



A Chinese Medicine Formulation, PHY906, Can Enhance The Therapeutic Index of CPT-11 and Other Anticancer Drugs Against Cancer in Mice

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ABSTRACT

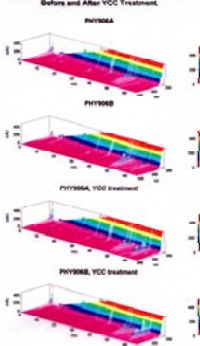
PHY906, a Chinese medicinal formulation, first described about 1500 years ago, is claimed to be useful for the treatment of diarrhea, subcardiac distention, abdominal spasms, fever, headache, vomiting, nausea and extreme thirst. Since some of these conditions are common side effects of chemotherapy, this raised the possibility of using PHY906 to alleviate these conditions, thereby improving the quality-of-life for cancer patients without compromising the anticancer effect. Tumor bearing mice treated with CPT-11 were used as a model to test this hypothesis. PHY906 was found useful to decrease the lethality and weight loss caused by CPT-11 in mice whereas antitumor activity of CPT-11 was enhanced. However, the pancytopenia caused by CPT-11 treatment was not altered. The schedule of drug administration has been shown to be very important. Pharmacokinetics studies indicated that PHY906 increased the AUCs of CPT-11 and its active metabolite, SN-38, in plasma, but not liver or tumor. This formulation was also found to enhance the therapeutic index of 5-FU, VP-16, L-OddC and 5-FU/LV. The impact of PHY906 on apoptosis, cell stress, cell survival and cell cycle progression was explored. Little variation in the quality of PHY906 batches have been ascertained by both chemical and biological analyses. Given the consistency of preparations, the claimed clinical usage for thousands of years and encouraging results of these laboratory studies, PHY906 will be further explored in the clinic to alleviate toxicity associated with chemotherapy in cancer patients.

Effect of PHY906 on Survival of CPT-11 Treated BDF-1 Mice Bearing Colon 38 Tumor

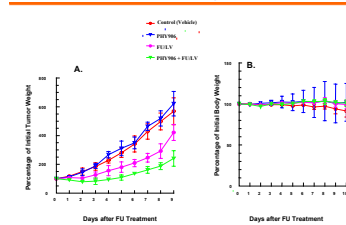
| Medication | Treatment * | | Total No. of Mice | | |
|----------------|--------------|--------------|-------------------|----------|------------|
| | CPT-11 | PHY906 | Treated | Survived | Survival % |
| Regimen | Dose (mg/kg) | Dose (mg/kg) | N | N | % |
| A | 0 | 0 | 35 | 35 | 100 |
| B [†] | 400 | 0 | 40 | 33 | 82.5 |
| C | 0 | 500 | 35 | 35 | 100 |
| D [‡] | 400 | 500 | 20 | 19 | 95 |

* CPT-11 was administered once on day 0, PHY906 was administered twice a day for 4 days.
[†] All the animals were observed for 14 days.
[‡] 7 of 40 mice with CPT-11 treatment died on day 5 (N=1), 6 (N=3), 7 (N=1) and 8 (N=2).
[§] With the combination treatment of CPT-11 and PHY906 treatment, one mouse died on day 6.

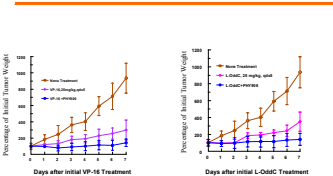
3-D HPLC Spectra of Different Batches of PHY906 Before and After YCC Treatment



Effect of PHY906 on (A) Tumor Growth and (B) Body Weight Change in FULV Treated BDF-1 Mice Bearing Colon 38 Tumor



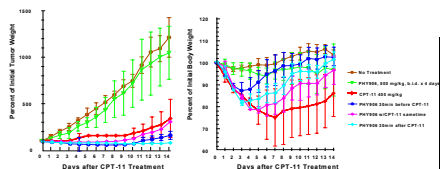
Effect of PHY906 on Tumor Growth in (A) VP-16 and (B) L-OddC Treated BDF-1 Mice Bearing Colon 38 Tumor



Indications for Traditional Use of PHY906 Formulation

- Diarrhea
- Subcardiac Distention
- Abdominal Spasms
- Fever
- Headache
- Vomiting
- Nausea
- Extreme Thirst

Administration Schedule of PHY906

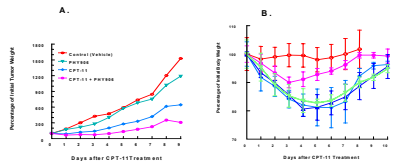


Effect of Different Herbal Formulations on CPT-11 Treated BDF-1 Mice Bearing Colon 38 Tumor

| Herbal Formulation (1 g/kg, bid, 5 days) | Protection from Body Weight Loss | P value ^{a,b} | Antitumor Effect | P value ^{a,c} | Animal Death ^d (Death/Total) |
|--|----------------------------------|------------------------|------------------|------------------------|---|
| None | | | | | 7/40 |
| PHY906 | Significant | 0.0004 | Enhancement | 0.0027 | 0/24 |
| PHY145T | No Change | 0.1072 | No Change | 0.2742 | 17/10 |
| PHY155T | No Change | 0.3259 | No Change | 0.6535 | 0/3 |
| PHY915 | Significant | 0.0306 | Decrease | 0.0885 | 0/5 |
| Loperamide | No Change | 0.9706 | No Change | 0.1595 | 31/10 |

^a based on the dry weight of formulations which contain excipient.
^b The p values were calculated using the Student's paired t-test.
^c All of the animals were observed for 14 days.
^d Calculated on the day that CPT-11-treated mice reached maximum body weight loss.
^e Calculated on the tumor size at day 6 after initial drug treatment.
^f One mouse died on day 6.
^g mice died on day 3 (N=1), 4 (N=1) and 5 (N=1).

Effect of PHY906 on (A) Tumor Growth and (B) Body Weight Change in CPT-11 Treated BDF-1 Mice Bearing Colon 38 Tumor

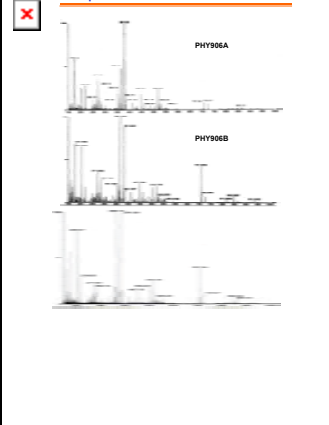


Effect of Different Batches of PHY906 on the Inhibition of β-Glucuronidase Activity and Cell Growth:

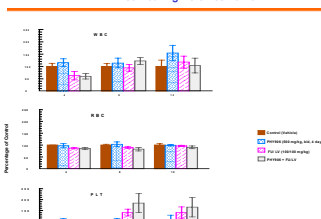
| Formulation Name | IC ₅₀ of β-Glucuronidase Inhibition (mg/ml) | IC ₅₀ of Cell Toxicity (mg/ml) | | |
|------------------|--|---|---------------------------|---------------------------|
| | | HepG2 | KB | Colon38 |
| PHY906-6 | 0.34 μg 0.01 [†] | 0.4 μg 0.20 [†] | 0.2 μg 0.04 [†] | 0.14 μg 0.07 [†] |
| PHY906-7 | 0.2 μg 0.01 [†] | 0.42 μg 0.07 [†] | 0.24 μg 0.04 [†] | 0.12 μg 0.07 [†] |
| PHY906-8 | 0.34 μg 0.04 [†] | 0.6 μg 0.24 [†] | 0.2 μg 0.04 [†] | 0.12 μg 0.04 [†] |
| PHY906-A | 0.34 μg 0.01 [†] | 0.4 μg 0.11 [†] | 0.24 μg 0.07 [†] | 0.14 μg 0.07 [†] |

[†] at diluted batch, contains 10% of excipient; concentration based on the dry weight of aqueous extract of raw herbs.
[‡] at research batch, contains 90% of excipient; concentration based on the dry weight of aqueous extract of raw herbs.

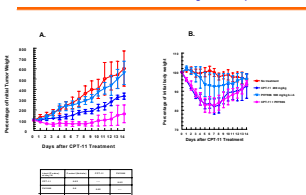
MS Spectra of Different Batches of PHY906



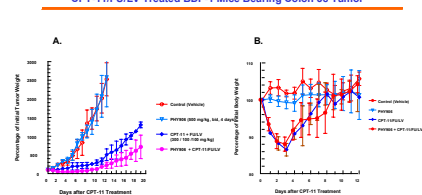
Effect of PHY906 on Hematological Change in FULV Treated BDF-1 Mice Bearing Colon 38 Tumor



Effect of PHY906 on (A) Tumor Growth and (B) Body Weight Change in CPT-11 Treated NCr-Nude Mice Bearing Human HepG2 Tumor



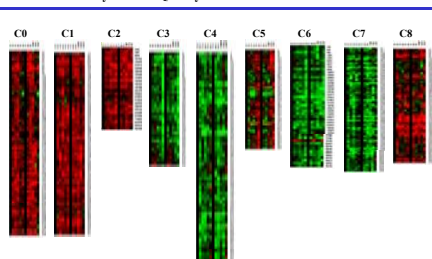
Effect of PHY906 on (A) Tumor Growth and (B) Body Weight Change in CPT-11/FULV Treated BDF-1 Mice Bearing Colon 38 Tumor



Summary

- CPT-11 treatment could cause side effects such as diarrhea, neutropenia, leukopenia, nausea and vomiting. Several herbal formulations with potential to alleviate these toxicities without compromising the CPT-11 anticancer activity were assessed. Among them, PHY906 is the best. Indeed, one herbal formulation, PHY915, was found to impair the antitumor activity of CPT-11.
- PHY906 increases the therapeutic index of CPT-11 in BDF-1 mice bearing Colon 38 tumor by enhancing the antitumor activity and alleviating the toxicities, including the prevention of body weight loss and reduction of lethality.
- PHY906 enhances the antitumor activity of FULV, CPT-11/FULV, VP-16 and L-OddC in BDF-1 mice bearing Colon 38 tumor.
- PHY906 can enhance the antitumor activity of CPT-11 not only in BDF-1 and B6C3F1 mice bearing Colon 38 tumor, but also in NCr-Nude mice bearing Human HepG2 tumor. There is no protection against body weight loss in NCr-Nude mice, suggesting that the protection comes from T cell activity which is missing in NCr-Nude mice.
- Quality assurance is the key issue in the development of herbal medicine. Using Phytomics analysis and YCC treatment, different batches of PHY906 display similar chemical fingerprints as well as biological fingerprints. This suggests that the consistency of PHY906 preparation will not be a major issue.
- With the consistent herbal quality and the potential enhancement of the therapeutic index for several classes of anticancer agents, PHY906 should be developed as a modulator for cancer chemotherapy.

DNA Microarray for The Quality Control of Different Batches of PHY906



* In the collaboration with Dr. Konan Peck's laboratory of Academia Sinica.