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Research Idea

Saxifraga aizoides extract: novel potential effects on tumor cell models

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Abstract

Background

Since ancient time nature has been used as the main source of herbal medicines. Even today many drugs derive or are elaborated from environmental flora. Of note natural products are fundamental sources of new anticancer drugs. Cancer is the second leading cause of death in the world and the fight against it still open and need every possible resources, including herbal product, which should be scientifically and deeply studied.

New information

Saxifraga aizoides extract (SAE) has been never tested in preclinical tumor cell models.

Keywords

Saxifraga aizoides, saxifragaceae, cancer cells, ethnopharmacology, phytotherapy

Overview and background

The use of herbal natural products is still the main source of medicines in many developing nations and is becoming increasingly popular among Western countries, especially over the past decades. Given the extraordinary interest about phytomedicine and ethnopharmacology, many researchers all over the world try to deepen and to understand the exact mechanism by which an herbal extract will interact with human body, and particularly with human diseases. In fact many successful uses of plant medicines have been derived from traditional/geographical use of plant, whose effects have been scientifically proved in recent years. In this context Traditional Chinese Medicine is gaining popularity and therapeutic efficacy, as numerous researchers (especially from China) scientifically investigated its role in human diseases (Leung et al. 2016, Zhang et al. 2010). The simplest way to use a plant is eating it, but of course this can be very dangerous. Consequenlty human skill developed different methods to benefit from the therapeutic effects of a plant. The first method was probably a simple aqueous solution, unfortunately suitable only for hydrophilic substances. Only later in history mankind discovered the use of special solutions, which can that can extract both hydrophilic and hydrophobic substance. Indeed a crude extract, which typically uses alcohol as a solvent, can be considered the simplest way to obtain a potential therapeutic mixture (Kiefer and Barrett 1999).

Saxifraga is the largest genus in the family Saxifragaceae, containing about 440 species of holarctic perennial plants, known as saxifrages or rockfoils (Wikipedia 2016). Their name derives from latin word "saxifraga" that means "stone-breaker" which is traditionally thought to indicate a medicinal use for treatment of urinary calculi (kidney stones). Among the genus, *Saxifraga aizoides* is a flowering herb and alpine plant (Fig. 1), that lives in cold and moist well draining neutral or basic bedrock, sand, or cliff environments (Pignatti 1982). It is common in North America, Greenland, Europe (including the Alps, Svalbard and Tatra Mountains) (Pignatti 1982). The flowers (Fig. 2) have with five sepals and petals (yellow—green) and the plant grows to 2–10 centimetres spreading by short rhizomes and mats of small colonies (Wikipedia 2016b). Only few works analyzed the effects of *Saxifragaceae* extracts on cancer cell models. Some authors demonstrated anti-cancer effects of different components of *Saxifragaceae* extracts (Lu et al. 2013, Luo et al. 2015, Kim et al. 2014, Chen et al. 2008), however up to now no research has tested the efficacy of *Saxifraga aizoides* (Fig. 1) extract on any cancer cell lines.

Objectives

The aim of this project is to study the phytochemical profile of the ethanolic crude extract of *Saxifraga aizoides* (Fig. 1) in preclinical tumor cell models. Moreover the research will investigate the effects of the crude extract on cell viability and vitality and the eventual alterations of morphologic profile of cell lines. In addition the clonogenic assay will allow to study the influence of the crude extract on the capacity of forming cell colonies. All together these experiments will give a clear and general hypothesis on the efficacy of the extract on

human ovarian, colon, prostate and breast cancer cell models, in which the crude extract will be tested. Cell lines to be used: SK-OV-3 (ovarian cancer), Caco2 (colon cancer), PC-3 (prostate cancer), MCF-7 (breast cancer).

Figure 1.

Saxifraga aizoides is a flowering herb of the genus Saxifraga, which lives in Europe and North America.

Figure 2.

Saxifraga aizoides: the characteristic yellow flowers punctuated by orange spots on the petals.

Impact

This research project will try to discovery the novel effects of a *Saxifraga aizoides* crude extract (SAE). In particular it will explore the anti-proliferative role of SAE in 4 human cell

models (ovarian, colon, prostate and breast cancer) and also in animal models (mice and rats) to evaluate anti-inflammatory and toxicity of SAE.

Implementation

Future perspectives

The research project aims at understanding the potential application of *Saxifraga aizoides* extract (SAE) on cancer cell models. In order to achieve this result, a scientific approach should be followed:

Materials and methods

Plant material

Saxifraga aizoides plant was collected in summer season 2015 at about 2000 meters above the sea level in Passo del Tonale (TN), Italy. Plant was identified according to Pignatti 1982. A voucher specimen number was deposited in the herbarium of the OU Endocrinology, University of Padova, with number 011SA. Plant was air-dried and powdered. 30g of powder was added to a mixture with ethanol (70%) for 24 h, then filtered. The solvent was removed by vacuum evaporator. A crude extract of *Saxifraga aizoides* was obtained (SAE).

GC/MS analysis of Saxifraga aizoides extract

The chemical analysis will be performed by re-solubilizing the dry extract in ethanol followed by gas-chromatography/mass spectrometry (GC/MS) analysis Cavaggioni et al. 2006).

Evalutation of anti-proliferative activity

Four different cell lines will be used: HeLa (ovarian cancer), HCT-116 (Colon cancer), PC-3 (Prostate cancer), MCF-7 (breast cancer) cell lines. Cell lines will be obtained by ATCC or will be a gift of other research labs. Cells will be cultured in DMEM/RPMI medium supplemented with 10% FBS, 100 U/ml penicillin, 100 ug/ml streptomycin in a humidified atmosphere with 5% CO_2 at 37°C. Cells will be plated on 96-well tissue culture microtiter plates at a density of $1x10^4$ cells/well and treated with SAE, at different concentrations. MTT cell viability (Sigma-Aldrich) will be tested after 72 h of treatment, as described elsewhere. For each drug, we measured the Inhibitory Concentration 50 (IC_{50}, defined as 50% of the inhibitory effect on cell viability). All experiments will be performed in quadruplicate and repeated three times.

The cytotoxic effects of SAE will be confirmed by the Trypan blue dye exclusion method. The assay will be performed at 72 h after treatment, using the single IC_{50} dose determined by MTT assay. At the end of treatment, cells will be collected by trypsinization, centrifuged and the cell pellet will be resuspended in 1 ml of PBS. Next, 10 ul of the resulting cell suspension will be admixed with 10 ul of Trypan blue (0.4% in PBS). The numbers of non-

stained viable cells (NSt cells) and stained dead cells (St cells) will be counted using a hemocytometer. Cell viability will be then calculated by the following formula: Viability (%)= ¼ (NSt cells)/(St cells + NSt cells) x 100. Experiments will be performed in triplicated and repeated three times. The results will be interpreted as the ratio of viable cells after drug treatments to that of the untreated control.

Cell morphology assessment with Wright's staining

Cells will be cultured on coverslips for 48h, incubated overnight in 0.1% FBS, and then treated with SAE for 72h. After that, cells will be washed in PBS, fixed in methanol for 5 min and Wright's stained for 5 min. Cell morphology will be assessed by light microscopy at x400 magnification. The experiments will be repeated twice.

Statistical analysis

Statistical analysis will be performed using GraphPad Prism 5 software (GraphPad Software, Inc., San Diego, CA) and Microsoft Excel software. Cell line data will be analyzed using a two-tailed paired Student's t-test. A $p<0.05$ will be considered statistically significant. Data will be expressed as mean ± standard error of mean (sem).

Expected results

We expect to obtain a SAE that will be used to explore its effects on cell cancer models. We will have a GC/MS profile of SAE. We will test the 4 cancer cell lines for viability after treatment with SAE, thanks to MTT and trypan blue assays. Furthermore cells morphology changes will be assessed by light microscopy. Clonogenic assay will be provide a specific insight on cell survival after treatment with SAE.

Implementation

The study of the *Saxifraga aizoides* extract (SAE) will be implemented by the *in vivo* experiments on animal models (both mice and rats).

Acute toxicity studies

The project will analyze the acute toxicity of the plant extract, evaluating the lethal dose (LD50) in mice. The method is described in Lorke (1983). Animals (at least 8 mice) will be deprived of food over night before testing. SAE will be administred *per os* in increasing doses (10, 100, 1000 mg/kg). Treated animals will be observed for 8 h looking for signs of toxicity. After 24h, if no animal will die, another scaling doses of SAE will administered (1500, 3000, 5000 mg/kg). Again animals will be observed for signs of toxicity or mortality.

Anti-inflammatory studies

Anti-inflammatory study will be performed using wistar rats. The method is derived by Winter et al. (1963). Aspirin (250 mg/kg) will be a positive control in a group of animals (at least 6 rats). Another group of animals (at least 6 rats) will be administred with *Saxifraga aizoides* extract. The treatments will start 1 hour before carrageenan injection (the drug will

induce an inflammatory reaction in the site of injection). Carrageenan will be prepared as 1% w/v solution in 0.9% w/v NaCl and 0.1 ml will be injected near rat's paw. The paw size will be measured will digital calliper in the 2 different groups and in the control group (no extract and no aspirin), at 0, 2, 4, 6 h after carrageenan injection.

Ethics

The request to use animal models will be submitted to Local Ethics Committee only after successfully completion of *in vitro* studies. Procedures involving animals and their care will be in conformity with institutional guidelines that comply with national and international law and policies (D.L. 116/92 and subsequent implementing circulars), and experimental protocols will be approved by the Local Ethics Committee of Padua University (CEASA).

Funding program

To be found.

Hosting institution

University of Padova, Departament of Medicine DIMED, UO Endocrinology

Ethics and security

Approved by Prof. Marco Boscaro, UO Endocrinology, DIMED, University of Padova, Italy

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Author contributions

Raffaele Pezzani: principal investigator.

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