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CARVEDILOL IN THE PHARMACOTHERAPY OF HYPERTENSION

I. OBJECTIVES AND BACKGROUND

a) Objectives

Conditioning is an inherent but ignored component of most pharmacotherapeutic regimens. Conceptualizing a drug treatment regimen as a series of conditioning trials suggests new strategies for assessing drug and placebo effects. In the present instance, we will capitalize on conditioned pharmacotherapeutic responses and partial schedules of pharmacotherapeutic reinforcement in an attempt to reduce the cumulative amount of medication used in the treatment of hypertension and, in the future, a variety of other diseases.

Adding a behavioral dimension to the design of drug treatment protocols changes the equation for understanding drug effects and is likely to stimulate new interdisciplinary research in neuropharmacology and behavioral pharmacology. Clinically, partial schedules of reinforcement might:

- Reduce the total amount of drug required for the treatment of a variety of disorders
- Reduce deleterious or noxious side effects and thereby increase adherence to a treatment protocol
- Extend pharmacotherapeutic effects (increase resistance to extinction)—and last, but not least,
- Reduce substantially the cost of long-term drug treatments

The study of conditioned pharmacotherapeutic effects (in contrast to conditioned pharmacologic responses) is an innovative approach to what remains a major contemporary challenge in biomedical research from a methodological and a clinical perspective—placebo phenomena. The conditioning model of placebo effects on which the proposed research is based challenges the very definition of a placebo response as a nonspecific response to an inert agent. The proposed placebo response is neither nonspecific nor is a placebo (a conditioned stimulus) inert. Instead of evaluating drug effects by treating with drug or placebo, we capitalize on conditioning principles and treat patients with drug *and* placebo. Titrating the amount of drug prescribed, not by varying the concentration of the therapeutic agent, but by holding dose constant and varying the schedule of pharmacotherapeutic reinforcement suggests new approaches to the study and application of the therapeutic effects of placebos. We are not aware of any laboratories in the United States or elsewhere that are pursuing this line of investigation of placebo phenomena or the clinical implications of conditioned pharmacotherapeutic responses.

Specifically, we will test the hypotheses that:

(1) patients treated under a partial schedule of antihypertensive medication will show a greater amelioration of symptoms than that achieved by patients treated with that same (reduced) amount of drug administered under a continuous schedule of reinforcement;

Conditions permitting, we will also test the predictions that:

(2) irrespective of initial treatment regimen, relapse will occur more quickly following withdrawal of active medication in patients who do not continue to receive conditioned stimuli (placebo) than in patients who continue to receive conditioned stimuli; and

(3) when active drug is withdrawn and replaced by conditioned stimuli alone, resistance to extinction will be greater (i.e., rate of relapse will be less) among patients treated under a partial schedule of reinforcement than patients treated with the same amount of drug administered under a continuous schedule of reinforcement (the partial reinforcement effect).

b) Background

Rationale.

Because the proposed research represents a departure from any prior research, we provide here a brief description of the background and rationale for the strategies underlying the proposed experiment(s).

Conditioned Pharmacotherapeutic effects. Clinical research and drug evaluation studies have, for the most part, adhered to the model in which a drug or placebo is administered in order to evaluate the efficacy of pharmacotherapies or to define the pharmacologic (as opposed to the psychologic) action of a drug. Thus, research has been directed to characterizations of the placebo, itself; characterizations of beliefs and expectancies, including those induced by the instructions to subjects; and characterizations of the subjects who respond to placebos. Much placebo research also derives from an effort to define the “true,” unadulterated action of a drug rather than an effort to understand the nature of the placebo effect and its therapeutic actions. There have been repeated but unanswered calls for studies of the placebo effect as a phenomenon that may have clinical implications in its own right. In this context, however, it is only the initial response to a placebo, whether in experimental subjects or patients, that are examined in the vast majority of placebo studies. This section will elaborate on a view of placebo effects that addresses the long-term therapeutic potential of this component of pharmacotherapy and provides a conceptual foundation for the proposed research.

The response to a placebo “looks like” the response to a conditioned stimulus. In behavioral terms, the physiological effects unconditionally elicited by pharmacologic or other therapeutic agents are unconditioned responses (UCRs), the agent itself being the unconditioned stimulus (UCS). Environmental or behavioral events or stimuli that are coincidentally or purposely associated with and reliably precede the voluntary or involuntary receipt of therapy—but are neutral with respect to eliciting the unconditioned effects of the active agent—are conditioned stimuli (CSs). These could include the environment (color or smell of the room) where medication is taken or administered (and by whom), and the color, shape or odor of the “pill” or injection, itself. Repeated association of CS and UCS eventually enables the CS to elicit a conditioned response (CR)—an approximation of the response unconditionally elicited by the UCS. Thus, the response to an inert or therapeutically irrelevant substance or placebo has been described as a conditioned response. One might ask whether any or all of the environmental stimuli that surround individual patients taking antihypertensive medication would complicate the definition of the conditioned stimulus in our studies. No attempt will or could be made to provide a uniform environment for all patients. We accept Rene Dubos’s observation that variability is the most ubiquitous finding in all biological research, and we accept the challenge that our intervention must be sufficiently robust to exert the hypothesized effects in spite of the variability among individuals. Actually, this has not been a problem in any of our previous research. *The most salient of potential CSs are those that are in close temporal relationship to the UCS, are novel, and reliably predict the effects of the UCS. Few of the stimuli of everyday living would meet all of these (and other) criteria.*

The notion that a placebo is a conditioned stimulus is not new. The placebo effect has been likened to a conditioned response and several investigators beginning in the 1950s have considered the influence of learning on the response to a placebo (2-10). It is argued that the entire ritual surrounding drug treatment can become a CS by virtue of the repeated association of such neutral cues with active drug administration in the history of the patient. Despite the provocative nature of existing data, the analysis of the placebo effect as a conditioned response has remained at a descriptive level. If the placebo effect is a conditioned response, what are the therapeutic implications and what are the implications for psychopharmacologic research?

Currently, research designed to evaluate drug effects involves only two basic groups: an experimental group that receives active drug and a control group that does not, receiving, instead, an inert or therapeutically irrelevant substance (the placebo). In all other respects, the stimuli that attend drug or placebo administration are, theoretically, “identical.” No matter how dose, route of administration, frequency, or duration of treatment may be varied, experimental subjects receive medication that is invariably followed (reinforced) by the unconditioned effects of the drug (a continuous or 100% reinforcement schedule). In contrast, control subjects who engage in the same behaviors under the same environmental conditions and receive placebo medication are never therapeutically reinforced; they are on a 0% reinforcement schedule. One is therefore prompted to ask: what about schedules of reinforcement between 0 and 100%?

There is, evidently, an alternative to evaluating drug effects by administering drug or placebo: one can administer drug *and* placebo. That is, one can introduce partial schedules of reinforcement in which “medication” and the attendant environmental cues are reinforced on some occasions but not on others. Schedule of reinforcement thus becomes another means for titrating cumulative drug dose. Instead of lowering drug concentration whenever therapy is administered, drug dose could be lowered under a long-term regimen of pharmacotherapy by prescribing a 90%, or 75%, 50% or 25% schedule of reinforcement in which only 90, 75, 50 or 25% of the medication received would actually contain the therapeutically active agent; the remainder of the time the patient would be receiving inert medication—CSs that have, in the past, been associated with the unconditional effects of the active medication. Capitalizing on conditioning effects, one might approximate the therapeutic effects of a continuous schedule of pharmacologic reinforcement, that is, suppress symptoms or maintain some physiologic state within homeostatic limits, using lower cumulative amounts of medication. This analysis would not apply to replacement therapies in which a chemical agent provides what cannot be produced or regulated endogenously, but it would apply in a myriad of other conditions, especially those in which the medication, itself, induced undesirable “side” effects.

This strategy of titrating cumulative drug dose by varying schedules of pharmacologic reinforcement rather than drug concentration has never before been attempted. There are, however, hints that may be derived from the literature to indicate the likely success of this approach. For example, the amount of phenothiazine given to schizophrenic patients could be reduced by substituting a placebo for active drug on a gradually increasing number of days per week (11). Other (12-14) studies have implicated learning processes in the therapeutic response to drugs by showing that placebo effects are greater when placebo treatment follows rather than precedes effective drug treatment (and drug effects are attenuated when preceded by placebo).

It is a common observation that patients switched from a regimen of active drug treatment to a period of placebo treatment, the conditions for which the drug was administered do not immediately return to the pre-treatment baseline. That is, the effects can persist for a period of time that exceeds the known residual effects of the drug. This phenomenon is most frequently attributed to indirect residual drug effects. But, the phenomenon could also reflect conditioning since the patients are being reexposed to a CS repeatedly associated with effective drug treatment in the immediate past (15).

Potential Problems.

Two issues have been raised in critique of a conditioning model of placebo effects. The first is that conditioned *pharmacologic* effects are sometimes opposite in direction to the responses elicited by the drug used as the UCS (conditioned compensatory responses). However, predicting the direction of drug-induced conditioned physiologic effects appears to depend upon a number of variables (16-23).

Operationally, there is a major difference between conditioned *pharmacologic* responses and conditioned *pharmacotherapeutic* responses. In the former, healthy subjects are brought into a laboratory where they are exposed to one or more pairings of a neutral CS and a pharmacologic agent that unconditionally elicits quantifiable physiological responses. Subsequent presentation of the CS, alone, elicits a conditioned response that typically mimics the direction and magnitude of the effects induced by the UCS. In some cases, however, the response elicited by the CS is opposite in direction to that evoked by the UCS. Such paradoxical or compensatory responses are presumed to occur in anticipation of and as a means of attenuating the effects of the UCS—a mechanism calculated to assure that the unconditioned response does not exceed homeostatic limits that could threaten the integrity of the organism.

In contrast, a conditioned *pharmacotherapeutic* response involves exposing *patients* to one or more pairings of a neutral CS and a pharmacologic agent that unconditionally elicit physiological responses calculated to correct the physiologic imbalance. Thus, while studies of conditioned pharmacologic responses involve an analysis of responses that deviate from a normal baseline, the study of conditioned pharmacotherapeutic responses involves responses designed to *restore* or maintain homeostasis. To our knowledge, there have been no reports of compensatory conditioning of therapeutic responses.

The second argument asks: if pharmacotherapy is a series of conditioning trials, why do some individuals show a placebo effect before they have experienced the therapeutic drug. That response, too, is considered to be the product of learning—an experiential history of which the clinician knows nothing. It is, however, a history that patients bring to the clinic and influences expectations and the like. Thus (to elaborate beyond the strategies for the current research), we would hypothesize a two-stage process. The initial response to a placebo satisfies the definition of a placebo response as a *nonspecific* response to a therapeutically neutral stimulus based, perhaps, on the individual's experiences with healers of one sort or another. After repeated occasions during which patients are treated with a particular therapeutic agent (the UCS), the patient now shows a *specific* conditioned therapeutic response in response to a conditioned stimulus that, by virtue of its association with the UCS is no longer "neutral."

Preliminary Studies

Conditioned pharmacotherapeutic Responses in Lupus-Prone Mice. In an effort to elaborate the biologic impact of conditioned immunosuppressive responses (24), Ader and Cohen (25) applied conditioning operations to the pharmacotherapy of spontaneously occurring autoimmune disease in New Zealand hybrid mice. A partial (50%) reinforcement schedule delayed the onset of disease using a cumulative amount of drug that was not, by itself, sufficient to influence progression of the autoimmune disorder in nonconditioned animals. Further, when active drug was withdrawn from conditioned mice, continued exposure to the CS, alone, delayed mortality relative to mice that were not re-exposed to the CS and did not differ from mice that continued to receive active drug (26).

A clinical case study. The treatment of a child with lupus was based on the above observations (27). After three pairings of distinctive gustatory and olfactory stimuli with cytoxan, the child became nauseous in response to the CSs, alone. During the course of 12 mo., half the chemotherapy sessions consisted of CS presentations without active drug infusions. A clinically successful outcome was achieved by providing only half the amount of CY that she would otherwise have received.

Conditioned Pharmacotherapeutic Effects in Hypertension. It was argued (15) that the classic crossover design does not provide sufficient information to enable one to differentiate between residual drug effects and conditioning effects as explanations of the ubiquitous observations of extended treatment effects after active drug therapy is discontinued. The crossover design needs to be supplemented by a "no treatment" group following the period of active and effective drug therapy. If residual drug effects can account for the persistence of active drug treatments when patients are shifted from active drug to placebo, then the results obtained from a "no treatment" group would parallel those obtained from the placebo group. If, as hypothesized, conditioning processes are involved in the persistence of drug effects when patients are switched from active drug to placebo (CS presentations only), then, as shown in Fig. 1, there would be a difference between the "no treatment" and placebo groups.

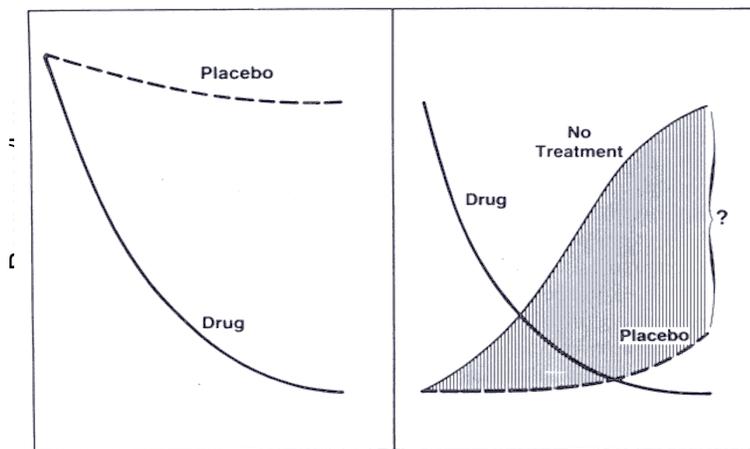


Fig. 1. Schematic representation of hypothetical results from a crossover study of drug effects that includes a "No Treatment" group. The hypothesized difference between the "Drug:No

We (28) have described the results of a study of drug therapy in hypertension that conforms precisely to the results predicted from this conditioning analysis of the placebo effect. Patients with mild to moderate essential hypertension were randomly assigned to one of three groups: Group P-D-N received placebo capsules daily for one week, capsules containing 50 mg atenolol daily for one week, and then no treatment; Group D-P received atenolol daily for one week and then placebo capsules; Group D-N received atenolol daily for one week and then received no treatments. There were no serious adverse outcomes in this study; only one of 25 participants withdrew from the study and the therapeutic regimens were apparently well tolerated. Patients treated with a placebo following active medication maintained normotensive blood pressure levels significantly longer than patients who were first treated with atenolol and then given no medication. The latter group defined the residual effects of these doses of atenolol, and, in the absence of active drug, the effects of continued exposure to the CSs exceeded these residual drug effects—a (psychopharmacologic) effect for which there is no pharmacologic explanation but which is hypothesized to be a reflection of conditioning processes operating during the period of pharmacotherapy. Also, it should be noted that in this study, a single week of treatment with atenolol was evidently sufficient to reveal the effects of conditioning as reflected by the delayed rate of relapse among patients reexposed to CSs after active drug was discontinued. These results are quite consistent with but provide no direct evidence that conditioning occurred during the initial period of pharmacotherapy. They do, nevertheless, provide grounds for expecting that the principles and strategies involved would be generalizable to other clinical situations. Our study on the conditioning of a cyclophosphamide (CY)-induced leukopenia in patients with multiple sclerosis (29) reinforces that expectation.

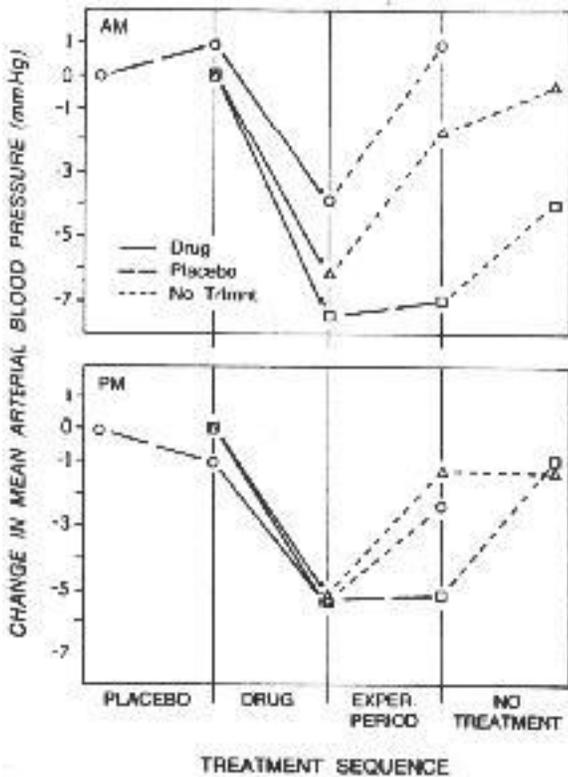
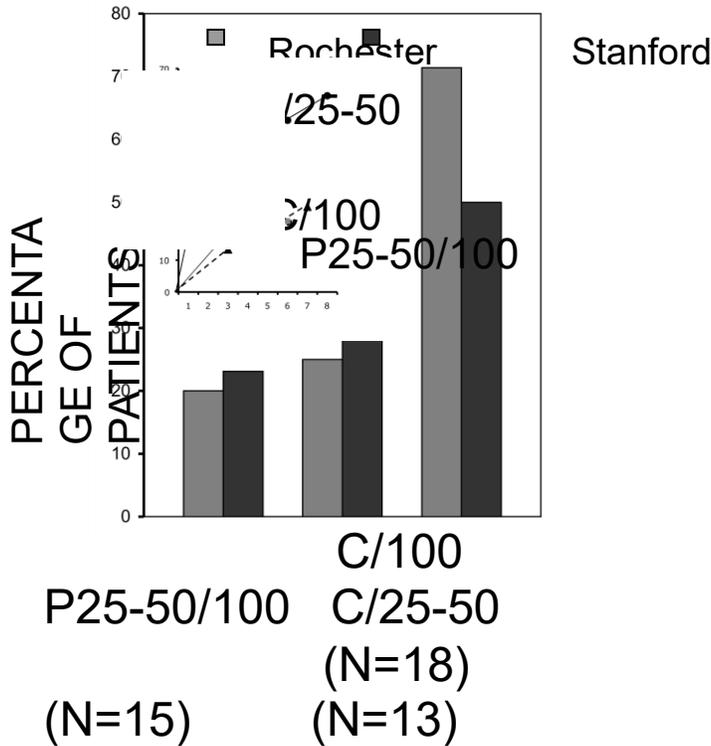


Fig. 2. Changes in morning and evening mean arterial blood pressure from baseline for Groups P-D-N (o), D-P (□) and D-N (D). Each point represents the change from baseline of the mean

Conditioned pharmacotherapeutic effects in Psoriasis. Of direct and immediate relevance to the proposed research are the results of a recently completed study (30) conducted at Stanford University and the University of Rochester. In this study, a conditioning component was added to a regimen of topical corticosteroid therapy for



for the reduced cumulative amount of active drug received by the Partial Reinforcement Group, received medication packets each of which contained 25-50% of the dose of steroid previously received. A physician, unaware of group assignments, conducted clinical evaluations weekly using a modified 9-point Psoriasis Severity Scale (PSS). As in the proposed research, it was hypothesized that “relapse,” defined here as a return to within two units of the initial PSS score, and disease severity would be less in patients treated under a intermittent schedule of pharmacologic reinforcement than in patients treated with the same cumulative amount of drug under a continuous schedule of reinforcement. As shown in Fig. 3, the results conformed precisely to predictions: the incidence of relapse in the Partial Reinforcement Group was significantly less than in Dose Control patients. There were no significant differences between the Partial Reinforcement and the Standard Therapy Groups.

There are a number of questions that need to be addressed to get beyond the inference that these results were due to conditioning. It could be argued, for example, that the reduced amount of drug received by the Partial Reinforcement Group was actually an effective dose of medication for psoriasis. However remote that possibility, we have, in the proposed research, added a second Dose Control Group that is treated with drug under the same conditions as the Partial Reinforcement Group but receives no intervening exposures to the CS. Nonetheless, the conclusion to be drawn from this first ever study of conditioned pharmacotherapeutic effects and the hints provided by the studies described above is that it is possible and feasible to introduce an intermittent schedule of pharmacotherapeutic reinforcement into a drug treatment regimen. And, it may indeed be possible to reduce the amount of drug required to control the symptoms of a disease by capitalizing on conditioned pharmacotherapeutic responses.

To date, then, the investigators have (a) demonstrated the therapeutic impact of conditioning in an animal model of autoimmune disease; (b) “successfully” treated a child with systemic lupus erythematosus using an intermittent schedule of immunosuppressive therapy; (c) confirmed predictions derived from this conditioning model of placebo effects in a crossover study of hypertensive patients; (d) obtained data indicating that the application of conditioning operations in a regimen of pharmacotherapy for another immunologic disease, psoriasis, is an effective means of maintaining these patients on reduced total amounts of medication.

The proposed research will study conditioned pharmacotherapeutic effects in the case of essential hypertension. This is a very common disease with a prevalence of 32% of all adults in the U.S. (CDC, Health United States, Table 71, 2008), and a significant economic burden on society highlighted by the expenditure of \$54 billion in 2001 (32). It is tempting to conclude that advances in drug therapy would solve the problem of rampant hypertension. The most recent National Health and Nutrition Examination Survey (NHANES) data (CDC, 2008) indicates that we are far from enjoying that success, with less than 40% of hypertensive Americans being successfully treated to goal. There are even more people with mild elevations in blood pressure, but also a growing number of people with severe hypertension who, because of their co-morbidities, must reach even lower goals. It is important to find therapies for the millions of Americans with borderline or Stage 1 hypertension who are limited in the amount of medication that they can take because of adverse side effects.

Based on principles of conditioning, using partial schedules of reinforcement in a pharmacotherapeutic protocol might:

- reduce the total amount of drug required for the treatment of hypertension, thereby maximizing benefits and reducing risks;
- reduce undesirable, deleterious or noxious side effects and thereby increase adherence to a treatment protocol;
- extend the effects of pharmacotherapy (i.e., increase resistance to extinction); and last, but by no means least,
- reduce very substantially the cost of long-term drug medications.

II. CHARACTERISTICS OF THE RESEARCH POPULATION

a) Eligibility Criteria:

- Subject population Characteristics: This investigation will apply the principles of conditioning described above in patients with mild (Stage 1) *hypertension*. These are patients who are normally started on a single drug and, most often, on the lowest dose recommended. In this case, we will be using, a drug currently used for the treatment of hypertension. Subjects will be 18-80 year old male and female patients **with mild hypertension** (systolic pressure between 140-170 and a **heart rate of 68 beats/minute or greater** . . .
- Number of available subjects: In the year 2004, the incidence of hypertension in the U.S. was 29% of the adult population, and rapidly rising. Patients will be recruited from the Medicine clinics of University of Rochester and affiliated Primary Care facilities. The pool of potential subjects is over 2,000.
- Acceptable disease status/condition (concomitant illnesses): Subjects should be in generally good health, and on few medications
- Must not suffer from severe or inhaler-dependent reactive airway disease (asthma or COPD).
- **Permitted or prohibited concurrent treatment:** the only classes of anti-hypertensive medications that can be used are ACE-inhibitors, angiotensin receptor blockers, calcium channel blockers, aldosterone antagonists, and diuretics. No other classes of anti-hypertensive medications are permitted.
- Time constraints for performance of pre-study (**eligibility**) tests: **If potential participant has not had blood work done within the** past year, a basic blood profile (one tube of blood) will be required to **ensure renal functions are safe for the use of carvedilol**. The blood draw can be done by research staff at the time of initial evaluation and available within 24 hours for review. Ability of the subject to provide informed consent: Only subjects able to provide informed consent will be recruited

b) Number of Subjects: 121. Sample size and Power Analysis: The proposed study will recruit 37 subjects for each of the ST, PR and DC1 treatment conditions, and 10 subjects for the CD2 group. The study has 93% power to detect a 50% difference in relapse rate between group 2 and group 3 over the 2-week assessment period, even account for 10% dropout rate based on a two-sided $\alpha = 0.05$ using the method of Lakatos, E. (35). We selected a 50% relapse rate difference for the power calculation based on our prior experience. For the primary continuous symptom measure blood pressure, we have 80% power to detect about a medium effect size 0.56 based on a longitudinal study design with 7 assessment points, a conservative estimate of within-subject correlation 0.5, and a two-sided type I $\alpha=0.05$ using the method of Tu et al. (36). Note that although blood pressure is assessed twice daily in Experiment 1, we conservatively set the number of assessments at 7 to improve the robustness of the power estimates. Thus, the proposed sample size not only has sufficient power to detect a clinically meaningful difference in relapse rates, but also to find meaningful change patterns for blood pressure over the treatment course.

c) Sex of subjects: The male to female ratio will follow the distribution of hypertension in the Rochester area community. Pregnant or nursing females will be excluded due to the nature of the intervention (carvedilol is Category C). Females of child bearing potential will be required to use 2 birth control methods during the study in order to participate.

d) Age of Subjects: 18 to 80 years of age. Carvedilol is not intended for use in children.

e) Racial and Ethnic Origin: No restrictions. It will follow the distribution of patients seen in the Rochester area.

f) Vulnerable Subjects: None will be enrolled.

III. METHODS AND PROCEDURES

a) **Study Design.** Randomized, single blind.

Medication. Hypertensive patients will be treated with distinctively colored, *odored and flavored* gelatine capsules (Capsuline, Inc, Pompano Beach, FL) containing 12.5 mg (generic version, available from the Strong Memorial Hospital Research Pharmacy). The “placebo” will be a capsule containing 12.5 mg of an inert substance with the same characteristics as the active drug capsules. Capsules will be packaged in a blister strip of single application packets by the Investigational Drug Studies program in the Strong Memorial Hospital Research Pharmacy. Preparation of the sequence of medication would be done from coded patient numbers and would depend upon the schedule of reinforcement that was being used. For example, if one were imposing a 25% reinforcement schedule (only one fourth of the packets of capsules containing active drug), a random sequence would be constituted with the proviso that the patient would receive no more than two doses of consecutive placebo capsules. Randomization will be performed by the research pharmacy in blocks of eight. Allocation concealment will be guaranteed, since only the research pharmacist will be aware of group distribution and all other members of the research team will be blinded. He will provide the research nurse with the pre-labeled packages.

Length of study: 8 weeks for those subjects that complete the full set of procedures. For those subjects who relapse early, or whose blood pressure does not remit (10-point decrease for a period of 5 days) prior to the Exp 1 phase, the minimal period in the study is 3 weeks.

Exp 1: Baseline Period: 2 weeks after a screening physical examination that will be performed within one week of admission. ; Experimental Phase: 2 weeks (or less for subjects' whose mild hypertension relapses). Therefore, maximal possible period receiving carvedilol: 4 weeks.

Exp 2: 4 weeks. In this experiment subjects either receive placebo or no treatment. We expect the latter group to relapse after a few days. Those receiving placebo are hypothesized to relapse later, and we are assuming that will relapse before 4 weeks on placebo. However, if we find that there are subjects that after 4 weeks have not relapsed, we plan to continue the administration of the placebo until they relapse (beyond the 4 weeks). Because we believe that this will occur rarely, for practical reasons we will state in the consent form that this period may last “up to 4 weeks”.

The choice of the β/α blocker for this study was based on:

a) targets receptors of the peripheral nervous system (PNS); it is a non-selective β -adrenergic blocking agent with alpha-1 blocking activity. It is approved for use in hypertension as well as heart failure. Other hypotensive drugs could have been chosen, however, their primary target is not “directly” aimed at the PNS or CNS. We are aware that ACE inhibitors decrease catecholamines, and angiotensin receptor blockers decrease vasopressin, however, for this initial study we chose a drug that is mostly associated to the sympathetic system. We believe that this will provide the best chances of success. Future studies will include alternative drugs.

b) Carvedilol has no adverse effect on glycemic control, and is the preferred β blocking agent for patients that have diabetes (as some of ours may).

c) We have preliminary published data (28) that strongly suggests the effectiveness of our conditioning paradigm in the pharmacotherapy of hypertension using a similar β blocker.

d) can be administered two times a day, leading to an enhanced number of “learned” exposures to the stimulus, and this regimen guarantees that subjects in groups that receive only one active drug

administration out of 4 will take adequate daily amounts of that will prevent them from the undesired rebound side effects of this medication that could occur if they were to have 3 days with no active drug.

Subjects will be started on the lowest recommended starting dose of 25 mg daily in 2 fractionated doses, which is reasonable given their level of hypertension (33,34). Once a reasonable blood pressure drop is achieved, they will be randomized to four experimental groups, as described in the methods section.

Potential participants will be informed that the purpose of the study is to determine if their blood pressure could be brought under control using lower amounts of medication and that, in the course of the study, the amount of medication they receive may be reduced. The medical staff with whom the patient comes in contact will not know if or when this occurs. Measurement of blood pressure during the study will be performed by the patients themselves, who will be instructed in the use of automated BP monitors (HEM-711AC, Omron Healthcare Inc., Bannockburn, IL) which will be provided. Subjects will obtain measurements twice (one after the other) in the AM and twice (one after the other) in the PM always at the same time of day.

A screening physical examination will be performed within one week of admission into the study and only patients, who are in good health, display no contraindications for the use of beta blockers and disclaim the use of antihypertensive medication for at least 2 wk. before the start of the study will proceed to the initial maintenance or baseline period.

Patients will be excluded from participation if they use illicit drugs, were using anti-hypertensive medications within the previous 2 weeks other than ACE-inhibitors, angiotensin receptor blockers, calcium channel blockers, aldosterone antagonists, or diuretics **Patients will be excluded from participation if they use illicit drugs; they use inhalers regularly to control reactive airway diseases like COPD and asthma;** if pregnant or if sexually active women are not using contraceptives; if they cannot be followed or monitored appropriately; or renal insufficiency are determined, which may require a blood draw at the time of screening physical examination if subject has not had basic blood profile done in the past 12 months.

Experiment 1. Effects of a partial schedule of pharmacotherapeutic reinforcement in maintaining the therapeutic effects of in the treatment of hypertension.

The proposed research will determine if, capitalizing on conditioned pharmacotherapeutic effects, patients suffering from hypertension can be effectively treated with smaller cumulative amounts of a beta blocker, . Some patients in this initial experiment will be treated on a partial rather than a continuous schedule of pharmacologic reinforcement. These patients will be compared to: (a) patients that continue to be treated on a standard regimen of pharmacotherapy at the baseline dose, and (b) patients that receive the same (reduced) total amount of medication under a continuous schedule of reinforcement as that received by experimental patients being treated under a partial schedule of reinforcement, and another Dose Control Group that receives at the same dose and frequency as the Partial Reinforcement Group, but receives no interspersed exposures to the CS.

It is possible that the therapeutic effects of a partial schedule of pharmacologic reinforcement (and, thus, a reduced amount of active drug) will be indistinguishable from a continuous (standard) regimen of pharmacotherapy. That outcome, however, is not critical for evaluating the role of conditioning in pharmacotherapies simply because these groups would have received different amounts of drug. Specifically, *it is hypothesized that patients treated under a noncontinuous schedule of medication will be less likely to show a recurrence of symptoms ("relapse") than patients treated with that same amount of drug administered under a continuous schedule of reinforcement.*

On the first visit, each potential subject will undergo a brief physical examination to record the medical history, sign an informed consent form, and receive instruction in the use of a blood pressure monitor. A strip of medication packets will be supplied at each clinic visit and will be taken by the subject at home, 2

times a day for the duration of the study.. The subject will return to the study center once a week or within 14 days of the drug dispensing cycle; this will allow flexibility for those with travel or work schedule challenges. The study coordinator will email or call to check on safety of the subject in the interim of a weekly clinic visit. Each drug card contains 15 days of study drug to ensure continual dosing in the event a weekly visit is missed. Packets containing medication will be dispensed by code so the patient and the physician will be blinded as to the nature of the medication. To consider the possibility that conditioning might be influenced by immediate stressful life experiences in these patients, we will examine this relationship using two measures of psychological distress administered at weekly intervals: the Hassles Scale, a frequently-used measure of daily stressors or “hassles” and the Impact of Events Scale (IES) (31).

The protocol for Experiment 1 is shown in Table 1. During a baseline period (of a maximum of 15 days), all patients will be treated BID with 12.5 mg. Those who satisfy the criterion of a 10-point decrease in blood pressure for a period of 5 days will be assigned randomly to experimental groups.

During the 15-day experimental period, the patients will be treated as follows:

Patients in a **Standard Therapy Group** will continue to receive 100% of the dose of medication on the same reinforcement schedule (100%) as they received during the baseline (maintenance) period. (12.5 mg) would be taken 2 times daily for 15 additional days. This is a continuation of their standard pharmacotherapeutic maintenance regimen and constitutes one control group.

The **Partial Reinforcement Group** will be treated under a partial (25%) reinforcement schedule. That is, patients will receive the same dose of drug previously received, but only a quarter of the daily packets will contain active drug (12.5 mg); the remaining capsules will contain only an inert substance. Packets will be arranged to ensure that a patient never receives the “placebo” for more than two consecutive times. The selection of a 25% reinforcement schedule is based on the fact that we are using the minimum recommended starting dose of , and lower doses (particularly this much lower) are likely to have minimal if any pharmacologic effect. Therefore, the total daily dose of 6.25 mg. would not be sufficient to sustain the normotensive levels achieved under continuous reinforcement during the baseline period.

Dose Control Group-1, a control for the cumulative amount of drug received by the Partial Reinforcement Group, will be treated twice daily under a continuous (100%) reinforcement schedule, but each packet will contain only 25% of the active drug received during the baseline period.

To be sure that the reduced amount of drug received by the Partial Reinforcement Group is not sufficient by itself to lower blood pressure, a **Dose Control-2 Group** will receive the same active dose as the Partial Reinforcement Group but will not receive CSs on the intervening doses (6.25 mg once every other day).

Table 1. Experimental Protocol

GROUP	BASELINE PERIOD (15 days)			EXPERIMENTAL PERIOD (15 days)		
	Drug Dose*	Reinforcement Schedule**	Freq.	Daily Drug Dose***	Reinforcement Schedule**	Freq.
Standard Therapy	25	100	2xDay	25	100	2xDay
Partial Reinforcement	25	100	2xDay	6.25	25	2xDay

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Dose Control-1	25	100	2xDay	6.25	100	2xDay
Dose Control-2	25	100	2xDay	6.25	100	every other day

* Daily Dosage in mg of antihypertensive medication

** Percentage of treatment occasions when active drug is received

*** Average Daily Dosage in mg of antihypertensive medication

The primary outcome measure will be the incidence and rate of “relapse,” defined as a blood pressure level that has risen to the baseline value (blood pressure value of the participant at the time of enrollment) for three consecutive days.

If, over the course of 15 days there is evidence of a gradual recurrence of symptoms, the experimental period can be extended. If there is no evidence of impending relapses, there are two options: (a) we can reduce the partial reinforcement schedule from 25% to 15%, for example, and adjust the dose of in the Dose Control Groups accordingly; or (b) proceed to a study of extinction effects (Experiment 2).

Timeline estimate. We propose a start-up period of 6 mo. (formulation of Consent Form, IRB approval of protocol, preparation of study personnel, data management procedures, preparation and placing of advertisements for study subjects, etc). Including an instructional session and testing of blood pressure measurements from the monitors provided to patients, patients enrolled in the study could be engaged for as many as 50-60 days. We calculate being able to process 40 patients/year. A final 6 mo. would be devoted to data processing and manuscript preparation and, thus, a total project period of 4 years.

Experiment 2. *Effects of CS exposure on the persistence of drug effects following the withdrawal of drug and the effects of partial schedules of pharmacotherapeutic reinforcement on resistance to extinction.*

Based on the time available and the outcome of Experiment 1, we may be able to gather additional preliminary data on the extinction of conditioned pharmacotherapeutic effects, adding evidence that results obtained in Experiment 1 are, in fact, the results of conditioning. We expect that patients in Dose Control Group-II from Experiment 1 will relapse within 2-3 days of the treatment imposed during the experimental period. A large percentage of Dose Control Group-I patients are also expected to relapse under a reduced dose of active drug during the experimental period. However, we expect that a significant percentage of patients in the Partial Reinforcement Group (as well as the Standard Therapy Group) would be available for further study of extinction effects.

The literature suggests and our own data (Suchman & Ader, 1992) indicate that the persistence of the therapeutic effects of at least some drugs after drug treatments have ended cannot be attributed solely to the residual effects of the drug. We infer that there is a component of learning in the repeated pairing of distinctive sensory cues and the therapeutic effects of drugs. Therefore, *it is predicted that, when active drug treatment is discontinued, reexposure to conditioned stimuli previously associated with effective drug treatment will extend the effects of the pharmacotherapy beyond that resulting from the residual effects of the drug.* That is, in hypertensive patients being treated with, the ameliorative effects of the pharmacotherapy will persist for a longer period of time (relapse will be delayed) in those patients who continue to be exposed to the conditioned stimulus (placebo “medication”) than in patients who receive no “medication” following the withdrawal of active drug.

Performance during a period of extinction—when subjects are reexposed to a CS that is *not* followed (reinforced) by the UCS (active drug)—constitutes a particularly sensitive measure of the strength of learned responses. The “partial reinforcement effect” in conditioning refers to the observations that responses acquired under partial schedules of reinforcement are more resistant to extinction than responses acquired under continuous reinforcement. Therefore, *it is predicted that when active drug is withdrawn and replaced by CSs alone, resistance to extinction will be greater (i.e., rate of relapse will be less) among patients treated under a partial schedule of reinforcement than patients treated with the same*

amount of drug administered under a continuous schedule of reinforcement. If this turns out to be the case, this strategy might profitably be applied in attempting to wean patients from certain drugs.

A comparison of groups that are or are not reexposed to the CS following the discontinuation of active drug therapy and an assessment of extinction effects as a function of reinforcement schedule are separate issues, but they could (should) be addressed in a single experiment.

If, as anticipated, there are a sufficient number of patients from Experiment 1 who show no evidence of relapse within the 15-day experimental period, we can proceed to Experiment 2. The remaining patients from the Standard Therapy and Partial Reinforcement Groups (and, perhaps, the Dose Control Group-1) would be divided into two subgroups. For one subgroup, all medication would be discontinued for a period of 2-4 wk; for the other subgroup, active drug would be discontinued but the patients would continue to receive the distinctive capsules containing inert substance. It is predicted that: (a) patients reexposed to the CS will be less likely to relapse or show a slower rate of relapse than patients in groups that are not reexposed to the CS; and (b) patients in the Partial Reinforcement Group will be more resistant to extinction (slower to relapse) than patients remaining from Dose Control Group-1 who were treated with the same cumulative amount of drug and may even be indistinguishable from the Standard Therapy Group that had received three times more drug. An alternative strategy, of course, would be to enlist a new population.

c) Statistical Analysis: Data analyses are described separately for each of the specific hypotheses. All statistical tests will be two-sided with $p \leq .05$. The principal analyses focus on the difference over 2-weeks between PR and two dose control groups (DC-1 and DC-2) in Experiment 1. The main independent variable is time to relapse as well as symptom reduction (blood pressure). These two outcomes will also be examined in Experiment 2 to see if they differ between the two groups (placebo and no-treatment groups). Because power for the comparisons in the second experiment may be undercut by high relapse rates in the first experiment, this second set of analyses is exploratory.

The Cox proportional regression model will be used to examine the time to relapse outcome, while mixed-effects (MM) model will be used to compare differences in symptom reductions over time across the treatment groups. The MM approach employed for longitudinal data analysis provides valid inference under the missing at random assumption (MAR), provided that the parametric distribution assumptions are met. The MAR model is quite general, accommodating almost all missing data mechanism that may arise in practical studies (37,38). However, in the presence of missing data, estimates in both cases may be biased if the data fails to follow the assumed statistical distributions, even with the use of the sandwich variance estimates (39). In cases where distribution assumptions are seriously violated, for example, if discrepancies between the MM and WGEE for fixed-effects show very different results, we will use clustered bootstrap methods for inference (40).

Experiment 1

AIM 1: a) PR will be more effective than DC-1 and DC-2 in reducing relapse rates. PR will have similar effectiveness to ST in reducing relapse rates.

Survival analysis will be conducted using Cox proportional hazards model. We will include dummy variables for the four treatment groups in addition to the covariates found to significantly differentiate the groups at baseline. Appropriate linear contrasts will be constructed to compare PR vs. each of the dose control groups, if a significant group difference is found.

Note that to formally confirm the equivalence between PR and ST, we must use non-inferiority tests. However, since the acceptable margin of tolerable difference is difficult to define due to the lack of insufficient literature on this topic, we will not conduct such a formal analysis. The effect size will provide similar indications of treatment difference.

b) PR will have higher (similar) symptom reductions than DC-1 and DC-2 (ST).

Longitudinal methods will be applied to compare the outcomes of pruritus and biologic measures across the four groups as well as the appropriate pairwise comparisons. We will dummy code the treatment conditions, include time and time by treatment interaction in addition to the covariates found to differentiate

the four groups at baseline. The hypothesis will be supported if a significant time by treatment interaction is found. Appropriate linear contrasts will be performed to compare PR vs. DC-1, DC-2 and ST.

Experiment 2

AIM 2: a) Subjects who continued to receive conditioned stimuli have a lower relapse rate than those who did not.

The same survival analysis approach in Aim 1/(a) will be conducted, with the exception that there are only two treatment groups.

b) Subjects who continued to receive conditioned stimuli have better symptom reductions than those who did not.

The same longitudinal methods in Aim 1/(b) will be applied to compare the blood pressure outcomes to compare the difference between the two groups.

AIM 3: a) Subjects who continued to receive conditioned stimuli have a higher relapse rate than those who did not.

The same survival analysis approach in Aim 3/(a) will be conducted, with the exception that there are only two treatment groups.

SCHEDULE OF EVENTS

Evaluation/ Procedure	Registration	Baseline	Study Period
Informed Consent	X		
Assess Eligibility	X	X	
Medical History		X	
Physical Exam		X	weekly
Vital Signs		X	weekly
Study Evaluations/ Assessments			weekly
Concomitant Medications		X	weekly
Dispense Study Agent		X	weekly
Review Diary/Record			weekly
Adverse Events			weekly
Randomization		X	
Blood Pressure Measurements			2 X day in the A.M.(one after the other) 2 X day in the P.M.(one after the other)

Criteria for investigator-initiated subject removal

- Inability to make study visits
- Unacceptable toxicity
- Progressive disease
- Inability to follow study directions

d) Reportable events: Serious Adverse Events will be reported by Dr. Bisognano to the RSRB within 24 hours of occurrence.

e) Data Storage and Confidentiality: All data will be stored in a computer with a secure network connection. All information will be coded to maintain confidentiality. Only Dr. Bisognano and the research pharmacist will have access to individual identifiers.

- f) **Transport of Study Drug:** As needed, study drug will be transported from the primary study center to Culver Medical Group by the study coordinator or the sub-Investigator. The minimum amount of study drug anticipated for use will be transported. It will be carried in a locked bag. Study drug will be logged in and out of each location. Study drug will be stored in a locked, temperature controlled room once on site at Culver Medical Group.

IV RISK/BENEFIT ASSESSMENT

- a) **Risk Category:** Greater than minimal
- b) **Potential Risk:** Low risk of transient low blood pressure. Subjects may have treatment for stage I hypertension delayed during the short duration of the study, but this is similar to approach that many newly diagnosed hypertensive patients receive (several months of attempts at non-pharmacological Therapy)
- c) **Protection against risks:** Close monitoring by investigators, Drs. Tausk and Bisognano will function as patient safety monitors as part of the study team ii) Data Safety Monitoring Board Meeting every 6 months.
- d) **Potential Benefit to Subjects:** none
- e) **Alternatives to Participation:** standard treatment of hypertension.

V. SUBJECT IDENTIFICATION, RECRUITMENT AND CONSENT/ASSENT

Method of Subject Identification and Recruitment:

- a) Subjects will be recruited by referrals from the physicians who practice in the general medicine outpatient clinics of Strong Memorial Hospital and all affiliated clinics.
- b) The study coordinator will work in collaboration with the Greater Rochester Practice Based Research Network (GR-PBRN) executive committee to expand recruitment opportunities. The relationship will be on-going for the duration of the study to seek best methods of recruitment from primary care practices. The objective is to make the primary care physician the point of contact with regard to announcing the research opportunity for our study.
- c) On an as needed basis, the study coordinator will work with the Clinical Translational Science Institute (CTSI) Research Support Center staff at the University of Rochester Medical Center.
- d) Primary care practice lists identifying potentially-eligible participants will be sent to either the study coordinator or CTSI Support Staff; depending on the preference of the patient care practice group.
- e) If a primary practice prefers the research letters are sent through CTSI Research Support Staff, the list of potentially-eligible participants will be processed and distributed through their staff. The letter will announce the research opportunity and direct interested parties to contact the study coordinator, not the primary care physician.
- f) If the primary practice group prefers to work with the study coordinator, the list of potentially-eligible participants will be sent to the coordinator. The coordinator will review and clean the data. Primary care practice physicians will be asked to review and approve the edited list before the research letter is sent on their behalf. Interested parties will be directed to contact the study coordinator, not the primary care physician.
- g) The study coordinator will work with URM Public Relations to help elevate both public and medical community awareness of the study and research opportunities for those who may be eligible for the

study. **a)** Internal press release opportunities: URM website, @Rochester, URM Facebook and Twitter pages. URM Public Relations can provide various venues of print and social networking to help enhance recruitment opportunities which include but are not exclusive to all sources: **b)** external press release opportunities: national media distribution lists which could lead to a whole host of different health/medicine websites. **c)** Local press opportunities may include: Messenger Post Media, In Good Health, City Newspaper, Rochester Healthy Living, Finger Lakes Community Health Magazine and Rochester Woman.

- h)** The study will be posted through [ResearchMatch.org](https://www.researchmatch.com/) on-line application to help with recruitment. RM is a national registry that help connect volunteers who wish to participate in studies to researchers conducting those studies.
- i)** The study coordinator will work with URM Marketing to place local radio and newspaper advertisements to enhance recruitment opportunities. Advertising materials may also be present at University sponsored health screenings/fairs. Study staff may also be present at these events.
- j)** Study coordinator will utilize the URM Cardiology Research Database [REDACTED] as an additional resource for subject recruitment.
- k)** Study coordinator will work with URM School of Nursing (SON) staff who administers employee biometric screenings which include services for: blood pressure, heart rate, weight, BMI, total cholesterol, HDL, triglycerides and glucose, and waist circumference. When appropriate, SON staff will provide a study flyer to URM employee. When possible, study staff will be present at the Healthy Living Center's check-out table to answer study related questions. No further follow up or communication will take place with URM employees regarding the study.
- l)** Study coordinator will utilize the Future Contact by Department of Public Health Sciences Database: [REDACTED] as an additional resource for subject recruitment.
- m)** The study coordinator will work in collaboration with the Healthy Living Center's doctors and staff to expand recruitment opportunities. The relationship will be on-going for the duration of the study to seek best methods of recruitment from the Healthy Living Center.
- n)** Electronic medical record search will be conducted to identify Dr. Bisognano's (PI) patients that meet study criteria.
- o)** Rochester Clinical Research (RCR) will be utilized for subject recruitment. RCR is an independent research company in Rochester, NY with a proven record for subject recruitment. RCR will recruit subjects from their database as well as advertisements and referred to URM. Potential subjects will be notified that the study is occurring through the University of Rochester. This relationship will be on-going for the duration of the study.
- p)** The study coordinator will work in collaboration with Culver Medical Group's doctors and staff to expand recruitment opportunities. The CMG's database will be utilized to identify potential study subjects. Any patients identified will be contacted at the discretion of the primary care physician utilizing the telephone script specific to Culver Medical Group. Study related appointments (conducted by the study coordinator and sub-Investigator) will also take place at Culver Medical Group as needed. The relationship will be on-going for the duration of the study to seek best methods of recruitment from Culver Medical Group.

- q) The study coordinator will work in collaboration with Clinton Crossings Internal Medicine Group's doctors and staff to expand recruitment opportunities. Any patients identified will be contacted at the discretion of the primary care physician. Study related appointments (conducted by the study coordinator and sub-Investigator) may also take place at Clinton Crossings Internal Medicine Group as needed. The relationship will be on-going for the duration of the study to seek best methods of recruitment from Clinton Crossings Internal Medicine Group.
- r)
- s) **Process of Consent:** Consent will be obtained by the research coordinator, study nurse, or Dr. Bisognano. Subjects will be offered ample time to reach a thoughtful decision making. We anticipate to screen approximately 1,000 subjects to reach 121 who will complete all procedures and phases (Experiment 1 and/or Experiment 2) of the study.
- t) **Screening:** (described above) Consent to participate in the study will be prior to screening for eligibility (Initial Evaluation, thus no screening consent). Identification of subjects that do not fulfill screening eligibility criteria will not be kept.

Subject Capacity: The research nurse will assess subject capacity to understand consent procedure. Their capacity to comprehend written language including the purpose of the study, its experimental nature, risks and anticipated benefits, the right to withdraw, alternatives to participation, confidentiality protections, and the safeguards used to minimize risks will be determined by discussion of the proposed research project with a prospective subject, e.g., during the consent process, followed by a series of questions to assess the person's understanding of key issues. Such questions might relate, for example, to the purpose of the research and the foreseeable risks and anticipated benefits of study participation. Other questions might explore the prospective subject's understanding of the voluntary nature of research and the elements of consent, including the right to be informed about appropriate alternative procedures or courses of treatment that may be available.

Debriefing Procedures: Subjects will be informed that this study will address different doses of administration of carvedilol, and that at times they may receive no medication at all. Because this is a study that will evaluate placebo effects, the actual word "placebo" cannot be used, since this will potentially decrease the effect. There is no direct deception involved, since the patients will be receiving active medication in all study groups. However, subject will be informed of the missing information after debriefing, which will occur once they complete any stage of the study.

Costs to subject: NONE

- u) **Payment for Participation:** Payment will be in weekly installments during the subject participation. Each subject will receive a maximum of \$300 prorated per study visit. .
- v) 1st payment: \$100.00 upon completion of study weeks 1-2.
2nd payment: \$100.00 upon completion of study weeks 3-4.
3rd payment \$100 upon completion of weeks 5-8. Not all subjects will participate in weeks 3-8.

VI. REFERENCES

1. Ader R. The role of conditioning in pharmacotherapy. Cambridge: Harvard Univ. Press, p. 138, 1997.

2. Wolf S. Effects of suggestion and conditioning on the action of chemical agents in human subjects--the pharmacology of placebos. *J Clin Invest* 29: 100, 1950.
3. Stanley WCS, H. The psychophysiological effects of tea. *J Psychol* 36: 435, 1953.
4. Ross S, & Schnitzer, S.B. Further support for the placebo effect in the rat. *Psychol Rep* 13: 461, 1963.
5. Lasagna L, Mosteller, F., von Pelsinger, J.M., & Beecher, H.K. A study of the placebo response. *Am J Med* 16: 770, 1954.
6. Kurland AA. The drug placebo: Its psychodynamic and conditioned reflex action. *Behav Sci* 2: 101, 1957.
7. Knowles JB. Conditioning and the placebo effect. *Behav Res* 1: 151, 1963.
8. Gleidman LH, Gantt, W.H., & Teitelbaum, H.A. Some implications of conditional reflex studies for placebo research. *Am J Psychait* 113: 1103, 1957.
9. Herrnstein RJ. Placebo effect in the rat. *Science* 138: 677, 1962.
10. Beecher HK. Measurement of subjective responses: Quantitative effects of drugs. New York: Oxford, 1959.
11. Greenberg LM and Roth S. Differential effects of abrupt versus gradual withdrawal of chlorpromazine in hospitalized chronic schizophrenic patients. *Am J Psychiat* 123: 221, 1966.
12. Batterman RC. Methodology of analgesic evaluation: experience with orphenadrine citrate compound. *Curr Ther Res Clin Exp* 7: 639, 1965.
13. Sunshine A, Laska, E., Meisner, M., & Morgan, S. Analgesic studies of indomethacin as analyzed by computer techniques. *Clin Pharmacol Ther* 5: 699, 1964.
14. Moertel CG, et al. Who responds to sugar pills? *Mayo Clin Proc* 51: 96, 1976.
15. Ader R. Conditioning effects in pharmacotherapy and the incompleteness of the double-blind, crossover design. *Integ Psychiat* 6: 165, 1989.
16. Eikelboom R and Stewart J. Conditioning of drug-induced physiological responses. *Psychol Rev* 89: 507, 1982.
17. Miller NE & Dworkin B. Different ways in which learning is involved in homeostasis. New York: Academic Press, 1980.
18. Roffman M, et al. Control of morphine-withdrawal hypothermia by conditional stimuli. *Psychopharmacologia* 29: 197, 1973.
19. Coussons ME, et al. Pavlovian conditioning of morphine-induced alterations of immune status. *J Neuroimmunol* 39: 219, 1992.
20. Siegel S. Classical conditioning, drug tolerance, and drug dependence. *Res Adv Alcohol Drug Prob* 7: 207, 1983.
21. Miller JS, et al. Conditioning of morphine-induced taste aversion and analgesia. *Psychopharmacology (Berl)* 101: 472, 1990.
22. Rozin Pand Kalat JW. Specific hungers and poison avoidance as adaptive specializations of learning. *Psychol Rev* 78: 459, 1971.
23. Ader R, Grotta, L. J., Moynihan, J. A., & Cohen, N. Behavioral adaptations in autoimmune disease-susceptible mice. New York: Academic Press, p. 685, 1991.
24. Ader R & Cohen N. Behaviorally conditioned immunosuppression. *Psychosom Med* 37: 333, 1975.
25. Ader R & Cohen N. Behaviorally conditioned immunosuppression and murine systemic lupus erythematosus. *Science* 215: 1534, 1982.
26. Ader R. Psychoneuroimmunologic contributions to the study of rheumatic diseases. New York: Plenum, pg. 669, 1985a.
27. Olness K and Ader R. Conditioning as an adjunct in the pharmacotherapy of lupus erythematosus. *J Dev Behav Pediatr* 13: 124, 1992.
28. Suchman AL, Ader R. Classic conditioning and placebo effects in crossover studies. *Clin Pharmacol Ther* 52: 372, 1992.
29. Giang DW, et al. Conditioning of cyclophosphamide-induced leukopenia in humans. *J Neuropsychiatry Clin Neurosci* 8: 194, 1996.

30. Ader R., et al., Conditioned pharmacotherapeutic effects: A preliminary study. *Psychosom. Med.*,2010, 72 (Published electronically 12/20/09, PMC Journal – In Process).
31. Horowitz M, et al. Impact of Event Scale: a measure of subjective stress. *Psychosom Med* 41: 209, 1979.
32. Balu S and J Thomas III: Incremental Expenditure of Treating Hypertension in the United States, *Am J Hypertens* 19:810, 2006.
33. Lucker P, et al.Pharmacokinetic and pharmacodynamic comparison of metoprolol CR/ZOK once daily with conventional tablets once daily and in divided doses. *J Clin Pharmacol*, 30:S17,1990.
34. Myers MG, Thiessen JJ. Metoprolol kinetics and dose response in hypertensive patients. *Clin Pharmacol Ther*, 27:756,1980
35. Lakatos, E. Sample sizes based on the log-rank statistic in complex clinical trials. *Biometrics*, 44, 229, 1988.
36. Tu X.M., Kowalski J, Zhang J, Lynch K, Crits-Christoph P. (2004) Power Analyses for LongitudinalTrials and Other Clustered Study Design. *Statistics in Medicine*. 23: 2799-2815.
37. Little RJA, Rubin DB: *Statistical Analysis with Missing Data*. New York: Wiley, 1987.
38. Kowalski, J and Tu, XM. *Modern Applied U Statistics*. New York: Wiley; 2008.
39. Lu, N., Tang, W., He, H., Yu, Q., Crits-Christoph, P., Zhang, H. and Tu, X.M.: On the Impact of Parametric Assumptions and Robust Alternatives for Longitudinal Data Analysis, *Biometrical Journal*, in press.
40. Field, C & Welsh, A. Bootstrapping clustered data. *Journal of the Royal Statistical Society: Series B (Statistical Methodology)*., 69:369,2007.