by improving the understanding of its biologic effect early in its development, validating markers that may predict subsets of patients who will benefit from this drug, and targeting select patients in subsequent clinical trials that are powered to detect changes in clinical outcome. The following are the specific Aims of the study:

Specific Aim #1: Evaluate the effect of the beta blocker propranolol on reducing the tumor proliferative index using Ki-67.

Specific Aim #2: Correlate ADRB expression and intra-tumoral catecholamine signaling to propranolol treatment efficacy.

Specific Aim #3: Assess the safety, toxicity and adherence to propranolol.

4. Research Design and Methods: This trial will be designed as a Phase II study using the "window-of-opportunity" (WOO) design in which the treatment-free window between breast cancer diagnosis and surgical tumor resection is used to study the biological effects of the beta blocker propranolol (Figure The proliferative index of the tumor after treatment will serve as the study's primary endpoint. We have strategically chosen the WOO design as it allows us to cost effectively evaluate molecular endpoints that support the efficacy of propranolol against breast cancer, and can be used to obtain subsequent larger grant funding from outside agencies for further clinical studies. WOO pre-surgical studies will expedite the process of testing propranolol in cancer patients by improving the understanding of its biologic effect early in its development, validating markers that may predict subsets of patients who will benefit from this drug, and targeting select patients in subsequent clinical trials that are powered to detect changes in clinical outcome.

Pathological Assessment:

Ki-67 assessment:

To measure the proliferation rate of each tumor before and after propranolol treatment, immunohistochemical staining on the tumor sections will be performed in the UMC lab as

standard of care on the pre-treatment tissue using an anti-Ki67 antibody (Abcam) based on protocols previously established in the Bryan Lab. The post-treatment Ki-67 will be performed in Dr. Bryan's lab. This procedure is a standardized IHC method consistent with those utilized by all in-house TTUHSC pathologists. Ki-67 based proliferative index will be quantified via manual counting by an individual who is blinded to the tumor data on the same specimen and to the corresponding Ki-67 staining in the sample pair. A fixed number of 400 tumor cells in both the initial biopsies and post-treatment surgical samples will be counted from representative areas of the tumor. Comparison of positive and negative controls will be performed for quality assurance. Ki-67 is one of the most robust biomarkers that measures proliferation and according to the International Ki-67 in Breast Cancer Working Group could be important both in standard clinical practice and, particularly, within clinical trials [6].We recently conducted a study to correlate between Ki-67, Recurrence score (RS) and Recurrence Score –Pathology – Clinical (RSPC) Assessment in Early Stage Breast Cancer and found significant concordance especially among the low and high risk groups [7]. Ki-67 staining shows relatively consistent measurements in specimens across a range of conditions used in routine fixation, tissue processing, and IHC analysis.

Tissue Microarray (TMA) From the original paraffin block of each tumor, so-called donor block (30 cases, post-treatment), a 3mm wide and 5mm deep punch biopsy, corresponding to the tumor site, will be cut. These punch biopsies will be re-embedded in a new paraffin block (recipient block) to create TMA (possibly all 30 cases in one block). An excel file will be created that identified all the 30 cases by only numbers. From this TMA, multiple 3-4 microns thick slides will be cut at the UMC histology lab for Immunohistochemistry and will be provided to DR Bryan's lab from the UMC Pathology (Dr. Torabi). **The team will need at least 4 slides from post-surgery tumor**. With access to each patient sample before and after propranolol treatment, we will have the ability to perform further analysis to understand at the molecular level how beta blockade is affecting the tumors, if the tumors are responding to the treatment, and if the tumors are actively taking steps to develop resistance to the treatment. We will begin by examining the expression of ADRB receptors in each tumor. We will subsequently examine the expression of several downstream effectors of ADRB signaling (as a positive control to confirm that propranolol is indeed blocking ADRB signaling in the treated tumors) and potential mechanisms of resistance

that the tumor might be modulating to overcome propranolol inhibition.

These molecular studies, which will08/ be performed in the Bryan Lab, are important in our understanding of how propranolol is affecting the growth of breast tumors and to identify potential compensatory resistance events that may be occurring in the tumors following beta blockade.

- To carry out the above tests, all tumor sections (pre- and post-propranolol treatment) will be obtained from UMC Pathology, tissue sectioning and immunohistochemical (IHC) analysis will be performed in Dr. Bryan's lab using established experimental protocols.
- For all experiments, scoring for the expression of each protein will be performed in a semi-quantitatively (0, +1, +2, +3) blinded fashion for 1) the staining intensity and 2) the physical area within the tumor which the proteins stain positive.
- Immunohistochemistry scores for each tumor section will be obtained by multiplying the staining intensity with the area of antigen positivity.

ADRB Receptor Analysis:

The three ADRB receptors that we will be looking at are ADRB1, ADRB2 and ADRB3 (which are the three beta adrenergic receptors expressed in humans, and the target of propranolol). We will attempt to statistically correlate treatment efficacy to the expression of each ADRB receptor. Tumor samples from the initial biopsy and surgical resection will be deidentified and stained via IHC for these specific ADRB receptors in Dr. Bryan's laboratory. We will require 3 tumor sections per collected tumor sample to accomplish this experiment. For this analysis, we will require 15 tumor sections pre-propranolol treatment and 15 tumor sections post-propranolol treatment. Each section will need to be 5 microns in thickness, thus we will need approximately 100-150 microns of tissue from each tumor to complete the analysis.

5. Eligibility Criteria:

Inclusion criteria - patients must fulfill all of the following to be enrolled on the study:

A) Ages 18- 65

B) A diagnosis of non-metastatic stage I-III breast cancer of at least 10 mm within the past 90 days, confirmed by a core biopsy

C) Patient who is planning to undergo definitive surgery including mastectomy or breast

conserving surgery

D) Medically able to abstain from alcohol and all illicit drugs, and limit caffeine intake to less than or equal to 2 cups of coffee per day for at least two weeks before and during the treatment period.

E) Potential subjects systolic blood pressure must be >90 mmHg and/or diastolic > 50 mmHg and no more than 95 mmHg.

F) Able to read, understand and sign a consent form

G) Normal baseline EKG

Exclusion criteria: - patients will be excluded for any of the following criteria:
A) Pregnancy; potential subjects of female bearing age will have to complete a pregnancy test during screening to ensure that they are not pregnant. Potential patients who are post-menopausal must have confirmed one year without menstrual cycle.

B) Free of major medical illnesses including:

- Cardiac diseases (history of cardiac valve disease, coronary artery disease, congestive heart failure, A-V block, peripheral vascular disease, any cardiac arrhythmia/bradycardia) with the exception of the diagnosed cancer.

C) Histories of asthma, bronchospastic disease, or obstructive pulmonary disease

E) Previously diagnosed thyrotoxicosis

F) Severe allergic reactions to medications which are included in the beta blocker family

G) Currently treated with a beta adrenergic receptor antagonist

H) Patients with breast cancer not amenable to surgical resection

I) Patients taking any of the following medications will be excluded:

Input

- Drugs that are categorized as beta-blockers
- Amiodarone
- Sotalol

J) If patient is scheduled to receive surgery in less than 3 weeks of biopsy, then patient will not be eligible.

6. Investigational Plan

This pilot study is looking to enroll 30 patients total at Texas Tech University Health Sciences Center.

- Once a patient is deemed eligible by the criteria listed above and they sign an informed consent, study treatment will begin. Since we are utilizing the window of opportunity method, it is imperative that we catch patients in the 3 week window from when the patient is diagnosed with breast cancer to when the surgery is scheduled to remove the cancer.
- Participants will remain on 1.5 mg/kg/day of Propranolol, which will be taken twice daily, for 2 weeks and 4 days. The patient will be asked to start a tapering period on day 5 of week 2 and their dose will be lowered by 50%. On day one of week 3, patients will be asked to lower their dose again by 25% of their original dose and they will take the medication at this dose for 4 days. After day 4, the patient will no longer take the medication.[11].
- Patient's first dose will be in the morning the day after the study medication is distributed to them so that it allows study staff to contact the patient 4-6 hours after they are scheduled to start taking the medication in order to assess toxicities.
- All data points that will be collected will be entered in to the database REDCap.
- All patient visits will be completed at the Garber Breast Care Center at the Texas Tech University Health Sciences Center.

Once a patient signs the appropriate consent and is enrolled in the study, treatment will begin. Dr. Bryan's lab will collect a sample of the biopsied mass and his lab will complete the biomarker assessment on the tissue. Ki-67 assessment is routinely done at the Pathology Department. The baseline study visit will include a physical exam, vitals, electrocardiogram and Eastern Cooperative Oncology Group (ECOG) performance assessment which will be completed by study staff. The subject will be administered the study drug, propranolol. The subject will also receive detailed instructions on how to self-administer the propranolol and be given a medication diary so that they can keep track of when they take the medication. The subject will be provided a blood pressure monitor with a pulse feature included in order to take their blood pressure and pulse twice daily while they are enrolled on the study. When patients are supplied the blood pressure machine

by study staff, a detailed training of how to use the machines will be provided to the patient. This will be overseen by Dr. Nahleh and a training log will be kept for each patient. Additional training will be provided during the follow-up visits upon patient's request. Study staff will also conduct a chart review to gather the patient's medical history.

7. Drug Information [10]

Propranolol hydrochloride is a synthetic beta-adrenergic receptor blocking agent chemically described as 2-Propanol, 1-[(1-methylethyl) amino]-3-(1-naphthalenyloxy)-, hydrochloride, (±)-. It's molecular and structural formulae are: Propranolol hydrochloride is a stable, white, crystalline solid which is readily soluble in water and ethanol. Its molecular weight is 295.80. The drug is available as 10 mg, 20 mg, 40 mg, 60 mg, 80 mg and 120 mg tablets for oral administration. The patient dose will be rounded so that the medication can be provided in the tablets previously listed. Store at controlled room temperature 20° to 25° C (68° to 77° F); excursions permitted to 15° to 30° C (59° to 86° F).

Clinical Pharmacology

General

Propranolol is a nonselective beta-adrenergic receptor blocking agent possessing no other autonomic nervous system activity. It specifically competes with beta-adrenergic receptor agonist agents for available receptor sites. When access to beta-receptor sites is blocked by propranolol, the chronotropic, inotropic, and vasodilator responses to beta-adrenergic stimulation are decreased proportionately.

Mechanism of Action

The mechanism of the antihypertensive effect of propranolol has not been established. Factors that may contribute to the antihypertensive action include: (1) decreased cardiac output, (2) inhibition of renin release by the kidneys, and (3) diminution of tonic sympathetic nerve outflow from vasomotor centers in the brain.

Although total peripheral resistance may increase initially, it readjusts to or below the pretreatment level with chronic use of propranolol. Effects of propranolol on plasma volume appear to be minor and somewhat variable.

Angina pectoris:
In angina pectoris, propranolol generally reduces the oxygen requirement of the heart at any given level of effort by blocking the catecholamine-induced increases in the heart rate, systolic blood pressure, and the velocity and extent of myocardial contraction. Propranolol may increase oxygen requirements by increasing left ventricular fiber length, end diastolic pressure, and systolic ejection period. The net physiologic effect of beta-adrenergic blockade is usually advantageous and is manifested during exercise by delayed onset of pain and increased work capacity.

Antiarrhythmic effect: Propranolol exerts its antiarrhythmic effects in concentrations associated with beta-adrenergic blockade, and this appears to be its principal antiarrhythmic mechanism of action.

Anti-migraine effect: The mechanism of the anti-migraine effect of propranolol has not been established. Beta-adrenergic receptors have been demonstrated in the pial vessels of the brain.

Anti-tremor effect: The specific mechanism of propranolol's anti-tremor effects has not been established, but beta-2 (non-cardiac) receptors may be involved. A central effect is also possible. Clinical studies have demonstrated that propranolol is of benefit in exaggerated physiological and essential (familial) tremor.

Pharmacokinetic and Drug Metabolism

Absorption
Propranolol is highly lipophilic and almost completely absorbed after oral administration. However, it undergoes high first-pass metabolism by the liver and on average, only about 25% of propranolol reaches the systemic circulation. Peak plasma concentrations occur about 1 to 4 hours after an oral dose. Administration of protein-rich foods increase the bioavailability of

propranolol by about 50% with no change in time to peak concentration, plasma binding, half-life, or the amount of unchanged drug in the urine.

Distribution

Approximately 90% of circulating propranolol is bound to plasma proteins (albumin and alpha1 acid glycoprotein). The binding is enantiomer-selective. The S (-)-enantiomer is preferentially bound to alpha1 glycoprotein and the R (+)-enantiomer preferentially bound to albumin. The volume of distribution of propranolol is approximately 4 liters/kg. Propranolol crosses the blood-brain barrier and the placenta, and is distributed into breast milk.

Metabolism and Elimination

Propranolol is extensively metabolized with most metabolites appearing in the urine. Propranolol is metabolized through three primary routes: aromatic hydroxylation (mainly 4-hydroxylation), N-dealkylation followed by further side-chain oxidation, and direct glucuronidation. It has been estimated that the percentage contributions of these routes to total metabolism are 42%, 41% and 17%, respectively, but with considerable variability between individuals. The four major metabolites are propranolol glucuronide, naphthyloxylactic acid and glucuronic acid, and sulfate conjugates of 4-hydroxy propranolol. In vitro studies have indicated that the aromatic hydroxylation of propranolol is catalyzed mainly by polymorphic CYP2D6. Side-chain oxidation is mediated mainly by CYP1A2 and to some extent by CYP2D6. 4-hydroxy propranolol is a weak inhibitor of CYP2D6.

Propranolol is also a substrate of CYP2C19 and a substrate for the intestinal efflux transporter, p-glycoprotein (p-gp). Studies suggest however that p-gp is not dose-limiting for intestinal absorption of propranolol in the usual therapeutic dose range. In healthy subjects, no difference was observed between CYP2D6 extensive metabolizers (EMs) and poor metabolizers (PMs) with respect to oral clearance or elimination half-life. Partial clearance of 4-hydroxy propranolol was significantly higher and of naphthyloxyactic acid significantly lowers in EMs than PMs. The plasma half-life of propranolol is from 3 to 6 hours.

Renal Insufficiency:

In a study conducted in 5 patients with chronic renal failure, 6 patients on regular dialysis, and 5 healthy subjects, who received a single oral dose of 40 mg of propranolol, the peak plasma concentrations (Cmax) of propranolol in the chronic renal failure group were 2 to 3-fold higher (161±41 ng/mL) than those observed in the dialysis patients (47±9 ng/mL) and in the healthy subjects (26±1 ng/mL). Propranolol plasma clearance was also reduced in the patients with chronic renal failure.

Chronic renal failure has been associated with a decrease in drug metabolism via downregulation of hepatic cytochrome P450 activity resulting in a lower "first-pass" clearance. Propranolol is not significantly dialyzable.

Hepatic Insufficiency: Propranolol is extensively metabolized by the liver. In a study conducted in 7 patients with cirrhosis and 9 healthy subjects receiving 80-mg oral propranolol every 8 hours for 7 doses, the steady-state unbound propranolol concentration in patients with cirrhosis was increased 3-fold in comparison to controls.

Drug Interactions:

Cytochrome P-450-

Because propranolol's metabolism involves multiple pathways in the cytochrome P-450 system (CYP2D6, 1A2, 2C19), co-administration with drugs that are metabolized by, or effect the activity (induction or inhibition) of one or more of these pathways may lead to clinically relevant drug interactions Substrates or Inhibitors of CYP2D6 Blood levels and/or toxicity of propranolol may be increased by co-administration with substrates or inhibitors of CYP2D6, such as amiodarone, cimetidine, delavudin, fluoxetine, paroxetine, quinidine, and ritonavir. No interactions were observed with either ranitidine or lansoprazole. Substrates or Inhibitors of CYP1A2 Blood levels and/or toxicity of propranolol may be increased by co-administration with substrates or inhibitors of CYP1A2, such as imipramine, cimetidine, ciprofloxacin, fluvoxamine, isoniazid, ritonavir, theophylline, zileuton, zolmitriptan, and rizatriptan. Substrates or Inhibitors of CYP2C19 Blood levels and/or toxicity of propranolol may be increased by co-administration with substrates or inhibitors of CYP2C19, such as fluconazole, cimetidine, fluoxetine, fluvoxamine, tenioposide, and tolbutamide. No interaction was observed with omeprazole.

Drug Metabolism Blood levels of propranolol may be decreased by co-administration with inducers such as rifampin, ethanol, phenytoin, and phenobarbital.

Cigarette smoking also induces hepatic metabolism and has been shown to increase up to 77% the clearance of propranolol, resulting in decreased plasma concentrations.

Cardiovascular Drugs:
Antiarrhythmics - The AUC of propafenone is increased by more than 200% by co-administration of propranolol. The metabolism of propranolol is reduced by co-administration of quinidine, leading to a two-three fold increased blood concentration and greater degrees of clinical beta-blockade.

The metabolism of lidocaine is inhibited by co-administration of propranolol, resulting in a 25% increase in lidocaine concentrations.
Calcium Channel Blockers - The mean Cmax and AUC of propranolol are increased, respectively, by 50% and 30% by co-administration of nisoldipine and by 80% and 47%, by co-administration of nicardipine. The mean Cmax and AUC of nifedipine are increased by 64% and 79%, respectively, by co-administration of propranolol.
Propranolol does not affect the pharmacokinetics of verapamil and norverapamil. Verapamil does not affect the pharmacokinetics of propranolol.

Non-Cardiovascular Drugs
Migraine Drugs Administration - of zolmitriptan or rizatriptan with propranolol resulted in increased concentrations of zolmitriptan (AUC increased by 56% and Cmax by 37%) or rizatriptan (the AUC and Cmax were increased by 67% and 75%, respectively).

Theophylline - Co-administration of theophylline with propranolol decreases theophylline oral clearance by 30% to 52%.

Benzodiazepines Propranolol - can inhibit the metabolism of diazepam, resulting in increased concentrations of diazepam and its metabolites. Diazepam does not alter the pharmacokinetics of

propranolol. The pharmacokinetics of oxazepam, triazolam, lorazepam, and alprazolam are not affected by co-administration of propranolol.

Neuroleptic Drugs - Co-administration of long-acting propranolol at doses greater than or equal to 160 mg/day resulted in increased thioridazine plasma concentrations ranging from 55% to 369% and increased thioridazine metabolite (mesoridazine) concentrations ranging from 33% to 209%. Co-administration of chlorpromazine with propranolol resulted in a 70% increase in propranolol plasma level.

Anti-Ulcer Drugs - Co-administration of propranolol with cimetidine, a non-specific CYP450 inhibitor, increased propranolol AUC and Cmax by 46% and 35%, respectively. Co-administration with aluminum hydroxide gel (1200 mg) may result in a decrease in propranolol concentrations. Co-administration of metoclopramide with the long-acting propranolol did not have a significant effect on propranolol's pharmacokinetics.

Lipid Lowering Drugs - Co-administration of cholestyramine or colestipol with propranolol resulted in up to 50% decrease in propranolol concentrations. Co-administration of propranolol with lovastatin or pravastatin, decreased 18% to 23% the AUC of both, but did not alter their pharmacodynamics. Propranolol did not have an effect on the pharmacokinetics of fluvastatin.

Warfarin Concomitant - administration of propranolol and warfarin has been shown to increase warfarin bioavailability and increase prothrombin time.

Alcohol - Concomitant use of alcohol may increase plasma levels of propranolol.

Indications and Usage

Hypertension
The drug is indicated in the management of hypertension. It may be used alone or used in combination with other antihypertensive agents, particularly a thiazide diuretic. Propranolol is not indicated in the management of hypertensive emergencies.

Angina Pectoris Due to Coronary Atherosclerosis

The drug l is indicated to decrease angina frequency and increase exercise tolerance in patients with angina pectoris.

Atrial Fibrillation
The drug is indicated to control ventricular rate in patients with atrial fibrillation and a rapid ventricular response.

Myocardial Infarction
The drug is indicated to reduce cardiovascular mortality in patients who have survived the acute phase of myocardial infarction and are clinically stable.

Migraine
The drug l is indicated for the prophylaxis of common migraine headache. The efficacy of propranolol in the treatment of a migraine attack that has started has not been established, and propranolol is not indicated for such use.

Essential Tremor
The drug is indicated in the management of familial or hereditary essential tremor. Familial or essential tremor consists of involuntary, rhythmic, oscillatory movements, usually limited to the upper limbs. It is absent at rest, but occurs when the limb is held in a fixed posture or position against gravity and during active movement. Propranolol causes a reduction in the tremor amplitude, but not in the tremor frequency. Propranolol is not indicated for the treatment of tremor associated with Parkinsonism.

Hypertrophic Subaortic Stenosis
The drug improves NYHA functional class in symptomatic patients with hypertrophic subaortic stenosis.

Pheochromocytoma:
The drug is indicated as an adjunct to alpha-adrenergic blockade to control blood pressure and reduce symptoms of catecholamine-secreting tumors.

Contradictions

Propranolol is contraindicated in 1) cardiogenic shock; 2) sinus bradycardia and greater than first degree block; 3) bronchial asthma; and 4) in patients with known hypersensitivity to propranolol hydrochloride.

Precautions

General
Propranolol should be used with caution in patients with impaired hepatic or renal function. Propranolol is not indicated for the treatment of hypertensive emergencies. Beta-adrenergic receptor blockade can cause reduction of intraocular pressure. Patients should be told that Propranolol may interfere with the glaucoma screening test. Withdrawal may lead to a return of increased intraocular pressure. While taking beta blockers, patients with a history of severe anaphylactic reaction to a variety of allergens may be more reactive to repeated challenge, accidental, diagnostic, or therapeutic. Such patients may be unresponsive to the usual doses of epinephrine used to treat allergic reaction.

Clinical Laboratory Tests
In patients with hypertension, use of propranolol has been associated with elevated levels of serum potassium, serum transaminases and alkaline phosphatase. In severe heart failure, the use of propranolol has been associated with increases in Blood Urea Nitrogen.

Drug Interactions
Caution should be exercised when Propranolol is administered with drugs that have an effect on CYP2D6, 1A2, or 2C19 metabolic pathways. Co-administration of such drugs with propranolol may lead to clinically relevant drug interactions and changes on its efficacy and/or toxicity.

Cardiovascular Drugs

Antiarrhythmics
Propafenone has negative inotropic and beta-blocking properties that can be additive to those of propranolol. Quinidine increases the concentration of propranolol and produces greater degrees of clinical beta-blockade and may cause postural hypotension. Amiodarone is an antiarrhythmic agent with negative chronotropic properties that may be additive to those seen with β-blockers

such as propranolol. The clearance of lidocaine is reduced with administration of propranolol. Lidocaine toxicity has been reported following administration with propranolol.
Caution should be exercised when administering Propranolol with drugs that slow A-V nodal conduction, e.g. digitalis, lidocaine and calcium channel blockers.

Digitalis Glycosides
Both digitalis glycosides and beta-blockers slow atrioventricular conduction and decrease heart rate. Concomitant use can increase the risk of bradycardia.

Calcium Channel Blockers
Caution should be exercised when patients receiving a beta blocker are administered a calcium-channel-blocking drug with negative inotropic and/or chronotropic effects. Both agents may depress myocardial contractility or atrioventricular conduction.
There have been reports of significant bradycardia, heart failure, and cardiovascular collapse with concurrent use of verapamil and beta-blockers.
Co-administration of propranolol and diltiazem in patients with cardiac disease has been associated with bradycardia, hypotension, high-degree heart block, and heart failure.
There have been reports of significant bradycardia, heart failure, and cardiovascular collapse with concurrent use of verapamil and beta-blockers.
Co-administration of propranolol and diltiazem in patients with cardiac disease has been associated with bradycardia, hypotension, high-degree heart block, and heart failure.

ACE Inhibitors
When combined with beta-blockers, ACE inhibitors can cause hypotension, particularly in the setting of acute myocardial infarction. The antihypertensive effects of clonidine may be antagonized by beta-blockers. Propranolol should be administered cautiously to patients withdrawing from clonidine.

Alpha Blockers
Prazosin has been associated with prolongation of first dose hypotension in the presence of beta-blockers. Postural hypotension has been reported in patients taking both beta-blockers and terazosin or doxazosin.

Reserpine
Patients receiving catecholamine-depleting drugs, such as reserpine, should be closely observed for excessive reduction of resting sympathetic nervous activity, which may result in hypotension, marked bradycardia, vertigo, syncopal attacks, or orthostatic hypotension.

Inotropic Agents
Patients on long-term therapy with propranolol may experience uncontrolled hypertension if administered epinephrine as a consequence of unopposed alpha-receptor stimulation. Epinephrine is therefore not indicated in the treatment of propranolol overdose

Isoproterenol and Dobutamine
Propranolol is a competitive inhibitor of beta-receptor agonists, and its effects can be reversed by administration of such agents, e.g., dobutamine or isoproterenol. Also, propranolol may reduce sensitivity to dobutamine stress echocardiography in patients undergoing evaluation for myocardial ischemia.

Non-Cardiovascular Drugs
Nonsteroidal Anti-Inflammatory Drugs
Nonsteroidal anti-inflammatory drugs (NSAIDS) have been reported to blunt the antihypertensive effect of beta-adrenoreceptor blocking agents. Administration of indomethacin with propranolol may reduce the efficacy of propranolol in reducing blood pressure and heart rate.

Antidepressants
The hypotensive effects of MAO inhibitors or tricyclic antidepressants may be exacerbated when administered with beta-blockers by interfering with the beta blocking activity of propranolol.

Anesthetic Agents
Methoxyflurane and trichloroethylene may depress myocardial contractility when administered with propranolol.

Warfarin
Propranolol when administered with warfarin increases the concentration of warfarin. Prothrombin time, therefore, should be monitored.

Neuroleptic Drugs
Hypotension and cardiac arrest have been reported with the concomitant use of propranolol and haloperidol.

Thyroxin
Thyroxin may result in a lower than expected T3 concentration when used concomitantly with propranolol.

Alcohol
Alcohol, when used concomitantly with propranolol, may increase plasma levels of propranolol.

Geriatric Use
Clinical studies of propranolol did not include sufficient numbers of subjects aged 65 and over to determine whether they respond differently from younger subjects. Other reported clinical experience has not identified differences in responses between the elderly and younger patients. In general, dose selection for an elderly patient should be cautious, usually starting at the low end of the dosing range, reflecting the greater frequency of decreased hepatic, renal, or cardiac function, and of concomitant disease or other drug therapy.

Adverse Reactions
The following adverse events were observed and have been reported in patients using propranolol.

Cardiovascular: Bradycardia; congestive heart failure; intensification of AV block; hypotension; paresthesia of hands; thrombocytopenic purpura; arterial insufficiency, usually of the Raynaud type.

Central Nervous System: Light-headedness, mental depression manifested by insomnia, lassitude, weakness, fatigue; catatonia; visual disturbances; hallucinations; vivid dreams; an acute reversible syndrome characterized by disorientation for time and place, short-term memory

loss, emotional lability, slightly clouded sensorium, and decreased performance on neuropsychometrics. For immediate-release formulations, fatigue, lethargy, and vivid dreams appear dose-related.

Gastrointestinal: Nausea, vomiting, epigastric distress, abdominal cramping, diarrhea, constipation, mesenteric arterial thrombosis, ischemic colitis.

Allergic: Hypersensitivity reactions, including anaphylactic/anaphylactoid reactions, pharyngitis and agranulocytosis; erythematous rash, fever combined with aching and sore throat; laryngospasm, and respiratory distress.

Respiratory: Bronchospasm.

Hematologic: Agranulocytosis, nonthrombocytopenic purpura, thrombocytopenic purpura.

Autoimmune Systemic Autoimmune: Systemic lupus erythematosus (SLE).

Skin and mucous membranes: Stevens - Johnson syndrome, toxic epidermal necrolysis, dry eyes, exfoliative dermatitis, erythema multiforme, urticaria, alopecia, SLE-like reactions, and psoriasiform rashes. Oculomucocutaneous syndromes involving the skin, serous membranes and conjunctivae reported for a beta blocker (practolol) have not been associated with propranolol.

Genitourinary: Male impotence; Peyronie's disease.

Overdosage

Propranolol is not significantly dialyzable. In the event of over dosage or exaggerated response, the following measures should be employed:

General: If ingestion is or may have been recent, evacuate gastric contents, taking care to prevent pulmonary aspiration.

Supportive Therapy: Hypotension and bradycardia have been reported following propranolol overdose and should be treated appropriately. Glucagon can exert potent inotropic and

chronotropic effects and may be particularly useful for the treatment of hypotension or depressed myocardial function after a propranolol overdose. Glucagon should be administered as 50-150 mcg/kg intravenously followed by continuous drip of 1-5 mg/hour for positive chronotropic effect. Isoproterenol, dopamine or phosphodiesterase inhibitors may also be useful. Epinephrine, however, may provoke uncontrolled hypertension. Bradycardia can be treated with atropine or isoproterenol. Serious bradycardia may require temporary cardiac pacing.
The electrocardiogram, pulse, blood pressure, neurobehavioral status and intake and output balance must be monitored. Isoproterenol and aminophylline may be used for bronchospasm.

Dosage and Administration

General

Because of the variable bioavailability of propranolol, the dose should be individualized based on response.

Hypertension

The usual initial dosage is 40 mg Propranolol twice daily, whether used alone or added to a diuretic. Dosage may be increased gradually until adequate blood pressure control is achieved. The usual maintenance dosage is 120 mg to 240 mg per day. In some instances a dosage of 640 mg a day may be required. The time needed for full antihypertensive response to a given dosage is variable and may range from a few days to several weeks. While twice-daily dosing is effective and can maintain a reduction in blood pressure throughout the day, some patients, especially when lower doses are used, may experience a modest rise in blood pressure toward the end of the 12-hour dosing interval. This can be evaluated by measuring blood pressure near the end of the dosing interval to determine whether satisfactory control is being maintained throughout the day. If control is not adequate, a larger dose, or 3-times-daily therapy may achieve better control.

Angina Pectoris

Total daily doses of 80 mg to 320 mg Propranolol, when administered orally, twice a day, three times a day, or four times a day, have been shown to increase exercise tolerance and to reduce ischemic changes in the ECG. If treatment is to be discontinued, reduce dosage gradually over a period of several weeks.

Atrial Fibrillation

The recommended dose is 10 mg to 30 mg Propranolol three or four times daily before meals and at bedtime.

Myocardial Infarction

In the Beta-Blocker Heart Attack Trial (BHAT), the initial dose was 40 mg t.i.d., with titration after 1 month to 60 mg to 80 mg t.i.d. as tolerated. The recommended daily dosage is 180 mg to 240 mg Propranolol per day in divided doses. Although a t.i.d. regimen was used in the BHAT and a q.i.d. regimen in the Norwegian Multicenter Trial, there is a reasonable basis for the use of either a t.i.d. or b.i.d. regimen. The effectiveness and safety of daily dosages greater than 240 mg for prevention of cardiac mortality have not been established. However, higher dosages may be needed to effectively treat coexisting diseases such as angina or hypertension (see above).

Migraine

The initial dose is 80 mg Propranolol daily in divided doses. The usual effective dose range is 160 mg to 240 mg per day. The dosage may be increased gradually to achieve optimum migraine prophylaxis. If a satisfactory response is not obtained within four to six weeks after reaching the maximum dose, Inderal therapy should be discontinued. It may be advisable to withdraw the drug gradually over a period of several weeks.

Essential Tremor

The initial dosage is 40 mg Propranolol twice daily. Optimum reduction of essential tremor is usually achieved with a dose of 120 mg per day. Occasionally, it may be necessary to administer 240 mg to 320 mg per day.

Hypertrophic Subaortic Stenosis

The usual dosage is 20 mg to 40 mg Propranolol three or four times daily before meals and at bedtime.

Pheochromocytoma

The usual dosage is 60 mg Propranolol daily in divided doses for three days prior to surgery as adjunctive therapy to alpha-adrenergic blockade. For the management of inoperable tumors, the usual dosage is 30 mg daily in divided doses as adjunctive therapy to alpha-adrenergic blockade.

How Supplied

Dispense in a well-closed, light-resistant container as defined in the USP. Protect from light. Use carton to protect contents from light. Store at controlled room temperature 20° to 25° C (68° to 77° F); excursions permitted to 15° to 30° C (59° to 86° F). Dispense in a well-closed, light-resistant container as defined in the USP. Protect from light.

Toxicity and Adverse Events

Known side effects of propranolol include hypoglycemia, hypotension, bradycardia, and bronchospasm. It was recently reported in a study of over 460 patients with infantile hemangioma that a dose of propranolol up to 3 mg/kg/day for up to 6 months resulted in no significant difference in adverse events between the placebo and treatment group [9]. Safety assessments will be performed at in-clinic visits by analysis of adverse events (i.e. any change in condition between the time of informed consent and the end of the trial); laboratory investigations (including measurement of glucose levels from finger-prick blood samples), physical examination (including pulmonary auscultation, liver palpation, assessment of vital signs, and assessment of cognitive function), and electrocardiography.

8. Toxicity Monitoring and Dose Modification

Patients will be closely monitored during the entirety of this study for known important risks associated with propranolol therapy. A baseline EKG will be done and read by the cardiologist

who will determine any abnormality that would preclude participation. Patients with a normal EKG will be allowed to participate on this trial. After taking the initial dose of propranolol, the study staff will contact the study participant via phone within 4-6 hours in order to follow-up and determine if the patient is having any side effects from the medication. If the patient is found to have side effects, the study staff that conducts the call will immediately notify the PI and/or co-investigators. They will then determine if the study treatment should continue or if the patient should stop treatment. Patients will also be asked to call the study coordinator at any time during the study if they have any further side effects.

In addition to in-clinic assessments, blood pressure and pulse monitoring will be performed and recorded by the participants in-home on a daily basis, twice daily, using supplied monitors to ensure that propranolol is not adversely affecting their blood pressure levels or pulse below established threshold levels. If the blood pressure of any participant drops below 100/60 and/or pulse is <55, propranolol will be stopped and the patient taken off protocol. The event will be counted as an adverse event.

A board certified cardiologist (A.A) who is on active staff at UMC and Texas Tech University Health Sciences Center will review the history and physical exam data, and the EKG to assure that the patient is unlikely to have pre-existing cardiac disease. The cardiologist will also be available throughout the study in order to see patients as needed if the PI and sub-investigators determine it would beneficial to the patient.

All toxicities will be graded according to CTCAE 4.0. The dose of Propranolol will not be modified. If a patient is found to have an AE grade of 2 or more, treatment will stop and the patient will complete the end of treatment visit.

Study treatment will continue 25 days until there is an unacceptable toxicity, the subject is non-compliant with trial procedures or if the principal investigator and co-investigators feel that it is unsafe for the patient to continue with the treatment.

9. Statistical Consideration and Data Analysis:

The primary endpoint of this study is the change in tumor proliferative index following propranolol treatment. We expect to have a moderate effect size in the tumor proliferative index following propranolol treatment. We anticipate having at least 10% mean change in the tumor proliferative index following propranolol treatment with a common standard deviation of 15% (a conservative estimate). Using this information, a total sample size of 25 patients achieves more than 85% power with 5% level of significance using a two tailed paired t-test. After adding an estimated 20% dropout rate, we propose to recruit a total of 30 patients in the study. This sample size is sufficient to determine the high correlation (80% or more) between change in proliferation marker and expression of beta adrenergic receptors with a confidence width equal to 30%. The sample size was determined using PASS software (www.ncss.com).

Expected results: Given the promising results of the recent retrospective clinical trials demonstrating that propranolol significantly reduces breast cancer metastasis and improves cancer patient survival, as well as the promising case studies showing rapid and complete remission of highly aggressive tumors following propranolol administration, we are highly optimistic that we will see a statistically and clinically significant reduction in the proliferative index of breast cancer tumors following the treatment. If our findings are promising, the data acquired could lay the foundation for using beta blockade as a safe, low cost therapeutic that could be especially useful in settings where many cancer patients do not possess the financial means for expensive novel therapeutics.

10. HIPAA and Confidentiality

All of the data that will be collected will be kept on REDCap (Research Electronic Data Capture), our electronic data capture system. REDCap is a secure web application that will allow us to enter patient information in accordance with HIPAA compliance, 21 CFR Part 11 and in a way in order to uphold patient confidentiality. REDCap provides audit trails to track data manipulation and user activity.

All study documents that will be collected during this trial will be kept under lock and key at the

Cancer Clinical Research Core at Texas Tech University Health Sciences Center. The study staff will conduct study practices in a manner to protect the patient's identity and comply with ICH and GCP guidelines.

For the purpose of the study, a data safety monitoring board will also be established to ensure that there is no safety or ethical concerns with the study. The board will be comprised of members who have no conflicts of interest with the study. The DSMB will meet periodically throughout the study and the results from the meeting will be submitted to the institutional review board. In addition to the DSMB, the study team will also conduct self-audits to ensure that the study is being conducted within the parameters of the approved protocol.

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