

#4758 Botanical Activity Relationship in Traditional Chinese Medicine: Studies of PHY906 as an Adjuvant Therapy with Cancer Chemotherapeutic Agents



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Cytotoxicity of PHY906 & Its Derivative Formula on Different Cell Lines

Introduction

PHY906, a Chinese medicinal formulation consisting of four different botanicals, has been shown in mouse models to enhance the therapeutic index of several anticancer agents such as irinotecan (CPT-11), 5-fluorouracil (5-FU), irinotecan/5-fluorouracil/leucovorin (LV), etoposide and L-Oddc by both potentiating the antitumor effects of the therapeutic agents as well as reducing various host toxicities. To investigate whether there is a need of all four botanicals to achieve the full observed biological activity, five formulations were studied: the complete formulation with all four botanicals and each of four formulations missing one of the botanical ingredients (A, B, C, D). In the BDF-1 mice bearing Colon 38 tumor model, the potentiation of the antitumor effect of CPT-11 was impaired when either botanical A or botanical B was removed from the PHY906 formulation. In contrast, botanicals C and D were not found to contribute to the potentiation of the antitumor effect of CPT-11. However, when examining weight loss as an undesired toxic side effect of CPT-11, all of the botanicals except for B, contributed in protecting against body weight loss. Thus, the biological activity of PHY906 observed require all four botanicals. Studies of the effects of botanicals on cytochrome P450 isozymes (CYP1A2, 2C9, 2C19, 2D6 and 3A4) *in vitro* have also been conducted to examine potential action of botanicals on isolated enzyme targets. One of these enzymes, CYP3A4, is known to be the major metabolizing enzyme for CPT-11. While studies in the BDF-1 mice bearing Colon 38 tumor model indicate that PHY906 has no effect on the pharmacokinetics or pharmacodynamics of CPT-11, it was observed that PHY906 was found to act as inhibitor of individual cytochrome P450 isozymes with different potency. Individual P450 inhibition studies of both the single botanicals as well as the four separate formulations of three botanicals, indicate that there are interactions between specific botanicals that modulate the individual enzyme inhibitory effects. These studies indicate the complex, compensatory and multi-factorial nature of botanical drug actions in both *in vivo* and *in vitro* studies in keeping with the philosophy of original Traditional Chinese Medicine. Currently, PHY906 is in Phase I/IIa clinical trials as a modulator of CPT-11/5-FU/LV in colorectal cancer patients.

Inhibitory Activity of PHY906 & Its Derivative Formula on CYP450

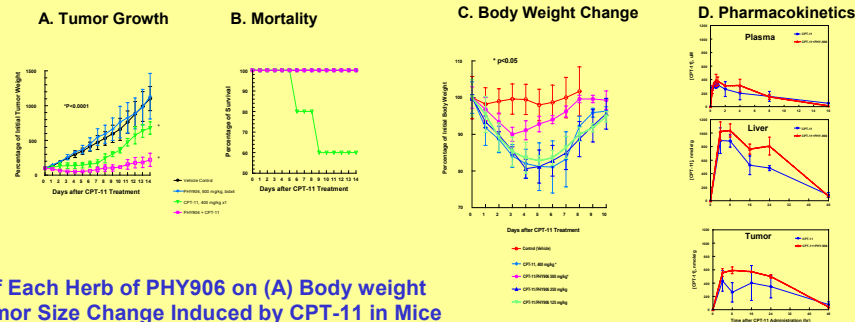
	IC ₅₀ (mg/ml) ¹				
	1A2	2C9	2C19	2D6	3A4
906-1	0.203±0.06	0.035±0.003	0.058±0.032	0.83±0.49	0.10±0.02
906-2 ⁽²⁾	0.025±0.01	0.013±0.009	0.023±0.015	0.13±0.03	0.04±0.01
906-3	0.017±0.002	0.010±0.008	0.012±0.005	0.08±0.01	0.03±0.01
906-4	0.019±0.011	0.027±0.011	0.015±0.009	0.09±0.02	0.04±0.02
906-5	0.022±0.006	0.008±0.002	0.017±0.011	0.11±0.04	0.03±0.02
906-6 ⁽²⁾	0.022±0.007	0.027±0.008	0.018±0.004	0.14±0.00	0.04±0.00
906-7 ⁽²⁾	0.021±0.010	0.025±0.001	0.019±0.000	0.12±0.02	0.04±0.01
906-8 ⁽²⁾	0.022±0.005	0.032±0.010	0.026±0.001	0.14±0.00	0.04±0.00
906-A ⁽²⁾	0.022±0.009	0.023±0.008	0.035±0.014	0.12±0.03	0.05±0.03
906-B ⁽²⁾	0.023±0.004	0.028±0.014	0.031±0.012	0.17±0.07	0.06±0.04
Herb A	0.008±0.001	0.009±0.004	0.009±0.005	0.04±0.01	0.03±0.00
Herb B	0.353±0.038	0.353	0.124±0.059	0.33±0.01	0.28±0.03
Herb C	0.033±0.001	0.005±0.002	0.010±0.006	0.13±0.05	0.01±0.00
Herb D	>4	>4	>4	>4	>4

(1) Herbs were extracted with water at 80°C for 30 min and concentration was based on the dry weight of herbs
(2) Contain all of four herbs

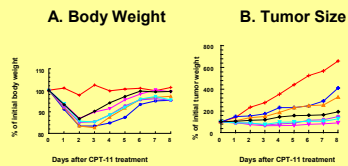
Formula	IC50 (mg/ml) ¹		
	HepG2	KB	Jurkat
PHY906-1	3.10±0.29	1.70±0.13	0.41±0.01
PHY906-2 ²	0.49±0.14	0.24±0.07	0.16±0.08
PHY906-3	0.37±0.11	0.19±0.07	0.046
PHY906-4	0.36±0.14	0.22±0.07	0.12±0.04
PHY906-5	0.58±0.12	0.24±0.02	0.12
PHY906-A ²	0.54±0.09	0.23±0.05	na
PHY906-B ²	0.57±0.16	0.23±0.09	na
Herb A	0.23±0.05	0.17±0.08	0.01
Herb B	0.96±0.42	0.30±0.09	0.29
Herb C	0.87±0.26	0.54±0.13	0.39±0.02
Herb D	>20	>20	>4

¹Herbs were extracted with water at 80°C for 30 min and concentration was based on the dry weight of herbs.
² contain all of four herbs

Effect of PHY906 on CPT-11 Treated Mice



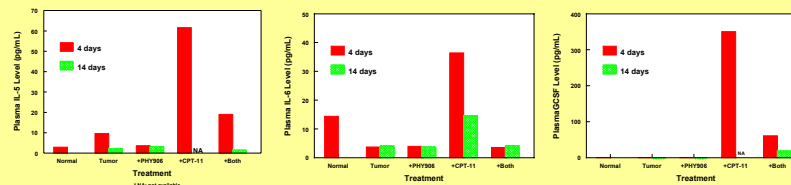
Role of Each Herb of PHY906 on (A) Body weight (B) Tumor Size Change Induced by CPT-11 in Mice



Plasma Cytokine Levels of BDF-1 Mice Bearing Colon 38 Tumor

PHY906 and its derivatives	Herb				Protection of Body Weight Loss	Enhancement of Antitumor Effect
	A	B	C	D		
PHY906-2	+	+	+	+	++	++
PHY906-1	-	+	+	+	-	-
PHY906-5	+	-	+	+	++	-
PHY906-4	+	+	-	+	-	+
PHY906-3	+	+	+	-	-	++

+ : effect, - : no effect



Summary

- PHY906 increases the therapeutic index of CPT-11 by
 - enhancing the antitumor activity;
 - decreasing the mortality and
 - protecting against the body weight loss.
- All four herbs are required in PHY906 formula for the full range of observed efficacy:
 - A** contributes to both body weight loss protection and antitumor potentiation (p<0.05) in the PHY906 formula.
 - B** contributed to the enhancement of antitumor activity of PHY906 (p<0.05), but not to the protection of cancer chemotherapy drug-induced body weight loss.
 - C** and **D** protected against the body weight loss induced by cancer chemotherapy agents.
- Different batches of PHY906 showed consistency in both human CYP450 inhibition and cell growth inhibition:
 - A** is a potent inhibitor for human CYP450 as well as for cell growth.
 - D** showed very low cytotoxicity in the cell lines examined and is a very weak inhibitor for human CYP450.
- PHY906 shows strong inhibitory activity against human CYP450 *in vitro*. However, animal data reveals that PHY906 does not affect the CPT-11 metabolism or impair the anticancer activity of chemotherapeutic agent.

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