Assessment of the antitumor activity of leech (Hirudinaria manillensis) saliva extract (LSE) in prostate cancer


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ABSTRACT

Background. Ancient traditional physicians from many countries used leeching to treat a wide range of diseases for thousands of years. A large number of peptides and proteins have been identified and characterized in leech saliva extract (LSE), an antithrombotic agent. Cancer metastasis inhibitors and anti-microbial agents. Currently, leech therapy is established as an important tool in microsurgery and reconstructive operations having demonstrated superior clinical outcomes for the optimal salvage of grafted tissues.

Methods. In the current study, we have determined the in vivo efficacy of LSE from (Hirudinaria manillensis) on castration resistant LNCaP and 22RV-1 xenograft mouse models. LNCaP study. Mice were divided into four groups of six, mice were subcutaneously injected (S.C) with either LSE (5 mg/kg), docetaxel (10 mg/kg), vehicle once or twice MDV (10 mg/kg) by oral gavage daily. PSA and tumor volume were measured weekly. 22RV-1 study. Mice were divided into four groups of eight, each group was assigned a different treatment, either LSE (5 mg/kg) once weekly, LSE (5 mg/kg) twice weekly, docetaxel (10 mg/kg) once weekly or vehicle twice weekly S.C. After four weeks of treatment, mice were euthanized, tumors and organs were collected for transcriptome and immunohistochemical (IHC) analysis.

Results. There was a significant decrease in the tumor volume and PSA in the LNCaP model compared to control, there was no significant difference in the anti-tumor activity between the three treatment groups (LSE, MDV and docetaxel). IHC showed significant increase in active caspase-3 and significant decrease in Ki-67 and P21 expression in the LSE treated mice compared to the control group. Similarly, in the 22RV-1 study, LSE caused a significant decrease in tumor growth at once and twice weekly dosing with no significant difference from docetaxel. IHC showed significant increase in active caspase-3 and in the group treated with LSE once weekly and a significant increase in Granzyme B with twice daily dosing compared to control. Interestingly, Transcriptome analysis by gene microarray showed that LSE had significant anti-inflammatory properties, along with significant effects on cell-cell adhesion, induction of glutathione transferase and inhibition of growth factors e.g. FGF and TGF-1. Conclusion. LSE has significant anti-tumor activity in two prostate cancer xenograft models with no apparent side effect.

INTRODUCTION

Hematophagous animals including leeches have been known to possess biologically active compounds in their secretions, especially in their saliva. The blood-sucking annelids, leeches have been used for therapeutic purposes since the beginning of civilization. The concept of the medical application of leeches can be traced back to the beginning of civilization. Ancient Egyptians, Indians, and Greek physicians used leeching as a treatment for a variety of diseases. In the last decades, comprehensive studies have investigated the therapeutic applications of leech products especially leech saliva. A wide variety of peptides and proteins that are antibacterial, antithrombotic and proteolytic, as well as components which have anti-inflammatory and anesthetic properties have been identified. Fractionation of leech saliva from different species identified multiple components including a proteinoid fraction and purified enzyme desubtilase, endo-x-(y-Glu)-Lys-isopeptidase which all have the ability to inhibit thrombus formation. Multiple studies were able to determine and isolate various anticoagulants including hirudin, a specific thrombin inhibitor; hemerin, a plasminogen activator, and hirumin, a fibrinogen- and fibrin-degrading enzyme. Inhibitors of various proteases were also identified in leech saliva. Having these effect on clotting and platelet activation, leeching was established as an effective treatment in salvage of grafted tissues and amputated digits. Regarding its applications in tumor and metastasis inhibition, the salivary extract of leech Haemantaria ghilli was found to contain a group of protease inhibitors which hinders clotting and platelet aggregation by tumor tissues and collagen while inhibiting tumor cells localization. In addition to this, LSE anticancer effects were delineated as an inhibitor of small cell lung cancer growth in vitro as a single agent and in combination with carboplatin and irinotecan. Despite the efficacious properties of leech therapy, the safety, and complications of leeching are still controversial.

MATERIAL AND METHODS

Sixty-five 4-week old athymic nude mice (Harlan Sprague Dawley, Inc.) weighing 25-30 g were subcutaneously inoculated with 2x10⁵ LNCaP or 22RV-1 cells at the posterior dorsal site. Weight and tumor volume were calculated once weekly in LNCaP and twice weekly in 22RV-1 model. In 22RV-1 model, mice were castrated five days after inoculation of cells and treatment started when the tumor volume reached 100mm³, mice were then randomly assigned a treatment, either LSE (5 mg/kg) once or twice weekly, docetaxel or vehicle. In LNCaP model PSA was measured weekly, when PSA exceeded 25 ng/ml mice were castrated, as a result, PSA decreased under 25 ng/ml until the tumor became resistant to the effect of androgens. Treatment started after the second rise of PSA above 25 ng/ml to resemble castration resistant prostate cancer. After second rise in PSA, mice were divided into four groups of six each. Mice were subcutaneously injected with either LSE (5 mg/kg), docetaxel (10 mg/kg), vehicle, all given S.C. once weekly, or MDV (10 mg/kg) orally every day, all given for four weeks.

RESULTS

LSE (5 mg/kg) once weekly regimen caused significant decrease in tumor volume compared to control in both studies, twice weekly was equally effective in 22RV-1 model (Fig.1, Fig.3.). There was no significant difference between LSE antitumor activity compared to docetaxel or MDV. In LNCaP model LSE treated group demonstrated significant decrease in PSA level compared to the control at all time points. Immunohistochemical staining (IHC) showed significant decrease in Ki-67, and P21 expression in the LSE treated groups compared to control in LNCaP model while caspase-3 was increased significantly in LSE group compared to both control and docetaxel groups (Fig.2.). Similarly, caspase-3 was significantly increased with LSE once weekly in 22RV-1 model, while Granzyme B was increased with LSE does twice weekly (Fig.4.). Transcriptome analysis by gene microarray showed that LSE had significant anti-inflammatory properties, along with inhibiting of cell-cell adhesion and growth factors such as FGF and TGF-1. Conclusion. In conclusion, this is the first report to evaluate the efficacy of LSE in treatment of prostate cancer and it is most likely that it acts through pleiotropic mechanisms including antiplatelet, anticoagulant, anti-proteolytic, anti-inflammatory, inhibiting growth factor, and suppressing tumor invasion leading to a significant increase in apoptosis along with cell cycle arrest and inhibition of proliferation offering a very promising, safe and convenient treatment for prostate cancer.