Pharmacologic Principles for Combination Therapy

Article *in* Proceedings of the American Thoracic Society · February 2005

DOI: 10.1513/pats.200504-037SR · Source: PubMed

2 authors:

Myron L Toews
University of Nebraska Medical Center
153 PUBLICATIONS **3,302** CITATIONS
SEE PROFILE

David B Bylund
University of Nebraska at Omaha
1,054 PUBLICATIONS **12,428** CITATIONS
SEE PROFILE

Pharmacologic Principles for Combination Therapy

Myron L. Toews and David B. Bylund

Department of Pharmacology, University of Nebraska Medical Center, Omaha, Nebraska

This article discusses the pharmacologic basis for understanding the therapeutic actions of drugs, particularly for their use in combinations. The focus is on principles underlying combination therapy in general, including examples from diseases other than chronic obstructive pulmonary disease (COPD). Pharmacodynamic aspects of drug action are covered, with an emphasis on recent advances in the understanding of drug–receptor interactions and of drug agonism. Pharmacokinetics and drug-induced adaptive changes in receptors and cell signaling pathways are summarized, emphasizing their importance for potential combination therapies aimed at prolonging drug action. An organizational framework for three different approaches to combination therapy is then proposed; the molecular rationales for each approach are described together with classic examples from other diseases, and then their application to combination therapy in COPD is discussed. Finally, terminology for the independent and interactive effects of drug combinations is discussed, and approaches to the quantitative analysis and visual display of the effects of drug combinations are introduced. The basic principles reviewed here provide the pharmacologic foundation for subsequent articles in this issue that address the combinations in current use for COPD, and they point to novel strategies for potential future approaches to combination therapy in COPD.

Keywords: desensitization; dose–response curves; drug–receptor interactions; pharmacodynamics; pharmacokinetics; synergism

A firm foundation in the basic pharmacologic principles that govern the actions of all drugs is essential to understanding the effects of combination drug therapy for chronic obstructive pulmonary disease (COPD). The specific actions of the drugs used in combination therapy must be understood first, but their individual actions are subject to modification by the presence of a second drug. Unique new actions may occur only with combination therapy and not with either drug given alone, adding yet another layer of complexity. The most important basic principles of pharmacology that are essential for understanding combination therapy are reviewed here, together with a new organizational framework for the diverse rationales for using drug combinations and a brief overview of terminology and analytical methods essential for understanding the individual and interactive effects of drugs used in combination therapy.

The field of pharmacology is divided into two broad areas: pharmacokinetics, which deals with safely getting the right amount of active drug to the right location for the right amount of time; and pharmacodynamics, which deals with understanding the effects of the drug at its site of action (1–3). Alternatively, pharmacodynamics has been described as the study of "what the drug does to the body" and pharmacokinetics as the study of "what the body does to the drug." Both pharmacokinetics and pharmacodynamics provide important targets and rationales for the use of combination therapy.

PHARMACODYNAMICS: DRUG ACTIONS AT THEIR TARGET SITES

Basic Aspects of Drug Action

Drug binding to receptors. The effects of nearly all drugs are mediated by interactions with specific receptors, either the classically defined receptors for hormones, neurotransmitters, and growth factors, or less classical drug receptors that nonetheless obey the laws of drug-receptor interactions (2, 3). These drug-receptor interactions can generally be described by the equation $[RD] = [R_T] \times [D] / (K_D + [D])$, where [RD] is the concentration of receptor-drug complex, $[R_T]$ is the total receptor concentration, [D] is the concentration of free drug (not bound to receptor), and K_D is the equilibrium dissociation constant for the binding of free drug D to free receptor R to form the RD complex, defined by the reaction $R + D \rightleftarrows RD$. The analogous form of the equation used in radioligand binding assays may be more familiar: $B = B_{max} \times F / (K_D + F)$, where B is the concentration of bound ligand, B_{max} is the total number of binding sites, F is the free drug concentration, and K_D is again the equilibrium dissociation constant for the binding reaction. Both of these equations yield the classical hyperbolic dose–response curve when data are plotted versus the free drug concentration and the classical sigmoid dose–response curve when plotted versus the log of the free drug concentration. In either plot, the K_D value is the concentration of drug that gives 50% of the maximal drug–receptor complex. K_D is a measure of the affinity of the drug for the receptor (or of the receptor for the drug), with smaller K_D values representing higher affinities and larger K_D values representing lower affinities. Both types of plots reveal the saturability of the reaction that arises from the finite number of receptors available for interacting with the drug.

Receptor-mediated drug effects. The end points of interest for treating COPD or other diseases are the cellular or clinical effects that result from formation of a drug–receptor complex, not the drug–receptor interaction itself. In the simplest case, the effect of a drug binding to its receptor is directly proportional to the concentration of the RD complex, and the equation for the drug's effect is essentially identical to that for its binding to the receptor. In mathematical terms, $E = E_{max} \times D / (K_a + D)$, where E is the effect at a given drug concentration D, E_{max} is the maximal effect when all of the receptors are occupied by active drug, and K_a is the concentration of drug that gives half of this maximal response and defines the drug's potency. In this simple case, the drug's potency for inducing a response is identical to its affinity for binding to the receptor; however, this is not usually the case.

Agonism and antagonism. Drugs that increase receptor activation when they form the RD complex are agonists. Drugs that bind but do not alter receptor activation are antagonists. Although antagonists do not alter the activation state of the receptor, their binding nonetheless can result in effects that are clinically important, because their occupancy of the receptor's

(*Received in original form April 14, 2005; accepted in final form July 6, 2005*)

This study was supported by The University of Nebraska Medical Center, Omaha, Nebraska.

Correspondence and requests for reprints should be addressed to Myron L. Toews, Ph.D., Professor, Department of Pharmacology, University of Nebraska Medical Center, 985800 Nebraska Medical Center, Omaha, NE 68198–5800. E-mail: mtoews@unmc.edu

Proc Am Thorac Soc Vol 2. pp 282–289, 2005
DOI: 10.1513/pats.200504-037SR
Internet address: www.atsjournals.org

binding site can prevent endogenous agonists from increasing receptor activation. A third group of drugs bind and activate the receptor in a manner similar to agonists but fail to cause the maximal response, even when sufficient drug is present to occupy all of the receptors. These drugs are partial agonists, and the extent to which they increase receptor activation is termed their "efficacy." The efficacy of partial agonists is defined in relation to the maximal response possible or the response to the historical reference drug for the receptor, that drug being called a full agonist to distinguish it from partial agonists. Full agonists have an efficacy of 1; antagonists have an efficacy of 0; and partial agonists have efficacy values greater than 0 but less than 1. Partial agonists with low efficacy frequently behave as antagonists in clinical use. For example, some β-adrenergic antagonists, such as pindolol, are partial agonists and are sometimes described as having intrinsic sympathomimetic activity. It is important to emphasize that potency is a measure of the amount of drug required to generate a given response, whereas efficacy is a measure of the magnitude of the response that is generated by the drug; these parameters are largely independent of each other.

Advanced Concepts in Drug Action and Receptor Theory

Complexities in drug–receptor interactions and responses. The simplifying assumption that a drug binds to a single state of its receptor to induce a single response that is directly proportional to its binding to the receptor is occasionally valid in the laboratory. It is especially valid when a specific receptor proximal effect is the end point being measured, such as G protein activation for G protein–coupled receptors or transcriptional activation of a specific gene for steroid receptors. It is now known, however, that receptors can have multiple active states that can send multiple signals, making receptor activation more complex. Also, in clinical studies and in medical practice, the endpoints being measured are generally many steps distal to the initial drug–receptor binding interaction, through complex signal transduction cascades and response mechanisms. In these cases, the final effect of the drug is still determined by its binding to its receptor, but both the drug's cellular or clinical potency and its cellular or clinical efficacy exhibit a much more complex relationship to the fraction of receptors occupied by the active drug. Several of the key factors that contribute to differences between simple receptor occupancy and actual receptor-mediated drug responses are highlighted here, together with the opportunities they may present for new approaches to COPD combination therapy.

Constitutive activity and inverse agonism. It is now firmly established that receptors can exhibit constitutive activity and activate cellular responses even in the absence of an activating ligand. Although these phenomena were first observed for mutated or highly overexpressed receptors in isolated cell systems, there is evidence to support their relevance in more physiologic systems and in some pathologies (4–6). This discovery has led in large part to the current concept that receptors can exist in an inactive conformation (usually represented by R) or an active conformation (R*) and that agonist ligands stabilize the R* conformation by binding to it. The extent to which agonists may also bind to the inactive R form of the receptor and actively drive the conversion to the active R* form remains to be established. In either case, the effect of agonist binding is to increase the fraction of receptors in the active R* form and to increase receptor-mediated signaling.

The identification of constitutively active receptors quickly led to the realization that many drugs previously considered to be antagonists could actually decrease the constitutive activity of these receptors. They were not simply antagonists with no effect of their own except to prevent agonists from binding and activating the receptor, but rather they were inverse agonists, drugs capable of altering receptors and signaling pathways in the opposite direction from classical agonists. Current understanding of inverse agonists is that they bind preferentially to the inactive R form of the receptor and stabilize it in the same way that agonists preferentially bind and stabilize the R* form. Furthermore, just as there are partial agonists that cause less than full activation of receptors even with full receptor occupancy, there are also partial inverse agonists that do not fully prevent the formation of R* even with full receptor occupancy. The current explanation for these partial effects is that these drugs can bind and stabilize both the R and R* conformations to various extent. Full agonists primarily bind and stabilize the R* state, full inverse agonists primarily bind and stabilize the R state, and the entire spectrum between these extremes is occupied by partial agonists, antagonists, and partial inverse agonists. These effects are quantified as the efficacy of the drug, with values ranging from 1 for a full agonist to -1 for a full inverse agonist. In current models, the only true antagonists are compounds with an intrinsic efficacy of exactly zero. Most drugs used as antagonists are likely to exhibit at least some partial agonist or partial inverse agonist activity; however, in clinical practice, many of these may not be significantly different from classically defined antagonists. For COPD therapy, it is important to consider the possibilities that some of the pathology may arise from receptor constitutive activity, that some of the effects of existing drugs could be mediated by inverse agonism rather than simple antagonism, and that new drugs with inverse agonist activity might provide novel therapeutic benefit.

Multiple active conformations of receptors. A related and almost certainly equally important concept is that many receptors can exhibit multiple active conformations, each of which can interact with different effectors to modify the activity of different downstream signaling targets to cause different effects. Drugs can bind to and stabilize these various active conformations selectively, making it possible to design drugs to target only a subset of the effects mediated by that receptor. Perhaps the best-characterized examples of this concept are the selective estrogen response modifiers, such as tamoxifen and raloxifene, which bind to estrogen receptors and act as agonists for some of the effects of estrogen but as antagonists for other estrogen receptor effects. Different estrogen response elements on DNA are preferentially activated or inactivated by these different estrogen receptor conformations, allowing a different set of genes to be expressed in response to each of these drugs, even though they all use the same receptor (7, 8). It seems likely that glucocorticoid receptors and response elements exhibit similar phenomena and that selective glucocorticoid response modifiers could become as important as those for estrogen receptors, with a likely beneficial increase in specificity of clinical effects as a result.

Similar multiple active conformational states have been demonstrated for G protein–coupled receptors, with different conformations activating different G proteins and different downstream signaling pathways. In the case of G protein–coupled receptors, this concept is sometimes referred to as "signal trafficking," with different ligands directing receptor responses down different intracellular signaling pathways (9, 10); "conformation-selective agonism" is proposed here as a more appropriate term. These concepts provide new opportunities for monotherapy with novel conformation-selective drugs. An additional exciting possibility is that combination therapies could target the interconversions among these various active states, with one drug serving as the activator and the other drug controlling the likelihood of the receptor being in the desired conformation for that drug to bind and stabilize.

Receptor expression levels and receptor reserve. Because of the signal amplification that can occur at many steps in the signal transduction pathways activated by receptors, and because factors other than the concentration of drug-receptor complex may be limiting for the final response, the maximal cellular or clinical response is often obtained with concentrations of drug far below those required to achieve maximal receptor occupancy. This results in phenomena referred to as "spare receptors" (so termed because the cell has more receptors than needed for a maximal response) or "receptor reserve" (because the cell can lose some of its receptors and still maintain full responsiveness). Receptor reserve shifts the dose–response curve for drug effects to the left, moving it away from the curve for drug binding to the receptor. The concentration of drug that gives half-maximal response in this context is usually referred to as the EC_{50} (effective concentration for a 50% response), because it is a complex factor affected by many variables rather than a true constant. The ratio of the EC_{50} to the K_D for binding is often used as a quantitative indicator of the extent of receptor reserve. The key outcome of these relationships is that the cellular or clinical effectiveness of a drug changes in a complex way as the number of receptors changes (e.g., because of disease states or drug-induced up-regulation or down-regulation of receptors or their coupling to downstream signaling pathways). In general, an increase in the number of receptors increases the maximal response in the absence of a receptor reserve but shifts the dose-response curve to the left (higher potency, lower EC_{50} value) in the presence of a receptor reserve (i.e., the original receptor number was already sufficient to generate a maximal response). Conversely, a decrease in the number of receptors leads to a rightward shift in the dose-response curve (decreased potency, higher EC_{50} value) if there are spare receptors; however, as the number of receptors becomes the limiting factor for the downstream effect, the maximal response also begins to decrease. This concept provides possibilities for combination therapy, with a second drug used to increase or decrease the expression of the receptors that are the target for the first drug. These concepts are also relevant to the adaptive upregulation and down-regulation of receptor expression that can occur in response to chronic drug exposure, discussed further later.

PHARMACOKINETICS: DRUG DELIVERY AND DURATION OF ACTION

Pharmacokinetic Principles for Combination Therapy

Pharmacokinetics is generally divided into four components: (*1*) absorption, (*2*) distribution, (*3*) metabolism, and (*4*) elimination (1). Before a drug can reach its target cells and receptors to mediate its effects, it must be effectively absorbed and then distributed to the desired site of action. The second drug for combination therapy could be a drug that improves the rate of absorption, extent of absorption, or the distribution of a first drug already known to be effective in causing the desired end point. A second drug might be used to target the effects of the first drug to the desired site of action, without the second drug having any actions of its own at that site. Metabolism and elimination of a drug can decrease either the amount or duration of action of active drug, and a second drug for combination therapy could be a drug that decreases either the metabolism or elimination of the active first drug. In these cases, the second drug has no direct effect of its own on the desired end point but only acts by increasing the effective concentration of the active first drug at its therapeutic target site.

Adaptive Changes in Receptors and Signaling Pathways as Targets for Combination Therapy

Drug receptors and their associated signaling pathways are not static entities, but rather are subject to adaptive changes in many of their properties. With constant or repeated exposure to agonist drugs, receptors can undergo a desensitization in their ability to bind their ligands or to initiate signals in response to ligand binding. Multiple mechanisms are often involved, and the time courses for onset and reversal of each component of the overall changes in responsiveness can differ. Covalent modification of the receptors or other signal pathway molecules is often involved and typically occurs rapidly. With somewhat longer treatment, many receptors undergo internalization into endocytotic vesicles or redistribution to other cellular compartments, such as movement into or out of caveolae or lipid rafts, where their accessibility to ligand or their ability to propagate signals may be different. With even longer exposure, there is often a decrease in the total number of receptors because of enhanced degradation, decreased synthesis, or both. This decrease in receptor number is referred to as downregulation, although it also can contribute to functional desensitization. Much recent work has focused on the multiple mechanisms for adaptive regulation of G protein–coupled receptors, with the β_2-adrenergic receptor that is the target of several COPD drugs being the best characterized (11). Many other types of receptors are subject to similar adaptive changes with chronic activation, although the specific molecular details are different. Conversely, drugs that block or decrease receptor activity are likely to induce receptor upregulation or increased signal capacity, allowing homeostatic maintenance of the proper level of sensitivity and responsiveness.

These adaptive changes provide novel possibilities for combination therapies. For example, a second drug could be used to decrease desensitization of the response to the first drug and prolong the first drug's actions. Alternatively, a second drug might be used intentionally to induce a tissue-selective desensitization or downregulation of the first drug's receptors, enabling the first drug to have more selective actions. Much effort has been placed on advancing the understanding of the mechanisms of receptor desensitization, and this work may someday lead to combination therapies with one drug to attain the desired response and a second drug to help maintain that response.

RATIONALES FOR COMBINATION THERAPY

Clinical Rationales for Combination Therapy

From a clinical therapeutics perspective, there are perhaps only two broad rationales for using drug combinations. The first and most obvious is to obtain a greater therapeutic effect with the combination than can be achieved with either drug alone. The second is to obtain the same therapeutic effect as could be obtained with only one of the two drugs, but with fewer deleterious side effects or dose-limiting toxicities. Presumably, an ideal combination therapy would accomplish both of these goals (12). In contrast to these two simple clinical rationales, there is a much larger range of pharmacologically based reasons for using a combination of two drugs rather than a single drug. These two-drug combinations target diverse mechanisms related to all aspects of drug action, as discussed in more detail in the following sections.

Mechanistic Rationales for Drug Combinations: An Organizational Framework

Overview. For the discussion below, the pharmacologic rationales for combination therapy are divided into three general approaches, referred to as classes. It should be stressed, however,

that this is not a rigorous classification scheme, because many drug combinations have multiple rationales and mechanisms of interaction and do not fall cleanly into one single class or another. This organizational framework may be useful as a guide for understanding current combinations, however, and as a stimulus for encouraging new approaches using novel combination strategies.

Class 1 combinations: rationales and examples. Class 1 combinations include two (or more) drugs that each target different aspects of the disease. This is perhaps the most obvious rationale for using drug combinations, because many diseases are known to be multicomponent or multifactorial. It is in this class of combinations where the actions of Drug A and Drug B are most likely to be independent, at least in their specific actions; however, they might have interactive effects on the overall improvement in patient status. Hypertension is a multicomponent disease treated with class 1 combinations, including drugs acting on completely different tissues or organs. Treatment of hypertension is likely to include some combination of diuretics acting on the kidney to decrease blood volume; vasodilators acting directly on vascular smooth muscle cells; α_2-adrenergic agonists acting centrally to reduce sympathetic activation; angiotensin-converting enzyme inhibitors acting on the endothelium to decrease angiotensin levels; and β-adrenergic antagonists acting on the heart to decrease myocardial contractility and on the kidney to inhibit renin release.

Class 1 combinations in COPD therapy. Class 1 combinations are a common approach to COPD therapy, because COPD is widely accepted as a multicomponent disease. A case can be made that more such combinations for COPD therapy are needed. The most obvious class 1 combination in COPD therapy is the use of β_2-agonists plus corticosteroids. The predominant therapeutic effect of β_2-agonists is bronchodilation by activation of β_2-receptors on the smooth muscle cells. In contrast, the predominant therapeutic effect of corticosteroids is to decrease inflammation, primarily by inhibiting the actions of inflammatory cells and inflammatory mediators. The use of muscarinic antagonists together with corticosteroids is a similar example of a class 1 combination, with muscarinic antagonists acting to decrease bronchoconstriction. Other aspects of these combinations are that each includes one agent primarily targeting acute aspects of the disease (β_2-agonists or muscarinic antagonists) and one agent primarily targeting longer-term elements of disease progression (corticosteroids), and that each contains one agent for symptomatic relief (the bronchodilators) and one that is perhaps truly disease-modifying (antiinflammatory corticosteroids). Additional mechanisms and target cell types are also involved in the effects of all three of these classes of drugs, as highlighted elsewhere in this issue. There is a need for additional drugs to target other aspects of COPD, most likely to be used in further combinations with the previously mentioned drugs. Aspects of COPD that are not currently targeted effectively are mucus production or secretion and cough, and the airway structural remodeling that occurs with disease progression and remains essentially irreversible.

Class 2 combinations: rationales and examples. Class 2 includes many combinations of drugs that target a single disease component and often a single cell type or even a single response pathway in that cell type, but with different sites of action. This specific targeting allows greater effects or reduced drug doses and reduced toxicities. In some of these cases, a single drug may be safe but insufficiently effective on its own. In other cases, drugs that are sufficiently powerful on their own may be available, but side effects and toxicity may prevent their use at fully effective doses. In either case, combining two effective drugs achieves the desired end point while avoiding toxicity. There are many different approaches within this class of combinations, as illustrated with typical examples next.

An example of using two drugs to obtain a greater response than with either drug alone, where the two drugs have similar targets and actions, is in Parkinson's disease. Levodopa is a prodrug precursor that is converted to dopamine, and it is now used together with bromocriptine, a direct-acting dopamine receptor agonist targeting the same receptor. The antibiotic cotrimoxazole uses two drugs acting at different points in a single pathway to achieve greater inhibition than either drug alone. It is a combination of sulfamethoxazole, which blocks folic acid synthesis by inhibiting dihydropteroate synthetase, and trimethoprim, which acts at a later step in nucleotide synthesis to inhibit dihydrofolate reductase.

Cancer chemotherapy provides multiple examples of the use of drug combinations to achieve the desired therapeutic end point while avoiding dose-limiting toxicities of the individual agents, each of which is theoretically sufficiently cytotoxic to kill cancer cells on its own. One such regimen combines cisplatin, dosage of which is limited by nephrotoxicity; etoposide or vinblastine, each of which are dose-limited by bone marrow suppression; and bleomycin, which is dose-limited by pulmonary toxicity. In this case, the individual drugs also have different mechanisms of cancer cell cytotoxicity, attacking the disease by multiple pathways while also avoiding damage to nonmalignant cells.

A variant of class 2 combination therapy is to use a second drug to decrease a single, specific, unwanted effect of the first drug. In prostate cancer, gonadotropin-releasing hormone agonists, such as leuprolide, are used chronically to effect downregulation of gonadotropin-releasing hormone receptors, inhibiting stimulation of testosterone synthesis. The androgen receptor antagonist flutamide is used in combination early in this therapy to block the actions of the increased testosterone that occurs initially with leuprolide, in the period before the gonadotropin-releasing hormone receptors become downregulated. Another example is the use of a potassium-sparing diuretic such as spironolactone, together with thiazides, to decrease the excess loss of potassium that can occur with thiazides alone.

Another class 2 approach is to use a second drug to decrease the workload for the first drug, making it more effective. In type 2 diabetes, the α-glucosidase inhibitor acarbose lowers glucose absorption from the gastrointestinal tract, decreasing the level of plasma glucose that other drugs, such as the sulfonylurea glipizide or injected insulin, must metabolize or store. Similarly in hypertension, angiotensin-converting enzyme inhibitors, such as captopril, decrease the concentration of angiotensin II, so that angiotensin receptor antagonists, such as losartan, have less angiotensin II to counteract.

Class 2 combinations in COPD. The use of bronchodilators in combination is an example of class 2 combinations for treating COPD. β_2-Agonists, muscarinic antagonists, and theophylline all target the bronchoconstriction component of COPD, act on airway smooth muscle cells, and elevate cyclic adenosine monophosphate as a key component of their mechanisms. β-Agonists and muscarinic antagonists both target the G protein–mediated input to the enzyme adenylyl cyclase, but β-agonists act to increase stimulatory input by G_s, whereas muscarinic antagonists act to decrease the inhibitory input by G_i. These two classes of drugs use different receptors and G proteins but share the same pathway from adenylyl cyclase to bronchodilation.

Combining theophylline with β-agonists can be viewed as using theophylline to decrease the workload for β-agonists, because theophylline prevents the breakdown of the cyclic adenosine monophosphate that is formed in response to β-agonist stimulation. From an alternate perspective, the use of other bronchodilators with theophylline serves as an approach to low-

ering the concentration of theophylline needed, thereby avoiding the significant side effects that occur with higher doses of theophylline. Although their shared ability to modulate cyclic adenosine monophosphate is the most widely accepted view of the actions of these agents, it is also possible that some of the benefits of using them in combination arise from unknown or underappreciated additional effects that may be different for each of the individual drugs.

Class 3 combinations: rationales and examples. Class 3 combinations include one drug that is useful on its own but is insufficiently effective or too toxic, and a second drug that does not share the same activity as the first drug and may have no useful effect on its own, but that can enhance the effectiveness of the first drug by either pharmacokinetic or pharmacodynamic mechanisms. It should be noted that these enhancers may modulate not only the magnitude of the response to the active drug but also the duration of action of the active drug, with either type of action being of potential therapeutic benefit.

The antibiotic Augmentin combines an active drug with an inhibitor of its degradation; the β-lactamase inhibitor clavulanate is included to prevent the target bacteria from inactivating the active drug amoxicillin. Another approach is to use a second drug to alter the tissue or cellular distribution of the first active drug, exemplified by Sinemet (carbidopa and levodopa), a combination used in Parkinson's disease. The therapeutic goal is to increase dopamine levels in the brain by having levodopa be converted to dopamine by dopa-decarboxylase in the brain. Dopa-decarboxylase in the periphery can metabolize levodopa to dopamine, however, decreasing the amount of levodopa delivered to the brain (dopamine does not cross the blood-brain barrier) and increasing unwanted side effects of dopamine in tissues outside the blood-brain barrier. Carbidopa is a dopa-decarboxylase inhibitor that does not cross the blood-brain barrier, selectively inhibiting dopa-decarboxylase in the periphery and indirectly targeting levodopa action to the brain.

Class 3 combinations in COPD. There are no clear examples of intentional class 3 combinations in current COPD therapy, although such effects may contribute to some extent to COPD therapy. Corticosteroids do not directly cause bronchodilation, but they can upregulate expression of β_2-receptors, potentially enhancing the effect of the β_2-agonist (13). There is mixed evidence on the question of whether corticosteroids may prevent or counter agonist-induced desensitization and downregulation of β_2-receptors, and these effects may vary with cell and tissue type or with specific receptor polymorphisms (14–16). Altering β-receptor expression and desensitization is clearly not the primary rationale for this combination, but these effects may contribute to some extent to the effectiveness of the combination.

There may be merit in considering the actions of some drugs used as monotherapy as being class 3 combinations with endogenous mediators. Theophylline as monotherapy can elevate cyclic adenosine monophosphate, presumably acting as an enhancer of the actions of endogenous agents that stimulate cyclic adenosine monophosphate formation, the constitutive activity of G_s-coupled receptors, or the basal activity of adenylyl cyclases. Similarly, muscarinic antagonists given alone can prevent the endogenous activation of muscarinic receptors that results from the presence of endogenous agonist or perhaps from constitutive activity of muscarinic receptors.

It is intriguing to think of salmeterol as a class 3 combination of two active ingredients within a single compound (17). Its saligenin headgroup is the active drug in β_2-receptor activation, whereas its arylalkyl-oxyalkyl tail is a built-in enhancer that targets the active drug to its specific site of action, where the drug then binds tightly to prolong the headgroup's duration of action at this site. Salmeterol provides an active drug plus two mechanisms for pharmacokinetic enhancement of its actions. This view of salmeterol may provide a useful model for potential new agents composed of a class 3 combination of active principles. Whether formulated as a single drug or used as separate constituents, combinations for selective targeting or for overcoming adaptive losses in drug sensitivity are approaches that should find greater use as these processes become better understood.

PHARMACOLOGIC ANALYSIS AND VISUALIZATION OF PHARMACOLOGIC EFFECTS OF DRUG COMBINATIONS

Basic Terminology for Analyzing Combination Effects

Using combinations of drugs necessitates considering the quantitative nature of the combination effects and the terminology for describing differences between the actual combination effects and what is expected based on the known effects of the individual drugs (18–20). In the simplest quantitative analysis, the effects of the combination can equal the sum of the expected effects of the two drugs alone, referred to as "additive"; less than the expected sum, "subadditive"; or greater than the expected sum, "superadditive." With regard to mechanisms of action, the effects of the two drugs can be either independent, if neither drug alters the action of the other, or interactive, if one drug in some way alters the action of the other drug. Some sources use the term "independence" to describe what is typically called additivity (20). The authors prefer to use the terms "additivity" for the quantitative nature of the combination and "independence" for the mechanistic (noninteractive) nature of the combination. The concept of interaction is inherent to the nature of the class 3 combinations described previously, where one drug by definition alters the actions of the other. In contrast, the targeting of different aspects of disease with the class 1 combinations does not necessarily imply independence in their actions, because either drug could alter the effects of the other on their different end points, in addition to its primary effect. Similarly, class 2 combinations can be either independent or interactive.

Mechanisms for Subadditive and Superadditive Effects of Drug Combinations

For drugs with independent actions, subadditivity is most likely to occur when tissue responsiveness rather than drug effectiveness is the limiting factor. If the sum of the responses to the two drugs individually is greater than the maximal response possible by the system, then subadditivity occurs, without any true interaction between the drugs (Figure 1, *top panel*). Subadditivity for interactive drugs occurs when one drug interferes with the action of the other to decrease its effect, for example by enhancing its degradation or by accelerating the downregulation of its receptors (Figure 1, *middle panel*). Classical antagonism is a special case of subadditivity caused by interference, where the second interacting drug has no action of its own except to block the action of either the first drug or the endogenous agonist.

Superadditivity can occur only for interactive combinations. Although many studies use the term "synergistic" for all effects of combinations that are greater than expected from simple additivity, the authors prefer to use "enhancement" and "synergism" as separate terms for superadditive effects, with these terms providing additional mechanistic information. Enhancement is used when the second drug has no effect on its own and only increases the effectiveness of the first drug (Figure 1, *bottom panel*, *left*). In contrast, "synergism" is reserved for the case in which each of the drugs has clearly demonstrable effects on its own but where the effects of the combination are clearly greater

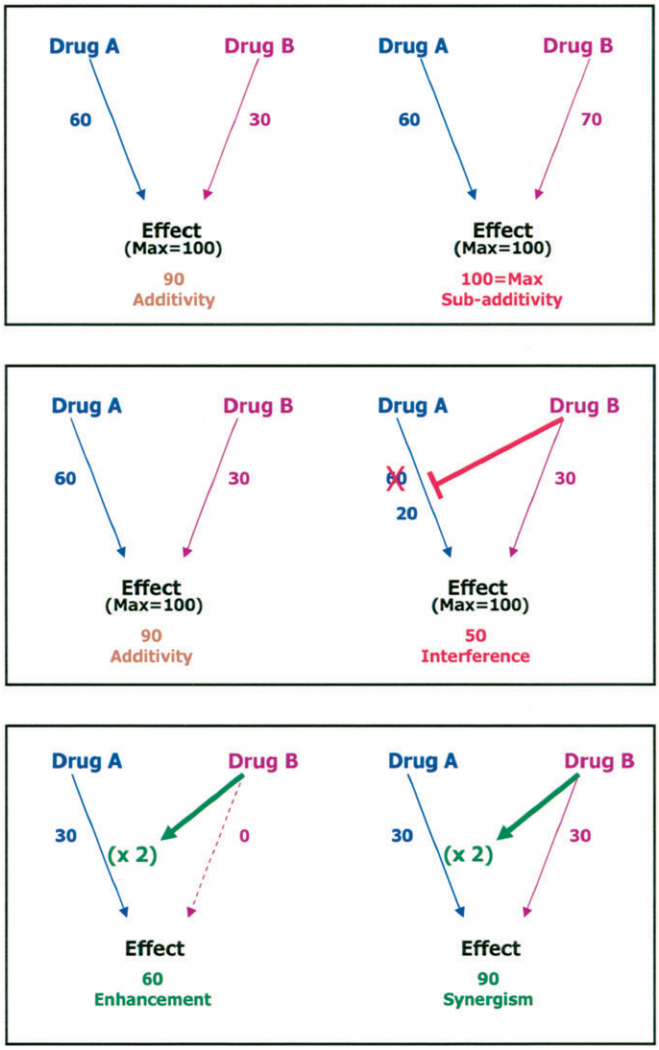

Figure 1. Mechanistic principles for additivity, subadditivity, and superadditivity of combination drug effects. The actions of the individual drugs are indicated beside their respective *arrows*, and the net effect of the combination is indicated at the bottom of each pair. *Top panel* illustrates mechanisms for drugs with independent actions, with additivity occurring if tissue responsiveness allows both Drug A and Drug B to exert their full effects (*left*), but with subadditivity occurring if tissue responsiveness is the limiting factor for the total magnitude of response possible (*right*). *Middle panel* illustrates additivity (*left*) versus subadditivity (*right*) for an interactive combination in which Drug B interferes with the actions of Drug A (a threefold reduction indicated by the *red inhibition line*), preventing it from exerting its full response, but Drug B nonetheless has a beneficial effect on its own. The case in which Drug B has no effect on its own and only interferes with Drug A action is simple antagonism. *Bottom panel* illustrates two possibilities for superadditivity for interactive combinations: enhancement for the left pair of drugs, in which Drug B increases the effect of Drug A by twofold (*green arrow*) but has no effect on its own, and synergism for the right pair of drugs, where Drug B exerts a useful effect on its own in addition to its twofold enhancement of the effects of Drug A.

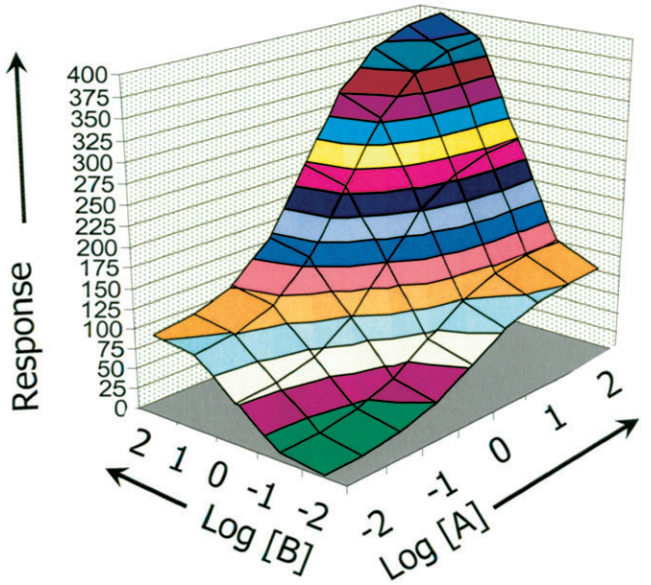

Figure 2. Fishnet diagram of the response surface for a synergistic drug combination. The *right front edge* of the diagram shows the response to Drug A alone, with an EC_{50} of 1 and a maximal response of 100. The *left front edge* shows the corresponding response for Drug B with the same EC_{50} and maximal response. The magnitude of the response at each pair of drug combinations is shown on the vertical axis. Drug A and Drug B also interact with each other to produce synergism, each increasing the magnitude of the response to the other. The response to Drug A in the presence of a maximal concentration of Drug B is shown on the left rear wall, and that to Drug B with maximal Drug A on the right rear wall, with an overall maximal response of 400 at maximal concentrations of both drugs.

than additive (Figure 1, *bottom panel*, *right*). Synergism arises from the summation of the individual drug effects plus additional mechanisms that lead to one or both drugs amplifying the effect of the other. In the organizational framework described previously, synergism occurs for drugs that are a class 3 combination in addition to either a class 1 or 2 combination. A value greater than 1.5 times the sum of the individual effects is often taken as the criterion for synergism. Note that enhancement can occur because of an increase in potency, an increase in efficacy, or both, and full dose-response curves in the absence and presence of the second drug are necessary to delineate fully these effects.

An example of synergism from the authors' work, albeit with endogenous agents rather than with drugs, is the stimulation of human airway smooth muscle cell DNA synthesis by the lipid mediator lysophosphatidic acid acting by a G protein–coupled receptor, and epidermal growth factor acting by its receptor tyrosine kinase pathway (21). Lysophosphatidic acid caused an 8-fold stimulation; epidermal growth factor caused a 17-fold stimulation; and lysophosphatidic acid plus epidermal growth factor caused a 98-fold stimulation, nearly four times the expected value from the sum of the agents separately. In contrast, in similar studies of this same response with endothelin and epidermal growth factor in another laboratory (22), there was no stimulation by endothelin alone, but the presence of endothelin increased the stimulation by epidermal growth factor from 23-fold to 86-fold, an example of enhancement. The authors of this latter study referred to their effect as "potentiation"; others have used the term "sensitization" for similar phenomena. Because these terms have connotations of increased potency or sensitivity, as opposed to increased efficacy or maximal response, and because both terms have specific uses for situations in which one agent is given as pretreatment rather than in combination, the authors prefer the more general term "enhancement" except in cases where there is clearly synergism. Several extensive treatises during the past 15 years have addressed the many different models that have been developed to understand the concepts

of independence, additivity, synergism, and antagonism (18–20). These works provide interesting insights and detailed descriptions of the mathematical analyses and theoretical models for such effects that are beyond the scope of this article.

Graphic Representation of Combination Drug Effects

The approach to graphic visualization and analysis of the effects of drug combinations depends on the class, as defined previously. For the different end points targeted by the agents in class 1 combinations, in which the effects on each end point are largely independent, separate dose-response curves for each response are adequate, because the response to each drug in these combinations is essentially flat for the different endpoint induced by the other drug. For class 3 combinations, in which one of the drugs has no effect on its own, a family of traditional dose-response curves in the absence and presence of increasing concentrations of the second drug should be adequate to analyze and illustrate the interactions. Changes in potency, efficacy, or both may be observed in these graphs.

For combinations in which both drugs affect the same end point on their own but may in addition have interactive effects ranging from antagonism to synergism, the most useful approach is a three-dimensional group of bar or line graphs of the dose–response curves for each agent in the absence and presence of increasing concentrations of the other drug. These curves can then be connected graphically to form a response surface diagram or fishnet plot, allowing convenient visualization of the measured or predicted effect of every pair of concentrations. In these graphs, the concentrations of each drug are plotted on the horizontal axes and the response is plotted on the vertical axis. The dose–response curves to each of the drugs alone are on the front edges of the diagram, and the dose–response curves to each drug in the presence of maximal concentrations of the other are on the back walls of the diagram. An example of such a plot is shown in Figure 2, for a case in which each drug enhances the efficacy but not the potency of the other. Standard spreadsheet programs and more specialized scientific graphing and analysis programs are capable of generating and analyzing these plots.

Two approaches are commonly used for visualizing and analyzing the occurrence of simple additivity versus synergism or interference. The first is an isobolograph, a plot of one or more of the isoboles, or equal-response lines (Figure 3). The dose of Drug A required to achieve a given level of response (e.g., 50%) is plotted on one axis, and the dose of Drug B required to achieve the same response is plotted on the other axis. All of the other concentration pairs of Drug A with Drug B that are expected to give the same response level, based on simply adding their individual effects, are then plotted to generate the isobole line. The actual measured response to each tested combination of Drug A given together with Drug B is then plotted on the same graph. Points that fall on the line are additive, whereas points that fall below the line of additivity represent combinations that are synergistic (requiring lower concentrations of the combination than expected to attain the given effect). Points that fall above the line are subadditive (requiring higher concentrations of the combination than expected to attain the given effect).

Although most discussions of this subject present these isoboles as straight lines (18–20), that is the expected outcome only in selected instances in which the two drugs act on the same receptor or other target in a competitive manner. For the more general case, where the two drugs act on different receptors or different pathways to the same end point, the predicted additivity isoboles are concave. The isobole lines for both assumptions are illustrated in Figure 3, highlighting the different conclusions depending on the assumption (or knowledge) regarding the targets of the two drugs.

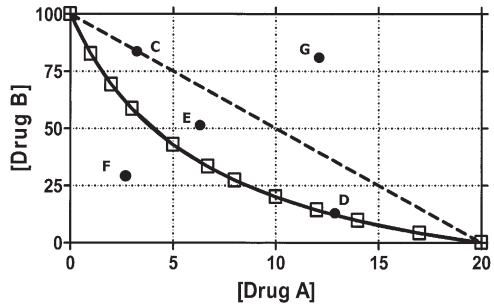

Figure 3. Isobolographic analysis of combination drug additivity, subadditivity, and superadditivity. Two different isoboles are shown for the 50% response level for Drug A, whose EC_{50} is 20, and for Drug B, whose EC_{50} is 100. The *two points on the axes* represent these 50% response levels for Drug A alone (at a concentration of 20) and Drug B alone (at a concentration of 100). The *isobole lines* represent all pairs of concentrations of Drug A and Drug B giving the same magnitude of response, the line of additivity. The *dashed line* is the linear isobole predicted from the assumptions used in most treatises, which is valid for cases in which the two drugs share the same receptor and response. The *solid line* shows the more general isobole for the case in which no assumptions about the receptors for the two drugs are required. The *square data points* on this line represent calculated pairs of concentrations whose individual responses add up to 50% of the maximal response; as examples, other concentration pairs giving the same response include 5 of Drug A plus 43 of Drug B, 10 of Drug A plus 20 of Drug B, and 17 of Drug A plus 4.2 of Drug B. *Points C–G* represent responses seen with various hypothetical drug combinations. *Combination C* falls on the dashed line and is additive in the first model, but in the second model it is above the line and subadditive (requiring higher than predicted doses to attain the indicated effect). Similarly, *Combination D* is additive in the first model, but in the second model it is superadditive (with the indicated effect level occurring at lower concentrations than predicted). *Combination E* is not additive in either model but is superadditive in the first model and subadditive in the second model. *Combination F* is superadditive using either model, whereas *combination G* is subadditive using either model.

Response surface or fishnet graphs are also useful for detecting synergism or interference. The surface of the predicted effects with simple additivity for all combinations of drug concentrations is plotted (i.e., not only for a single effect level). Here, combinations that exhibit synergism fall above the additivity surface (lower concentrations required to achieve a given response) and combinations that exhibit interference lie below the surface. An interesting plot that can be generated from these data, at least in theory, is a plot of the difference between the actual response surface for the combinations and the surface predicted by simple additivity. This plot generates a new surface revealing the pattern of concentrations exhibiting synergism or interference and the extent of this interaction for each pair of concentrations; readers are referred to more extensive treatises for examples and additional details (20).

ADDITIONAL PHARMACOLOGIC ASPECTS OF COMBINATION THERAPY

This discussion of combination pharmacology has focused on rationales and mechanisms for the beneficial effects of drug combinations. It is critical to recognize, however, that combining two or more drugs is always associated with the danger of greater side effects. These can include additivity of the known side effects of each of the drugs or completely unexpected side effects caused by interactions between them.

A topic of considerable interest to pharmacologists that is not discussed here is the question of using drug combinations in a fixed-dose single preparation versus using the drugs separately at their individually most effective doses. Also beyond the scope of this discussion are the potential advantages or disadvantages of combining the activities of the components of a combination preparation into a single molecule with multiple beneficial effects.

Note that combination therapy need not be limited to combinations of drugs. Single drugs can be used with various interventions for additional benefit. This is particularly relevant to COPD, in which smoking cessation, pulmonary rehabilitation, oxygen therapy, and lung-volume–reduction surgery may all be beneficial in addition to pharmacologic approaches (23).

CONCLUSIONS

There are many different rationales for the use of combination therapy, and the pharmacologic principles for evaluating and understanding their actions are in hand. There are already multiple examples of the use of combination therapy for COPD, with evidence for beneficial effects. It is clear, however, that better drugs are needed to treat COPD, perhaps including new combinations. By applying the pharmacologic principles and considering additional possibilities based on the diverse mechanistic rationales summarized here, it may very well be possible to develop novel combinations that are more efficacious or have fewer side effects than existing agents and combinations.

***Conflict of Interest Statement*:** M.L.T. is the recipient of a research grant from GlaxoSmithKline to study salmeterol and fluticasone, alone and in combination, on regulation of airway cell epidermal growth factor receptors, in comparison with other related agents, and he received honoraria from HealthMatters Communications for serving on the steering committee for this Symposium. D.B.B. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

References

1. Rescigno A. Foundations of pharmacokinetics. New York: Kluwer Academic/Plenum Publishers; 2003.
2. Limbird LE. Cell surface receptors: a short course on theory and methods. Norwell, MA: Kluwer Academic Publishers; 1996.
3. Foreman JC, Johansen T. Textbook of receptor pharmacology. Boca Raton, FL: CRC Press; 2002.
4. Kenakin T. Efficacy as a vector: the relative prevalence and paucity of inverse agonism. *Mol Pharmacol* 2004;65:2–11.
5. Milligan G. Constitutive activity and inverse agonists of G protein-coupled receptors: a current perspective. *Mol Pharmacol* 2003;64:1271–1276.
6. Seifert R, Wenzel-Seifert K. Constitutive activity of G protein-coupled receptors: cause of disease and common property of wild-type receptors. *Naunyn Schmiedebergs Arch Pharmacol* 2002;366:381–416.
7. McDonnell DP, Connor CE, Wijayaratne A, Chang C-Y, Norris JD. Definition of the molecular and cellular mechanisms underlying the tissue-selective agonist/antagonist activities of selective estrogen receptor modulators. *Recent Prog Horm Res* 2002;57:295–316.
8. Krishnan V, Heath H, Bryant HU. Mechanism of action of estrogens and selective estrogen receptor modulators. *Vitam Horm* 2001;60:123–147.
9. Kenakin T. Drug efficacy at G protein-coupled receptors. *Annu Rev Pharmacol Toxicol* 2002;42:349–379.
10. Clarke WP, Bond RA. The elusive nature of intrinsic efficacy. *Trends Pharmacol Sci* 1998;19:270–276.
11. Gainetdinov RR, Premont RT, Bohn LM, Lefkowitz RJ, Caron MG. Desensitization of G protein-coupled receptors and neuronal functions. *Annu Rev Neurosci* 2004;27:107–144.
12. Reid JL. Pharmacokinetic and pharmacodynamic aspects of the choice of components of combination therapy. *J Hum Hypertens* 1995;9:S19–S23.
13. Collins S, Bolanowski MA, Caron MG, Lefkowitz RJ. Genetic regulation of β-adrenergic receptors. *Annu Rev Physiol* 1989;51:203–215.
14. Moore PE, Calder MM, Silverman ES, Panettieri RA Jr, Shore SA. Effect of dexamethasone on β_2-adrenergic desensitization in airway smooth muscle: role of the ARG19 polymorphism. *Chest* 2003;123:368S–369S.
15. Tan KS, McFarlane LC, Lipworth BJ. Concomitant administration of low-dose prednisolone protects against in vivo β_2-adrenoceptor subsensitivity induced by regular formoterol. *Chest* 1998;113:34–41.
16. Hjemdahl P, Zetterlund A, Larsson K. β_2-agonist treatment reduces β_2-sensitivity in alveolar macrophages despite corticosteroid treatment. *Am J Respir Crit Care Med* 1996;153:576–581.
17. Coleman RA, Johnson M, Nials AT, Vardey CJ. Exosites: their current status, and their relevance to the duration of action of long-acting β_2-adrenoceptor agonists. *Trends Pharmacol Sci* 1996;17:324–330.
18. Tallarida RJ. Drug synergism: its detection and applications. *J Pharmacol Exp Ther* 2001;298:865–872.
19. Berenbaum MC. What is synergy? *Pharmacol Rev* 1989;41:93–141.
20. Greco WR, Bravo G, Parsons JC. The search for synergy: a critical review from a response surface perspective. *Pharmacol Rev* 1995;47:331–385.
21. Cerutis DR, Nogami M, Anderson JL, Churchill JD, Romberger DJ, Rennard SI, Toews ML. Lysophosphatidic acid and EGF stimulate mitogenesis in human airway smooth muscle cells. *Am J Physiol* 1997;273:L10–L15.
22. Panettieri RA, Goldie RG, Rigby PJ, Eszterhas AJ, Hay DWP. Endothelin-1-induced potentiation of human airway smooth muscle proliferation: an ET_A receptor-mediated phenomenon. *Br J Pharmacol* 1996;118:191–197.
23. Barnes P, Drazen J, Rennard S, Thomson N, editors. Asthma and COPD: basic mechanisms and clinical management. London: Academic Press; 2002.