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Identifying Botanical Mechanisms of Action

May Fern Toh^a and Joanna E. Burdette^a

May Fern Toh: joannab@uic.edu

^aDepartment of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, 833 S. Wood Street, Chicago, IL 60612. U.S.A.

Abstract

The biological mechanism of action for any botanical extract is a necessary part of discovery to determine pharmacological use and safety. Interestingly, many activities that are governed by endogenous compounds are not fully understood making the characterization of mechanisms elusive. For example, phytoestrogens are being consumed for menopausal symptoms while the biological action of estradiol are still being investigated. Therefore, long term efficacy and safety issues are a challenge in the field. As new activities are associated with new biological pathways, an important component of therapeutic discovery will need to be the re-evaluation of negative or less active natural products to determine their relative use as medicines.

Keywords

botanicals; mechanism; activity

Introduction

One of the major challenges in medicinal use of natural products is understanding the mechanism of action for novel compounds. When trying to understand how a botanical extract functions in the human body, an investigator often predicts a particular biological activity based on ethnobotanical human consumption or based on a chemical structure present in the plant extract. The challenge comes in using either the known biological endpoint or the known chemical structure to identify the target of interest. Alternatively, libraries of extracts and compounds are tested against a series of enzymes or cells, and this approach can provide a challenge in later experiments when trying to determine if the compound is properly moved across the cell membrane, metabolized to something active or inactive, or toxic to alternative enzymes or cell types when introduced *in vivo*. Interestingly, many studies attempt to correlate a new compound with a known activity or a new activity with a known compound. A flaw in this logic is that it necessitates the investigator to replicate an existing activity or compound. When looking for new biological mechanisms of action, it is critical to consider that new targets and new compounds might be present in botanical extracts and these might ultimately provide a new molecule that is safer and more efficacious than an existing therapy.

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Correspondence to: Joanna E. Burdette.

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Is Red Clover Estrogenic?

One of the first logical targets for the treatment of menopausal hot flashes would be to stimulate the estrogen receptor. Supplementing estrogen to a menopausal woman significantly reduces hot flashes [1]. The endogenous hormone estradiol signals primarily by binding to its receptor to initiate gene transcription either by directly binding estrogen responsive elements in DNA or by interacting with proteins to modify DNA [2–4]. A secondary method of signaling is by activating membrane receptors and by the stimulation of growth factors that phosphorylate estrogen receptors on serine 116 [4,5]. Numerous studies have confirmed that the isoflavones in red clover interact with and activate estrogen receptors to initiate gene transcription [6–8]. However, the pleiotropic effects that estrogen receptors can induce in each specific tissue based on the coactivators and corepressors that are present are less well understood [9–12]. The evidence that red clover exerts behavior similar to an “estrogen” is murky given the fact that estrogenic compounds all seem to have different effects in the hormonally responsive tissues. However, this difference between estrogens is what allows isoflavones from red clover to be relatively safe for the uterus whereas estradiol can increase the risk for developing endometrial cancers[13]. A similar phenomenon can be noted in the mammary tissue where an isoflavone-rich red clover extract failed to proliferate mammary ducts of ovariectomized rats and did not increase mammary density in women [14–16]. Results consistently link estrogenic effects of red clover in the vagina epithelium, but this is not associated with being unsafe [7,17]. Red clover has not demonstrated clinical efficacy for reducing vasomotor symptoms and in this regard it is not as efficacious as estrogen [6,18–22]. However the very fact that red clover is not strong enough to reduce hot flashes might be the factor allowing it to be considered safe in the uterus and breast. Simply identifying something as interacting with the estrogen receptor is not sufficient to determine how it will function biologically to help relieve menopausal symptoms.

Is Red clover more than estrogenic?

While many of the traditional endpoints for estrogenic activity are negative when red clover is investigated in vivo such as uterine weight, mammary gland proliferation, or hot flashes, red clover has displayed some additional health benefits different from estrogen. For example, clinical trials on the use of estrogen to increase cognitive function in menopausal women are inconsistent while red clover clinical trials depict a distinct improvement in some clinical cognitive symptoms[23,24]. As secondary endpoints are measured in clinical trials, the usefulness of red clover in a menopausal woman might ultimately be for its bone sparing activity and lowering low-density lipoprotein levels in serum [25–27]. While estrogens are also known to increase bone density, they do it while also increasing the risk of mammary and uterine proliferation, which is not associated with red clover[28–30]. The safety of red clover has also been demonstrated in women with a history of breast cancer, although much more must be done to determine if these phytohormones might increase the risk for breast cancer metastasis [31]. Estrogens are actually associated in some clinical trials with increasing the risk for blood clot and heart disease in menopausal women indicating that red clover’s isoflavones act beneficially as compared to endogenous estrogen on the cardiovascular system [32–35]. The placebo effect for reducing hot flashes can be anywhere between 30–60%, and this alone might justify taking something like red clover, which does not seem to reduce these flashes but is safe and offers other health benefits [18,22].

How to define estrogenic?

Studying estrogenic activity implies that the molecule estrogen performs one specific function that can be monitored consistently in different tissues. Yet a true understanding of

all the biological mechanisms and effects of estrogen are not known. For example, the creation of new models of estrogen receptor signaling using transgenic animal models has helped to reveal that estrogen can have several different activities [11,36,37]. One animal model that might be particularly interesting for identifying how botanical extracts signal is the estrogen non-responsive estrogen receptor knock-in (ENERKI) mouse[38,39]. The ENERKI mouse has a mutant form of estrogen receptor that cannot bind endogenous estradiol but can bind more flexible ligands [38]. By introducing new ligands at different periods of development or during different stages of disease, the role of the (phyto)estrogenic molecule can be studied. Interestingly in this model, the proliferation of the uterus occurs by addition of IGF-1 instead of estrogen, providing proof that the AF1 portion of the receptor can drive uterine cell proliferation in the absence of estrogen, albeit to a lower degree [39]. The additional role of growth factors to modulate and amplify estrogen signaling in vivo could provide some rationale for the various effects of different ligands in tissues like the uterus. Specifically, genistein has not been reported to increase uterine weights. If a complex botanical mixture contained both an IGF-1 activator or growth factor mimetic and a plant based estrogen, this extract might increase uterine proliferation similar to estrogen alone and this might reduce the safety of the estrogen if taken alone[17,40]. A complementary relationship could also persist allowing the estrogen-like activity to provide critical bone-sparing abilities that are supported by clinical and pre-clinical models given red clover extracts.

How to chase biological activity?

An interesting aspect of studying red clover for its estrogenic properties is the identification of metabolism as a critical part of generating biological activity. The isoflavones in red clover are biochanin A, formononetin, daidzein, and genistein [6,41,42]. While genistein and daidzein are the most active molecules in vitro because of their high affinity for the estrogen receptor, biochanin A and formononetin can both be demethylated to form genistein and daidzein respectively and this activation step allows them to be more stable and active in vivo [43]. Without exposing red clover to metabolic enzymes, the activation or prodrug-like activity of the extract would not have predicted that the whole extract would be the same as it is after metabolism[44]. Equol is another example of a potent estrogen receptor ligand that is only formed after metabolism [44]. The high rate of glucuronidation might eliminate equol from the system before it can act, but recent evidence indicates that local glucuronidases are present in tumor tissue that could increase estrogen activity locally [45]. Therefore, one cannot determine if botanical extracts will function properly as an estrogen in the absence of understanding the metabolic processes that might encourage or discourage bioactivation such as both liver p450 enzyme mediated and gut flora mediated metabolism.

Conclusions

Finding new phytochemicals from botanicals simply to recapitulate existing biological activity might fail at identifying novel mechanisms of action and might fail to take advantage of unique biology to improve safety. Mechanisms need to be evaluated at the enzyme or receptor, cell, and whole animal levels because many aspects of drug activation or elimination can only be studied in a living organism. Finally, looking at single target assays might miss complementary relationships between compounds in plant extracts. The power of botanical medicine might reside in identifying mechanisms by evaluating beyond model compounds and existing structures. Specifically, in the case of identifying phytoestrogens, a broader understanding of how the estrogen receptor signals when bound to novel ligands in the presence of tissue specific coactivators and corepressors will be necessary to fully characterize the biological mechanisms.

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Figure 1.
Estrogen and red clover modulate different biological activities in the postmenopausal woman based on potency, metabolism, and tissue specific biology.