Investigation of a Traditional Chinese Medicine Herbal Therapy protocol for Treatment of Dogs with Stage II Splenic Hemangiosarcoma after Splenectomy

Multi-Institutional Clinical Study
Background information and Study Summary

Canine Splenic Hemangiosarcoma (HSA) is an aggressive disease with short survival times when treated with surgery alone. The addition of chemotherapy provides only modest improvement in survival, with recent studies suggesting chemotherapy treatments may not provide a statistically significant survival benefit. Novel therapies for this disease are needed. The purpose of this study is to evaluate the impact of a standardized herbal therapy protocol on survival of patients with stage II splenic hemangiosarcoma after splenectomy. We hypothesize that dogs with stage II splenic hemangiosarcoma treated with this standardized protocol after splenectomy will have improved survival times over historical controls treated with splenectomy alone and long-term survival rates comparable to or better than historical controls treated with splenectomy and chemotherapy.

Background:

Given the frequency with which splenic HSA occurs in canine patients, the almost uniformly fatal nature of the disease and the minimal overall survival benefit seen with the addition of chemotherapy, novel treatment options are needed for this aggressive disease. New options for management of this disease are desperately needed. In light of the evidence that treatment with chemotherapy agents is minimally effective, a more comprehensive focus on the tumor microenvironment seems logical and scientifically defensible. Conventional medicine, unfortunately, has few tools which effectively address the complex dynamics in the tumor microenvironment, in particular the inflammatory and angiogenic (formation of new blood supply) pathways which are important processes in the pathogenesis of this disease.[1]

Research has shown that inflammation and angiogenesis are important processes in the pathogenesis of HSA and that this modulation of the tumor microenvironment promotes tumor growth and survival in canine HSA. Of even greater interest is the fact that HSA patterns of inflammation, angiogenesis and cell signaling are unique compared to other cancer types, and reflect more than just a state of malignant transformation. These characteristics also illustrate how HSA cells communicate with their environment in a way that is different from non-malignant endothelial cells. Enrichment of cytokines such as interleukin-8 (IL-8) and marked up-regulation of angiogenic factors (factors that promote formation of new blood vessels to tumor cells) including VEGF, MMPs/TIMPs, PDGF/PDGFR were characteristics determined to be important in the pathogenesis of canine HSA.[2]

Additionally, a number of specific pathways have been elucidated for HSA. For example, vascular endothelial growth factor (VEGF) is a major player in both development and metastasis of HSA[3]. Angiogenesis mediated by VEGF has been proposed as a new target for anticancer therapy.[4,5] Other angiogenic growth factors that are over-expressed in HSA along with their associated receptors are basic fibroblast growth factor (bFGF) and angiopoietin-1 and angiopoietin-2 (Ang-1 and Ang-2).[6,7] This concomitant expression of growth factors and growth factor receptors results in autocrine stimulation of tumor growth. There are numerous other signaling pathways which have been evaluated for their role in HSA progression, including Interleukin-8 (IL-8) and MEK signaling pathway. IL-8 promotes HSA progression through effects on the tumor microenvironment. These findings were supported in both in vitro and in vivo studies and showed that IL-8 was important in providing a "permissive microenvironment" early in the course of HSA tumorgenesis.[8] MEK signaling inhibition prevents growth of HSA cells.[9] Any of the above pathways may be important in the role of adjuvant therapy for HSA. Herbal therapies, in particular, may offer a novel approach to modify these tumor pathways in a relatively non-toxic way for use as maintenance therapy after other conventional treatments such as surgery, with or without chemotherapy.

Research in herbal medicine has increased ten-fold over the past ten years, with over 600 studies now being published annually. Many herbal medicines have demonstrated mechanisms of action relevant to tumor progression

pathways known to be important in development, progression and metastasis of canine HSA. For example, the following herbs or herbal compounds have been shown to inhibit activity of IL-8 either by reducing IL-8 secretion, competitively binding IL-8 receptors or by selectively binding to IL-8 ligands: baicalin from Scutellaria (Huang Qin),[10,11] tanshinone from Salvia (Dan Shen),[12] berberine from Bupleurum (Chai Hu),[13,14] Ginger (Sheng Jiang),[15] glycyrretinic acid from Licorice (Gan Cao),[16] tetramethylpyrazine from Ligusticum (Chuan Xiong),[17] anthocyanins from Bilberry.[18] Additionally, Scutellaria (Huang Qin), Angelica Root (Dang Gui), Schisandra (Wu Wei Zi) and Licorice (Gan Cao) have been shown to exert anti-neoplastic activity through inhibition of MEK-ERK signaling mechanisms.[19-23] Scutellaria (Huang Qin), Bupleurum (Chai Hu), Licorice (Gan Cao), Astragalus (Huang Qi), Salvia (Dan Shen), and Rhubarb (Da Huang) have been shown to contain compounds with anti-neoplastic action related to abrogation of FAK signaling.[24-32] FAK signaling has been suggested as an important factor contributing to HSA persistence and progression.[33]

Yunnan Pai Yao is an important formula in the management of HSA and allows for improvement in survival time in many cases through its ability to minimize the severity of hemorrhage from HSA tumors. This formula has been recently reported to exhibit *in vitro* antineoplastic action in canine HSA cell lines in concentrations likely to be obtainable with oral administration.[34] Panax notoginseng (San Qi), the main herb in this formula, contains ginsenosides which have shown antitumor activity including inhibition of tumor angiogenesis via VEGF inhibition, blocking tube-like network formation and decreasing MMP-2 and MMP-9 which are overexpressed in HSA and involved in metastasis.[35-37]

Mushroom Polysaccharides

Mushroom polysaccharides have been used for their immune stimulating activity for centuries. Medicinal mushrooms exert their antineoplastic effects by activating immune responses. Hot water extracts of mushroom polysaccharides have been shown to act through T-cell, natural killer (NK) cell, B-cell and macrophage-dependent immune responses.[38] Of relevance to aggressively metastatic neoplasms like HSA are studies that show the potential to prevent metastasis. A blend of mushroom polysaccharides was shown to inhibit lung metastasis through immune regulation involving cytokine signaling and immune response in the tumor microenvironment.[39]

A clinical trial evaluating a single polysaccharide extracted from the Coreolus versicolor mushroom, also known as Turkey Tail Mushroom and Yun Zhi, showed improved survival time for dogs diagnosed with splenic HSA treated with splenectomy alone. This study evaluated 15 patients diagnosed with splenic HSA divided into three groups which differed in the dosage of Coreolus PSP (standardized to 46% PSP polysaccharides) administered: 25mg/kg/day, 50mg/kg/day, 100mg/kg/day. Each group contained three dogs with metastasis at the time of diagnosis and two dogs without evidence of metastasis. All dogs were treated with splenectomy and did not receive chemotherapy. Location of metastasis and whether metastatic disease was surgically resected was not stated. Median survival times for the low, intermediate and high dose groups were 2.8 months, 3.9 months and 6.6 months respectively. The difference between groups was not statistically significant. Although this was a very small study, the survival times reported here, exceed the published median survival times of 1-2 months for patients with stage II/III disease treated with surgery alone. And, in the high dose group, compare favorably to the 2-6 month median survival times reported for surgery and chemotherapy.[40]

Coreolus PSP has been shown to activate peritoneal macrophages both *in vivo* and *in vitro* via the Toll-Like R 4 (TLR4) signaling pathway.[41] Coreolus PSP also increases the number of monocytes in circulation without affecting NK, T-cell and B-cell numbers.[42]

While studies like the above tend to attract attention to specific polysaccharides, it is important not to lose sight of the complexities of whole plant herbal medicine and the potential benefits of other compounds within the medicinal plant. While Coreolus PSP has gained attention because of the above studies and numerous others reporting immune modulating activity, other studies exist showing potential anticancer activity of other compounds within the Coreolus versicolor mushroom. For example, PSK isolated from Coreolus shows anti-neoplastic activity both *in vitro* and *in vivo* which is unrelated to immune modulation but involves cell cycle arrest and induction of apoptosis.[43] Thus, there may be benefit to administering whole mushroom medicinals in order to provide a broader spectrum of anti-neoplastic action.

A Meta-analysis of the literature published in 2012 reported strong evidence that Coreolus (Yun Zhi) resulted in survival benefits for cancer patients, specifically those with carcinomas. The study highlighted the need for

prospective studies to guide treatment protocols.[44] A careful search through the available literature will produce a vast body of research outlining the role medicinal mushrooms play in supporting the cancer patient and, with an absence of significant side effects, there is little down side to this treatment in the management of cancer.

Very few other laboratory or clinical studies have examined the impact of herbal medicines on canine hemangiosarcoma, but given the herb actions identified above there is a scientific basis for investigating the clinical benefit of these plants in management of canine HSA. A retrospective evaluation of 14 dogs with stage II splenic hemangiosarcoma treated with TCM herbal therapies after splenectomy, resulted in a median survival time of over 253 days (4 patients still alive at 292, 302, 533 and 2255 days past splenectomy) with a 36% 1-year survival rate and 14% 2-year survival rate (as of 7/29/17 with four patients still ongoing) (unpublished data, Erin Bannink and Steve Marsden). One main limitation to the evaluation of the efficacy of TCM herbal treatments is that prescribing according to patient-specific TCM prescribing principles results in a non-homogenous treatment regimen. Not only does this make evaluation of the impact of specific formulas more difficult, it also prevents the use of this treatment by practitioners who are not trained in TCM herbal medicine. The goal of this study is to prospectively evaluate the impact of a standardized Bupleurum-based herbal therapy protocol, administered with vitamin D supplementation, Yunnan Pai Yao and Coreolus PSP, on survival of canine patients diagnosed with stage II splenic HSA following splenectomy.

Vitamin D

A number of mechanisms have been outlined involving vitamin D, vitamin D receptor (VDR) expression and cancer progression. And the protective effect of vitamin D against development and progression has been documented in people with a variety of cancer types.[45]

In a 2016 veterinary study, low serum vitamin D_3 levels were shown to be associated with an increased risk of developing cancer in canine patients. The optimal serum vitamin D_3 level was determined to be 100 -120 ng/mL based on iPTH and c-CRP variations plateauing at this level.[46] In the author's practice, serum vitamin D3 levels are routinely monitored and supplementation with oral vitamin D_3 initiated with a target range of 100-120 ng/mL, although higher serum concentrations have been maintained in individual patients with no accompanying hypercalcemia to date.

Risk of vitamin D toxicity is often cited as a concern in oral vitamin D_3 supplementation, especially because optimal dosing and clear information about serum levels at which vitamin D toxicosis occurs in dogs is lacking. Although the precise threshold of $25(OH)D_3$ associated with hypercalcemia in animals has not been established, based on a number of animal studies the plasma vitamin D_3 concentrations associated with toxicity have all been in excess of 930 ng/mL. Current human data support the viewpoint that, for people, plasma 25(OH)D concentration must rise above 750 nmol/L (well over 1500 ng/mL) to produce vitamin D toxicity although an upper limit of 250 nmol/L (624 ng/mL) has been suggested as a more prudent limit to ensure a wide margin of safety.[47] In another review article published in 2008, it was stated that efficacy of vitamin D_3 for many health goals in humans requires serum vitamin D_3 levels above 80 nmol/L (which equals 199.68 ng/mL) or higher. In this study, toxicity was reported to occur (in humans) at serum levels of 500 nmol/L (1248 ng/mL) or higher. To achieve these levels, supplementation of 1000-2000 IU daily is typical and toxicity reported to be associated only with extremely excessive oral intakes, typically above 20,000 IU/day. [48]

Based on this information and the fact that documentation of vitamin D toxicity associated with oral supplementation of vitamin D_3 has not been extensively evaluated, it may be that concern for iatrogenic hypervitaminosis D due to oral vitamin D_3 supplementation in dogs is overemphasized. Until more clear dosing regimens are established periodic monitoring of serum vitamin D_3 and calcium levels is prudent in patients receiving oral vitamin D_3 supplementation in the upper limit of the "normal" range.

Summary:

Herbal therapies may assist in management of aggressive cancers like canine splenic HSA through their anti-inflammatory, anti-angiogenic (anti-blood vessel formation) and immune modulating activity. The goal of this clinical trial is to evaluate whether a standardized herbal medicine and vitamin D3 supplementation protocol is effective at improving survival time in canine patients treated by splenectomy for stage II HSA.

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