Cancer Chemother Pharmacol (2004) 53: 384–390
DOI 10.1007/s00280-003-0746-1

ORIGINAL ARTICLE

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Interactions of a herbal combination that inhibits growth of prostate cancer cells

Received: 31 July 2003 / Accepted: 7 November 2003 / Published online: 17 January 2004

Abstract *Purpose*: PC SPES is an eight-component herbal product marketed for the treatment of prostate cancer. The manufacturer of PC SPES claims that the herbal combination is a synergistic blend, but the purported synergy has never been tested. We examined the interaction in cell culture of these eight individual herbal components by the use of an isobologram. *Methods*: US patent no. 5,665,393 (1997) for PC SPES was acquired, and each of the eight herbal components described was acquired, properly identified, and extracted by 95% ethanol. The extracts were tested for cytotoxicity to PC 3 human prostate cancer cells in culture by the MTT (3-[4,5-dimethythiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay. Seven combinations of herbal extracts were made, varying in the proportion of the most cytotoxic herbal extract, that of *Panax notoginseng*. The interactions of *P. notoginseng* with the other seven herbs were evaluated through the use of an isobologram. *Results*: In all seven herbal combinations, *P. notoginseng* was found to be antagonistic with the other seven herbal components in the cytotoxicity assay (*P* values: 0.09, 0.12, 0.12, 0.33, 0.45, 0.56, and 0.76). *Conclusions*: The interaction between the most cytotoxic herbal component of a widely used herbal product and the other seven components was antagonistic. Herbal combinations are no different from traditional combination pharmacotherapy. If herbal combinations are able to achieve antagonism, then theoretically they can achieve synergism if combined properly.

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Keywords PC SPES · Synergy · Isobologram · Prostate cancer · Chinese herbal medicines

Introduction

"Synergy" achieved through a combination of herbs is an important principle of traditional Chinese medicine (TCM). Synergy is defined as the interaction between two or more individual components such that the combined effect is greater than the sum of the effects of the individual components. Synergy can result when two drugs have overtly similar effects but have independent mechanisms. One drug may also synergistically augment the effectiveness of another drug by increasing drug bioavailability via enhancing systemic absorption or decreasing metabolism.

PC SPES (BotanicLab, Brea, Calif.), a popular herbal formula used widely for the treatment of prostate cancer, has been claimed to be "a synergistic blend of high quality herbal extracts based on an ancient Chinese remedy" (on product label). However, the purported synergy has not been documented. The benefits patients gain from the product, if real, could be due to one or more active herbs that is/are cytotoxic to prostate cancer cells or supportive of prostate function. At US $108 per bottle plus shipping and handling, this expensive product can cost prostate cancer patients over $400 per month. If synergy does not exist within the patented formula, patients should be able to achieve similar benefits from consuming similar dosages of the much cheaper individual herbal extracts.

PC SPES is composed of eight herbs (*Dendranthema morifolium*, *Ganoderma lucidum*, *Glycyrrhiza uralensis*, *Isatis indigotica*, *Panax notoginseng*, *Rabdosia rubescens*, *Scutellaria baicalensis*, and *Serenoa repens*). *Serenoa repens* has been widely used for improvement of prostate

function in patients with benign prostatic hyperplasia [1, 27]. The other herbs are known for their purported anticancer and antiinflammatory properties [2, 14, 15, 16, 19, 22, 26]. It has been found in 30 published studies on PC SPES, including several small clinical trials and case reports, that PC SPES benefits prostate carcinoma patients with both hormone-sensitive and hormone-insensitive tumors [5, 6, 17, 18, 23]. PC SPES suppresses the growth of hormone-sensitive (LNCaP) and hormone-insensitive (PC 3 and DU 145) prostate cancer cell lines in vitro and in a mouse model [5]. PC SPES has been reported to alleviate symptoms in patients with advanced prostate cancer, including those in whom conventional therapy has failed [4]. Some proponents of PC SPES argue that the formula may achieve synergy by acting at multiple targets to cause cytostatic and cytotoxic effects on cancer cells [9]. However, all of the published studies were conducted on the commercial combination without testing the individual components for synergy.

For isoeffective dosages of a two-drug combination ($d_A + d_B$) and of the individual drugs alone (D_A, D_B), drug interactions are defined by the following three conditions: additivity ($d_A/D_A + d_B/D_B = 1$), antagonism ($d_A/D_A + d_B/D_B > 1$), and synergism ($d_A/D_A + d_B/D_B < 1$) [7]. If synergy exists then a lower concentration of d_A and/or d_B would be required to achieve the same effect as that of the theoretical dosages for additivity. If antagonism exists then a greater concentration of either or both compared with the theoretical dosages would be required for the same effect. An isobologram can depict these three conditions graphically. This graph allows assessment of biological responses induced by mixtures of agents based upon individual activities of the individual agents. Biological responses can be greater than, equal to, or less than the theoretical response, depending on whether the interactions of the two agents are synergistic, additive, or antagonistic, respectively [10]. Figure 1 depicts five different combinations of drug A and drug B all at the same biological response (e.g. ID_{50}). The theoretical (additive effect) isobol is a straight line connecting D_A, the concentration of drug A where ID_{50} is achieved, to D_B, the concentration of drug B where ID_{50} is achieved. This line of additivity or line of zero drug interaction, illustrates the outcome where the potency of a combination is simply the additive effect of the individual agents. Additivity means that each constituent contributes to the effect in accordance with its own potency [25]. Points above the line indicate antagonistic interactions and points below the line indicate synergistic interactions.

Studying drug interactions through the use of an isobologram has been popular for investigating antiepileptic drug polytherapy [20, 21]. It has been used for radiosensitizing antineoplastic drugs [11, 13]. A synergistic combination of drugs can achieve therapeutic effects with lower drug toxicities than if one of the drugs was used alone. A search of Medline revealed that the use of an isobologram has apparently not previously been applied to studies of herbal combination products.

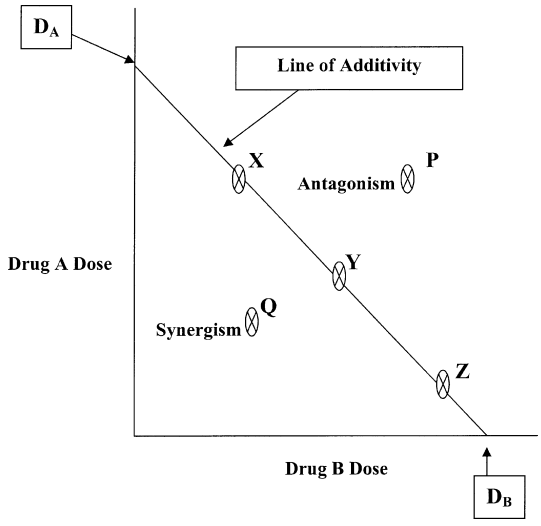

Fig. 1 All points on this isobologram are isoeffective, for example attaining ID_{50}. The line of additivity, defined as $d_a/D_a + d_b/D_b = 1$, connects point D_A to point D_B. Points *X*, *Y*, and *Z* lie on the line of additivity, so the combinations are additive. Points *Q* and *P* refer to synergistic and antagonistic combinations, respectively

Materials and methods

Solvents, chemicals, and buffer

Solvents used were of analytical reagent grade or were redistilled. Unless specified otherwise, chemicals were purchased from Sigma-Aldrich (St. Louis, Mo.).

PC 3 Cell Line

The PC 3 cell line was provided as a gift from Dr. Qihan Dong of the Department of Medicine, University of Sydney. It was originally isolated from a bone metastasis of a 62-year-old patient with grade IV prostatic adenocarcinoma [12]. The cells were maintained in RPMI 1640 medium supplemented with 10% non-dialyzed fetal calf serum (FCS), L-glutamine and gentamicin. The cells were grown at 37°C in a humidified atmosphere comprising 95% air and 5% CO_2. When the cells reached 80% confluency they were subcultured into a new flask. After washing with phosphate-buffered saline (PBS), the cells were removed by trypsinization with 0.05% trypsin in 0.02% ethylenediaminetetra-acetic acid (EDTA) and transferred into a new flask containing fresh RPMI 1640 medium with 10% FCS.

Acquisition of patented PC SPES formula

The manufacturer of PC SPES is BotanicLab. The now inactive company website, http://www.botaniclab.com, revealed that Sophie Chen, PhD, is the founder. Therefore a search for "prostate cancer" and "herbs" as keywords and "Chen" as author was performed on the online US Patent and Trademark Office—Patent Full-Text and Full-Image Databases (http://patft.uspto.gov). The search yielded a 9 September 1997 patent entitled "Herbal composition for treating prostate carcinoma."

Table 1 Proportions (by weight of dried extract) of the components of PC SPES as reported in the patent

Plant	Proportion
Panax pseudoginseng[a]	1
Isatis indigotica	4
Ganoderma lucidum	6
Dendranthema morifolium	4
Glycyrrhiza uralensis	4
Scutellaria baicalensis	4
Rabdosia rubescens	6
Serenoa repens	6
Total	35

[a]On the bottle label, *Panax notoginseng* was listed instead of *P. pseudoginseng*. *Panax pseudoginseng* is a synonym of *P. notoginseng*. Since TCM practitioners whom we have consulted prefer to use the name *P. notoginseng*, in this paper the same name will be used

The herbal composition discussed in the patent contains the same eight herbs as in PC SPES [3]. The patent also reveals the proportions of the dried extracts of the eight herbs in the combination (Table 1).

Plants

All of the plants were purchased as packaged dried products. Specific parts of the plants as indicated in the patent and on the bottle label were acquired. *Dendranthema morifolium* (*Chrysanthemum morifolium*), *Glycyrrhiza uralensis* (*G. glabra*), *Ganoderma lucidum*, *P. notoginseng*, and *Scutellaria baicalensis* were purchased from a reputable Chinese medicinal herb shop in Sydney. *Isatis indigotica* and *R. rubescens* were purchased from Guangzhou by staff of the Guangzhou TCM University. *Serenoa repens* (*S. serullata*) berries were provided as a gift from Woods and Woods Pty. (Sydney, Australia).

All TCM herbs were identified by Associate Professor W. Li of the Pharmacognosy Section of Guangzhou University of Traditional Chinese Medicine in Guang Zhou, China. Voucher specimens were deposited at the Herbal Medicines Research and Education Centre of the Faculty of Pharmacy, University of Sydney.

One bottle containing 60 capsules (320 mg each) of PC SPES was purchased from BotanicLab (Brea, Calif.) through the manufacturer's website, www.botaniclab.com. The lot number was 5431219 and the expiration date was 08/2003. The bottle was stored in a cool and dry area.

Extraction of herbs and PC SPES

Each of the dried plants (100 g) and the powdered contents of 12 PC SPES capsules (3.8 g) were immersed in 95% ethanol at room temperature overnight. After the solution was decanted off, the extraction was repeated on the residual solid three times. The decanted ethanol extracts were filtered and concentrated on a rotary evaporator. The concentrated extracts were dried at room temperature under a stream of nitrogen. When not in use, the extracts were kept in a dark cool place.

Stock solutions of individual herbal extracts

The plant extracts were dissolved in a solution containing 6% dimethyl sulfoxide (DMSO) and 94% double-distilled water, yielding various concentrations of "drug" solutions. All of the drug solutions were stored at 4°C.

Stock solutions of combinations of herbal extracts

Taking "P" to represent the drug solution of *P. notoginseng*, and "M" to represent a mixture of the remaining seven herbs in the same proportions by weight as indicated in the patent [*D. morifolium* (4), *I. indigotica* (4), *Glycyrrhiza uralensis* (4), *Ganoderma lucidum* (6), *R. rubescens* (6), *Scutellaria baicalensis* (4), and *Serenoa repens* (6)], the following nine fixed-ratio P:M solutions were made: 0:1, 1:50, 1:34, 1:15, 1:1, 15:1, 34:1, 50:1, and 1:0. The solution 1P:34M is equivalent to the patented PC SPES formula.

MTT assay

In this colorimetric assay, 3-[4,5-dimethythiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT) is added to the growth medium. When MTT is oxidized by the mitochondria of living cells, the reaction releases a colored substrate that can be detected by spectrophotometry. MTT was dissolved in serum-free RPMI 1640 to yield a concentration of 1 mg/ml. The solution was filtered with a 0.45 μm Millipore filter to remove undissolved blue formazan crystals.

After verifying cell viability by the trypan blue dye exclusion assay, the cells were resuspended in RPMI 1640 containing 10% FCS at concentrations of $6–10\times10^4$ cells/ml. The cell suspension was distributed into the wells of a 96- well flat-bottomed microtiter plate, 90 μl to each well, yielding approximately $6–10\times10^3$ cells/well. Two to four wells were left empty. The plate was incubated for 24 h at 37°C in an atmosphere containing 95% air and 5% CO_2, allowing the cells to attach to the bottom of the wells. The next day 10 μl of the drug solution or vehicle alone was added into the wells, and the cells were incubated for 72 h. During each experiment, three or four wells were used for each drug concentration.

The medium in the wells was decanted from the actively growing cells, and 50 μl of 1 mg/ml MTT was added to all wells (final concentration 50 μg/ml) followed by incubation for at least 4 h at 37°C. The MTT solution was carefully decanted to remove untransformed MTT. DMSO (150 μl) was added to all wells to dissolve the formazan crystals. The plate was shaken gently for a few minutes until a purple color appeared. The absorbance was determined by an ELISA (enzyme-linked immunoabsorbent assay) plate reader at 540 nm. The absorbance of the initially empty wells was used to provide a background absorbance value (A_0). Thus the calculation reflected the absorbance of only converted MTT.

Determination of ID_{50}

Cell growth 72 h after exposure to drugs was measured by the MTT assay. At least three different points (drug concentration = X, cell growth = Y) were plotted using Microsoft Excel to derive a trend curve and equation. From the equation, the point at which the drug concentration inhibited 50% of cell growth (ID_{50}) was determined.

Isobologram analysis

Herb component interactions were analyzed with the use of an isobologram as described by Deckers et al. [7]. The ID_{50} values of P (*P. notoginseng*), M (reproduced formula of PC SPES without *P. notoginseng*), and several P:M fixed dose ratios were calculated by the MTT growth inhibition assay. The ID_{50} of M was plotted on the X-axis, and the ID_{50} of P was plotted on the Y-axis. The line connecting these two points is defined as the line of additivity.

Before the ID_{50} values of each drug combination could be plotted, the individual M and P components were derived. For example, if the ID_{50} value for the 1P:15M combination was 100 μg/ml, then the P component would be:

$$100 \times 1/(1 + 15) = 6.25\ \mu g/mL$$

and the M component would be:

$100 \times 15/(1 + 15) = 93.75$ μg/mL

For each point, the M component corresponded to a value on the X-axis, and the P component corresponded to a value on the Y-axis. The ID_{50} of each of the following P:M fixed ratios was plotted on the same plane with the line of additivity. Points on the line, above the line, and below the line were considered to represent additivity, antagonism, and synergism, respectively.

Herb interaction values analysis

The interactions of two different drugs can be defined by the following conditions:

- Additivity: $d_M/D_M + d_P/D_P = 1$
- Antagonism: $d_M/D_M + d_P/D_P > 1$
- Synergism: $d_M/D_M + d_P/D_P < 1$

where D_P is the ID_{50} of *P. notoginseng* by itself, d_P is the ID_{50} of the *P. notoginseng* component in the fixed ratio combination, D_M is the ID_{50} of M by itself, and d_M is the ID_{50} of the M component in the fixed ratio combination. The values of $d_M/D_M + d_P/D_P$ are referred to as the "interaction value" in this paper.

Results

Growth inhibition by individual herbs

All eight herbs individually and the PC SPES extract were assayed for growth inhibition of PC 3 cells. The extract of *P. notoginseng* was the most potent, while that of *Serenoa repens* was the least potent among the extracts. PC SPES extract was of average potency (Table 2).

An isobologram depicts the interactions of only two drugs. Since the herbal combination under study contained eight different herbs, the most potent growth-inhibiting herb was selected based upon the above data. Therefore, the interaction of *P. notoginseng* with a mixture of the other seven herbs was then analyzed.

Table 2 Growth inhibition of PC 3 cells. Cell growth (%) is expressed as the mean from four individual wells

Extract	Cell growth (%) at 72 h		Estimated ID_{50} (μg/ml)[a]
	1000 μg/ml	100 μg/ml	
Panax notoginseng	5.4	48.9	385
Rabdosia rubescens	6.2	51.8	402
Scutellaria baicalensis	24.9	45.8	493
Isatis indigotica	22.7	54.4	515
Ganoderma lucidum	8.1	86.0	530
PC SPES	11.1	90.1	559
Dendranthema morifolium	6.8	121.0	610
Glycyrrhiza uralensis	6.6	128.0	624
Serenoa repens	44.8	69.0	850

[a]The estimated ID_{50} values are overestimates of the actual ID_{50}values. The ID_{50}values were derived from the best-fit curve. Using 1000 μg/ml as a data point moves the curve to the right and artificially inflates the estimated ID_{50}. The estimated ID_{50} values in these tables are used strictly to rank the potency of the eight herbs and should not be considered the actual ID_{50}

Growth inhibition by fixed ratio combinations

For each of the fixed dose combinations, growth inhibition was evaluated at three different concentrations. From the three different concentrations the ID_{50} was derived. The experiments were run three times, so that a mean and standard deviation could be obtained. P (*P. notoginseng* extract) was the most potent agent with an ID_{50} of 121.4 μg/ml. It also had the smallest standard deviation of 2.4 μg/ml. The combination 1P:15M (ID_{50} 290.7 ± 68.4 μg/ml) was the least potent agent and had a relatively large standard deviation (Table 3). For each drug combination, the individual M and P components had to be determined to plot the data points for the isobologram. The individual M and P components were derived from the ID_{50} of each combination (Table 4).

Isobologram analysis

Plotting the M and P components gave the isobologram shown in Fig. 2. All the data points lay above the line of additivity, indicating antagonism. However, of the nine points, only the points representing the combinations 34P:1M, 15P:1M, and 1P:1M lay one standard deviation outside the area of additivity. None of these points lay more than 1.96 standard deviations outside the area of additivity. Therefore, the antagonism found in these drug combinations cannot be considered statistically significant.

Table 3 Effect of herbal extracts on growth of PC 3 cells. The data are the average (±SD) of three separate experiments

Drug combination	PC 3 cell growth (%) at 72 h			ID_{50} (μg/ml)
	200 μg/ml	100 μg/ml	50 μg/ml	
M	54.1 ± 8.9	75.5 ± 10.3	87 ± 2.7	221.5 ± 45.8
1P:50M	61.5 ± 4.2	84.2 ± 15.9	93.2 ± 12.4	264.8 ± 30.7
1P:34M	55.7 ± 9.4	82.6 ± 2.7	89.5 ± 11.1	236.8 ± 45.2
1P:15M	65 ± 9.8	79.3 ± 9.1	110 ± 18.2	290.7 ± 68.4
1P:1M	57.8 ± 11.4	74.4 ± 15.3	73.4 ± 12.3	243.2 ± 71.3
15P:1M	45.0 ± 6.7	58.1 ± 8.2	67.3 ± 7.0	159.8 ± 23.6
34P:1M	46.7 ± 8.3	58.3 ± 2.1	75.4 ± 13.0	167.8 ± 26.6
50P:1M	36.4 ± 6.6	54.2 ± 6.0	62.8 ± 5.9	134.9 ± 20.5
P	33.0 ± 2.8	54.1 ± 2.7	55.8 ± 8.3	121.4 ± 2.4

Table 4 Components of M and P in each drug combination at the concentration achieving ID_{50}. The data presented are means ± SD

Drug combination	ID_{50} (μg/ml)	M (μg/ml)	P (μg/ml)
M	221.5 ± 45.8	221.5 ± 45.8	0
1P:50M	264.8 ± 30.7	259.6 ± 30.0	5.2 ± 0.6
1P:34M	236.8 ± 45.2	230.0 ± 43.9	6.8 ± 1.3
1P:15M	290.7 ± 68.4	272.5 ± 64.1	18.2 ± 4.3
1P:1M	243.2 ± 71.3	121.6 ± 35.7	121.6 ± 35.7
15P:1M	159.8 ± 23.6	10.0 ± 1.5	149.8 ± 22.1
34P:1M	167.8 ± 26.6	4.8 ± 0.8	163.0 ± 25.8
50P:1M	134.9 ± 20.5	2.6 ± 0.4	132.3 ± 20.1
P	121.4 ± 2.4	0	121.4 ± 2.4

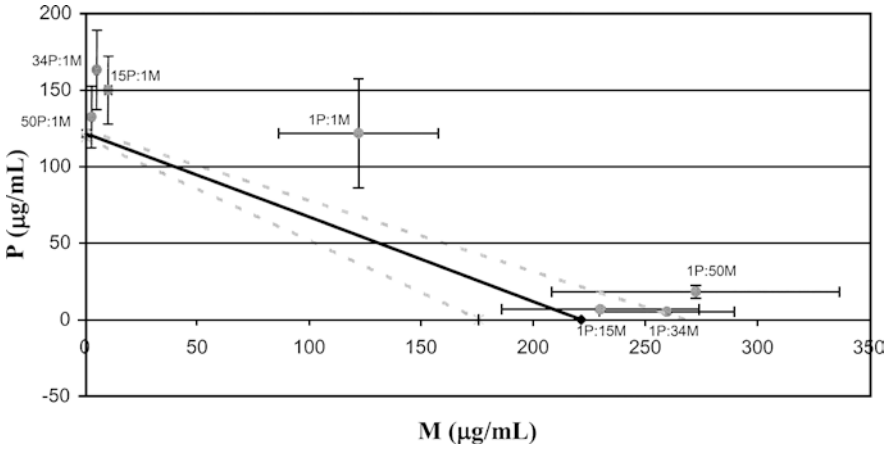

Fig. 2 Isobologram depicting interaction of the fixed-ratio drug combinations. The *solid black line* is the line of additivity. The two *dashed lines* represents one standard deviation above and below the line of additivity. The data points represent the seven drug combinations. *Error bars* represent one standard deviation

Table 5 Interaction values for each of the drug combinations. The *P* values indicate the significance of the deviation of the interaction values from 1. The data presented are means ± SD

Drug combination	d_M	d_M/D_M	d_P	d_P/D_P	Interaction value	*P* value
1P:50M	259.6 ± 30.0	1.17 ± 0.28	5.2 ± 0.6	0.04 ± 0.01	1.21 ± 0.28	0.45
1P:34M	230.0 ± 43.9	1.04 ± 0.29	6.8 ± 1.3	0.06 ± 0.01	1.09 ± 0.29	0.76
1P:15M	272.5 ± 64.1	1.23 ± 0.39	18.2 ± 4.3	0.15 ± 0.04	1.38 ± 0.39	0.33
1P:1M	121.6 ± 35.7	0.55 ± 0.20	121.6 ± 35.7	1.00 ± 0.29	1.55 ± 0.35	0.12
15P:1M	10.0 ± 1.5	0.05 ± 0.01	149.8 ± 22.1	1.23 ± 0.18	1.28 ± 0.18	0.12
34P:1M	4.8 ± 0.8	0.02 ± 0.01	163.0 ± 25.8	1.34 ± 0.21	1.36 ± 0.21	0.09
50P:1M	2.6 ± 0.4	0.01 ± 0.003	132.3 ± 20.1	1.09 ± 0.17	1.10 ± 0.17	0.56

Interaction values analysis

Using the ID_{50} values of P and M (121.4 ± 2.4 and 221.5 ± 45.8 μg/ml, respectively; Table 4), the interaction values shown in Table 5 were derived. All of the values were greater than 1, suggesting antagonism. The levels of significance of the calculated interaction values deviating from 1 were assessed. The drug combination 34P:1M was found to be the most significantly antagonistic (1.36, $P = 0.09$), followed by 1P:1M (1.55 ± 0.35) and 15P:1M (1.55 ± 0.35). These are the same three drug combinations shown by the isobologram to deviate the most from the line of additivity.

The interaction values are shown graphically in Fig. 3. The interaction value of drug combination 1P:15M lay approximately one standard deviation above 1, while the values for drug combinations 1P:1M, 15P:1M, and 34P:1M lay more than one standard deviation above 1, but less than 1.96 standard deviations outside the value of 1. A graph of the best-fit curve shows a smooth curve with a peak at drug combination 1P:1M (1.55, $P = 0.12$). As the combination approached either extreme the interaction value decreased until it reached 1 at M and P. This graph shows that synergism could be attained in any P:M combination, since P and M are the theoretical limits.

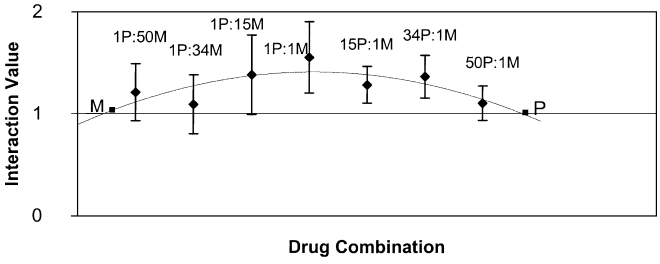

Fig. 3 The best-fit curve for the interaction values

Discussion

When this study was in its final stages, we became aware of the recall on PC SPES products. The California Department of Health Services Food and Drug Branch found that PC SPES contains warfarin [8]. A different group found that PC SPES previously contained diethylstilbestrol and indomethacin [24]. It is unclear whether BotanicLab intentionally added these prescription drugs or whether the contamination came from adulterated raw materials purchased from China. The patent reveals that the alcohol extracts of the seven Chinese herbs were purchased from the Shanghai Medical College of Traditional Chinese Medicine as a powder [3]. The adulteration of herbal products from China with drugs or heavy metals is not uncommon. Investigation is in progress, and consumers have been instructed to stop using all PC SPES products. Adulteration with drugs may have affected the results of all previous studies on PC SPES products. However, in this project we investigated the possible interactions between the eight components of a herbal combination in vitro, not the effectiveness of the PC SPES product in treating prostate cancer. Each of the eight herbs used in this work was purchased

individually, properly identified, and extracted in our laboratory.

Some manufacturers of herbal products claim indiscriminately the synergistic benefit from combination herbal polytherapy. On the other hand, critics of herbal medicine may consider herbs as pharmacologically inert agents and dismiss offhand possible interactions in herbal combinations. This project was an exploratory attempt to scientifically document pharmacological interactions within one herbal combination. In this preliminary in vitro study using one prostate cancer cell line, synergism was not found between *P. notoginseng* and the other seven herbs in the PC SPES combination. The results suggested antagonism, but they were not statistically significant.

Analysis of the isobologram and the interaction values revealed that all the drug combinations showed antagonism between *P. notoginseng* and the other seven herbs (*P* values: 0.09, 0.12, 0.12, 0.33, 0.45, 0.56, 0.76). These results, though not statistically significant, suggest the presence of a pharmacologically significant interaction between *P. notoginseng* and the other seven herbs. If herbal combinations are able to achieve antagonism, then they can theoretically achieve synergism if combined properly. However, combining herbs together in any proportion does not guarantee synergy. Some herbal combinations for which synergy is claimed but without scientific proof may be inactive, additive or antagonistic.

The results of this work are intriguing, but only limited conclusions about the PC SPES formula can be drawn. Apart from the limitation of using only one cancer cell line, this work tested only the interaction between *P. notoginseng* and the other seven herbs. Potential synergy among any of the other seven herbs would not have been detected. For a combination of eight different herbs there is an overwhelming number of permutations. Testing each permutation is not feasible. This is why the most cytotoxic herb, *P. notoginseng*, was chosen and the interaction with the other components studied.

In this work, interaction between two agents was measured strictly by growth inhibition of one prostate cancer cell line in vitro, and a trend to antagonism was detected. An in vitro experiment does not predict the outcome in vivo. In vitro studies will not detect drug interactions that increase bioavailability or decrease metabolic catabolism through the cytochrome P450 system. These factors can be studied through animal or human experiments. Future work in this project could be carried out with in vivo models, where tumor growth is measured for each of the drug combinations. Evaluating the ID_{50} for multiple different drug combinations in mice or humans would be labor- and time-intensive. It is conceivable that the antagonistic effects detected in vitro may lead to enhanced activity against an in vivo heterogeneous tumor cell population, in contrast to the homogeneity which characterize normal prostatic tissue or tumor cells growing in vitro after multiple passages.

Herbal polytherapy can have multiple actions, including immune stimulation, antiinflammation, reduction of pain, and improvement of digestive or urinary function. The eight herbs in PC SPES are reported to have many of these activities, and patients report nonspecific benefits, such as improved quality of life. By ''treating'' multiple symptoms related to cancer, instead of merely killing cancer cells, a herbal combination could ''synergistically'' improve quality of life. As an in vitro study, this project focused on potential synergy in the killing of cancer cells.

Since antagonism detected in this work was noteworthy but not statistically significant, future work could be carried out using a different cell line (e.g. DU 145, L1210, or LNCaP, among others). If antagonism between *P. notoginseng* and the other seven herbs was reproducible in a different cell line, there would be more evidence to suggest that antagonism does exist in this herbal combination. Additionally, this work could be repeated to detect interaction between a different herbal extract (e.g. *Scutellaria baicalensis*) and the remaining seven herbs.

Herbal medicine is deeply rooted in tradition, but it can also be scrutinized using modern scientific rigor. When performing experiments under controlled circumstances, one can find consistencies in the pharmacological properties of herbs. For example, *P. notoginseng*, *R. rubescens*, *Scutellaria baicalensis*, and *Glycyrrhiza uralensis* were found to be among the most cytotoxic herbs. This finding is in agreement with previously reported results. On the other hand, commercial PC SPES was consistently among the least potent growth inhibitory agents for three prostate cancer cell lines that we studied (unpublished observation). There is nothing inherent in herbal medicine that renders it inaccessible to scientific examination. If care is taken in identifying, preparing, and testing the herbs, their pharmacological activities are reproducible.

Acknowledgements We would like to thank Dr. Q. Dong for providing the PC 3 prostate cancer cell line and Assoc. Prof. W. Li for identification of the Chinese herbs. We also thank Dr. David Eisenberg for being V.Q.C.'s faculty sponsor at Harvard Medical School. This work was completed as V.Q.C.'s postgraduate research fellowship that was funded by the Fulbright Foundation.

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