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Common phytochemicals are ecdysteroid agonists and antagonists: a possible evolutionary link between vertebrate and invertebrate steroid hormones

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Abstract

Many plant compounds are able to modulate growth and reproduction of herbivores by directly interacting with steroid hormone systems. In insects, several classes of phytochemicals, including the phytoestrogens, interfere with molting and reproduction. We investigated whether the anti-ecdysone activity may be due to interaction with the ecdysone receptor (EcR) using a reporter-gene assay and a cell differentiation assay of an ecdysone-responsive cell line, Cl.8 + . We tested rutin (delays molt in insects); four flavones: luteolin and quercetin (metabolites of rutin), and apigenin and chrysin; and three non-flavones, coumestrol and genistein (both estrogenic) and tomatine (alters molt in insects). None of the phytochemicals tested were ecdysone agonists in the reporter-gene assay, but the flavones were able to significantly inhibit EcR-dependent gene transcription. In the Cl.8 + cells, quercetin and coumestrol were mixed agonists/antagonists, while genistein, tomatine and apigenin showed a synergistic effect with ecdysteroid in the reduction of cell growth. We suggest that the rutin effects on molting in insects are most likely due to the metabolites, luteolin or quercetin, while tomatine acts via a non-EcR pathway. Flavones not only interact with EcR and estrogen receptor (ER), but also signal nitrogen-fixing bacteria to form root nodules. The NodD protein which regulates this symbiosis has two ligand-binding domains similar to human ERα. The evolutionary significance of these findings are discussed. © 2001 Published by Elsevier Science Ltd.

Keywords: Phytoestrogen; Ecdysone; Steroid hormone receptor; Cl.8 + cell line

1. Introduction

Recently much attention has been centered on the potential of phytochemicals to interact with vertebrate sex steroid hormone systems. Although the focus of these studies has been the estrogenic potential of these compounds, progestorenic, androgenic and corticosteroidal activity have also been reported [1]. There are several ways in which phytochemicals can interact with the estrogen system. First, they are able to modulate estrogen action by interfering with steroid hormone binding proteins in serum, which alters the rates of hormone delivery and metabolism/elimination. Several phytochemicals, in particular the flavones such as quercetin, inhibit estrogen binding to rat α-fetoprotein (AFP) [2]. This inhibition of binding is much stronger in flavones than in the isoflavones (e.g. genistein), suggesting that there is specificity of AFP for the flavone structure. Similarly in human serum, it has been shown that the isoflavones, coumestrol and genistein, do not bind, or only weakly bind, the human sex steroid binding globulin [3,4]. Another mechanism for phytochemicals to interfere with hormonal action is by interacting with various cytochrome P450 isozymes which metabolize steroids [5,6]. Most significantly, aromatase

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(CYP19), which converts testosterone to 17β-estradiol, is inhibited by many phytochemicals, including chrysin, quercetin, and apigenin. Phytochemicals can act directly as either estrogens or anti-estrogens by interacting with the estrogen receptor (ER) isoforms [4,7]. There is evidence that the steroid hormones themselves, in a variety of animals, act through non-genomic pathways in addition to the more traditionally accepted mode of action via receptor-mediated control of gene transcription [8]. A model has been proposed which not only suggests an additional pathway but also that the non-genomic pathway may exercise some control over the genomic component of the hormonal effect [9]. Finally, many phytochemicals have effects on signal transduction pathways which may be independent of hormone mimicking activity but have synergistic effects with true hormone action [10,11].

It has been hypothesized that plants produce these flavones and isoflavones to deter mammalian herbivores [12]. At least one phytochemical, coumestrol, is capable of histopathological changes in the ewe uterus [13]. Clover disease in sheep has been well studied, and the infertility problems have been linked to high levels of phytoestrogens in the subterranean clover feed [12].

Evolutionarily, insects arose prior to vertebrates, and the first herbivores were arthropods. Insects are capable of the destruction of vast areas of crops and other vegetation, and over time plants have produced a wide array of feeding-deterrent compounds against insects, including high levels of ecdysteroids which cause abnormal molting or decreased fertility and fecundity [14,15]. In arthropods, molting hormone (ecdysone) is the steroid hormone which controls not only molting, but also development. Ecdysone is metabolized by specific cytochrome P450 isozymes to the active form, 20-hydroxy ecdysone (20 HE). 20 HE is then transported through the hemolymph to the target cells, where it binds to the ecdysone receptor (EcR) to cause gene transcription. To date, three EcR isoforms have been identified during various developmental stages in insects [16–19]. In addition to EcR, orphan receptors, MHR3, E75A and DHR78, play a role in ecdysteroid actions [16,20–23]. EcR is in the same gene family as another vertebrate hormone receptor, the thyroid receptor (TR). Like TR, the EcR needs a heterodimeric partner to function maximally. This heterodimeric partner, ultraspiracle (USP), is homologous to the vertebrate Retinoic Acid Receptor (RXR), which makes the EcR/USP system comparable to the vertebrate TR/RXR [24,25].

Interestingly, steroidal estrogens are not ecdysteroid agonists or antagonists [26], and the lack of a role of vertebrate sex steroid hormones in physiological processes in Arthropods has been reviewed extensively [27,28]. However, some non-steroidal environmental estrogens are ecdysteroid antagonists, including lindane, bisphenol A, diethylphthalate and *p,p′*-DDT [26]. In addition, several classes of phytochemicals are able to antagonize ecdysone activity both in vitro and in ecdysone-responsive cell lines [19,26,29,30]. Plants and insects have co-evolved for a much longer period of time than have plants and mammals, and it is not surprising that there are many more classes of phytochemicals which interfere with the insect steroid hormone system. These include the flavonoids, brassinosteroids and the cucurbitacins, of which the latter two have been shown to interact with the EcR [31,32]. The flavones, which are potent at interacting with mammalian and bacterial receptors, have not been investigated in the ecdysteroid system. We hypothesized that these chemicals also may be interacting with the invertebrate ecdysone receptor (EcR).

This study was done to determine whether several classes of phytochemicals, some of which are known to interact with the vertebrate estrogen receptor, can also act as agonists or antagonists at the EcR (Table 1). Rutin is a flavone-glycoside known to alter molting in insects, causing death [14]. There are several species of bacteria which are found on plants and in insect guts which specifically cleave the sugar molecule from rutin to form the flavones luteolin or quercetin [33,34]. One of these metabolites, luteolin, is a potent anti-estrogen [35] Other flavones, such as apigenin and chrysin, are also potent anti-estrogens [36,37]. Although these chemicals interfere with estrogen binding to its receptor, some flavones also inhibit the P450 enzymes which metabolize steroids to their active forms. Apigenin, chrysin and quercetin inhibit both mammalian and insect cytochrome P450 isozyme expression and activity [6,38–41]. Tomatine is a plant alkaloid which is extremely potent at inhibiting insect herbivory [42], but has not been studied for its ability to interact with steroid hormone receptors. Coumestrol and genistein are in different classes of phytochemicals, the coumestrins and isoflavones, and interact with estrogen receptors [43–45]. These eight diverse phytochemicals, some of which have activity in insects and vertebrates, and some of which affect only one or the other system, were studied in the ecdysone-dependent reporter gene assay using Chinese hamster ovary (CHO) cells and cell proliferation and differentiation assay using Cl.8 + cells.

2. Materials and methods

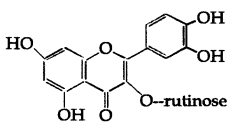
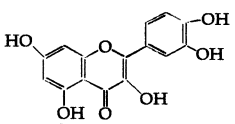
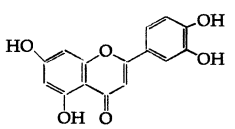
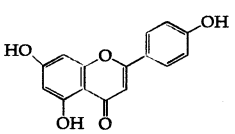
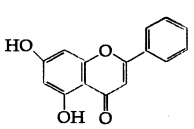
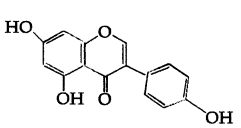
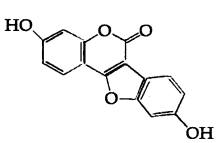
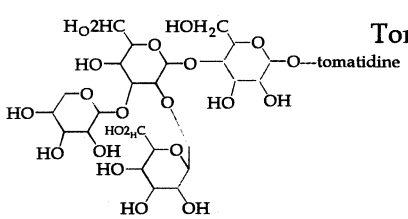
2.1. Reporter gene assay

CHO cells which stably express the modified *Drosophila* ecdysone receptor VgEcR/RXR, the *PIND/lacZ* plasmid, muristerone A, and zeocin were obtained from InVitrogen, Carlsbad, CA. CHO cells were cul-

tured as described elsewhere [46]. To maximize signal:noise ratios, muristerone A was used in experiments at 1 μM concentration, which is the lowest dose level where maximal reporter gene expression is seen (Fig. 1).

Vg EcR/RXR CHO cells were plated in 6-well plates at 1×10^6 density, and grown in media with charcoal-stripped serum for 36 h prior to experiments. Cells were transfected for 5 h with *PIND/lac z* and *pGL3* plasmids in 1 ml serum-free media per well using 9 μl lipofectAMINE reagent/well (Gibco BRL, Gaithersburg, MD). *PIND/lac z* (InVitrogen) contains modified ecdysone/glucocorticoid response elements linked to the β-galactosidase reporter gene and was transfected at 0.5 μg/ml, while *pGL-3* promoter plasmid (Promega,

Table 1
Phytochemicals used in this study

Structure	Name / Class	Activity
	Rutin/Flavone-glycoside	inhibits caterpillar molt
	Quercetin/Flavone	phytoestrogen Inhibits caterpillar molt Rutin metabolite
	Luteolin/Flavone	phytoestrogen Rutin metabolite
	Apigenin/Flavone	phytoestrogen
	Chrysin/Flavone	phytoestrogen
	Genistein/Isoflavone	phytoestrogen Clinical usage
	Coumestrol/Coumestrin	phytoestrogen
	Tomatine/Alkaloid	inhibits caterpillar molt

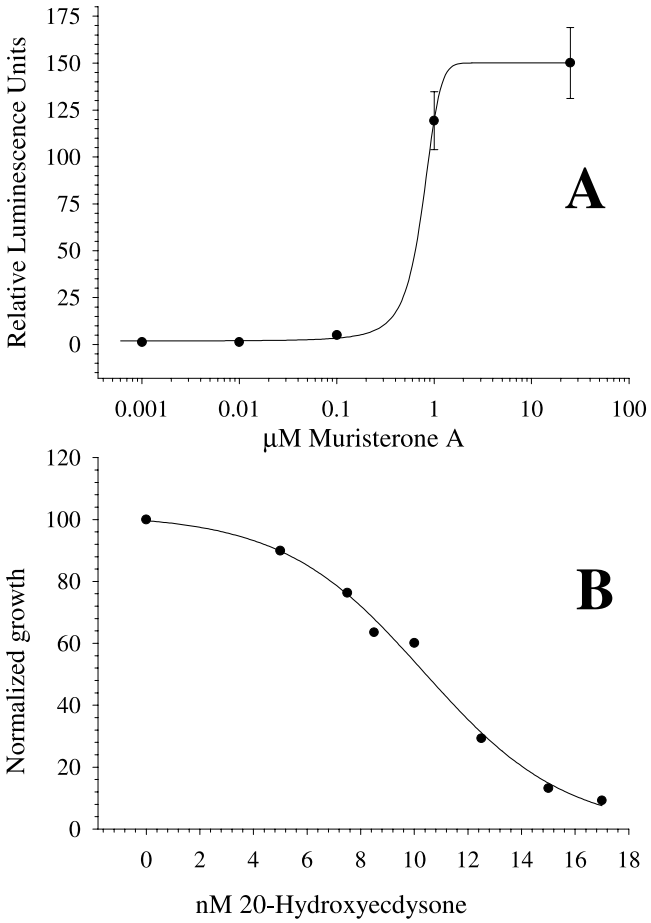


Fig. 1. Dose–response curves of muristerone A and ecdysone in the reporter gene assay (A) and Cl.8 + cell assay (B). Muristerone A is a more effective ecdysteroid in the reporter gene assay, whereas 20 HE is best in the Cl.8 + cells. Bars are S.E.M.

Madison, WI) was used at 0.1 μg/ml as a transfection efficiency marker with luciferase as the reporter gene.

2.2. Phytochemicals

Phytochemicals were obtained from Indofine, Somerville, NJ. CHO cells were washed once with charcoal-stripped serum media and dosed in triplicate either with or without 1 μM muristerone A. Chemicals were dissolved in DMSO, and controls as well as dosed cells received the same amount of total DMSO (1% of media for reporter gene assay, 0.1% for Cl.8 + cell culture assay). For the reporter gene assay, the phytochemicals were each dosed at 1, 10 and 25 μM, except tomatine which was extremely cytotoxic and was, therefore, dosed at 10 and 100 nM and 1 μM. After a 16 h incubation period, cells were checked to ensure normal growth, and β-galactosidase and luciferase were measured with Dual-Light System kit, TROPIX, Bedford, MA. Protein was measured using the BCA kit from Sigma, St. Louis, MO.

2.3. Differentiation of Cl.8 + cells

Cl.8 + cells were cultured as per Cullen and Milner [47]. Cl.8 + cells are a stable cell line established from *Drosophila melanogaster* imaginal wing discs. These cells respond to ecdysteroid by differentiating and halting proliferation [48]. This response can be used to study the effects of xenobiotics on the endogenous EcR/USP, as opposed to the VgEcR/RXR of the CHO cell assay. Cl.8 + cells were plated in 5 cm tissue culture dishes at a density of 3×10^6 cells/dish (4×10^5 cells per ml). Cells were dosed in 5 ml media with 20-hydroxy ecdysone (20 HE) between 5–17 nM, or phytochemicals (1, 5, 10 or 25 μM) alone or in combination with 20 HE. The toxicity of the various phytochemicals proved different in Cl.8 + cells than in CHO cells; the maximum dose of rutin, tomatine, coumestrol and apigenin was 25 μM, for luteolin, quercetin, chrysin, and genistein the maximum dose was 10 μM, though this was still toxic in the case of luteolin. The 20 HE concentration was adjusted to suit the stock cells being used, as the aim was to use two concentrations of 20 HE showing at the lower dose some morphological and (usually statistically non-significant) growth effects, and at the higher dose marked morphological change and a statistically significant reduction in cell growth. Prior to harvesting, cells were inspected for signs of cytotoxicity and photographs were taken to record changes in morphology associated with the differentiation effect. Cells were harvested after 4 days by pipetting with a magnesium- and calcium-free saline ($D^=$) wash, centrifuged at 250 rpm for 5 min, and resuspended in 1 ml $D^=$, and counted using a hemocytometer.

2.4. Statistical analysis

For reporter gene assays, cells were dosed in triplicate for each assay, averaged, and normalized to controls where 1 μM muristerone A ≡ 100%. For the Cl.8 + cells, cells were dosed in triplicate for each assay, averaged, and normalized to controls where Cl.8 + cells receiving only 0.1% DMSO (solvent control) were set at 100% growth. Each assay was repeated three times, and the average from each assay was used as one data point. Data was analyzed using ANOVA (SYSTAT for IBM) and if significant ($P < 0.05$), followed by a post-hoc Tukey test to determine which doses were different from control.

3. Results

Dose–response curves of both the reporter gene assay and Cl.8 + cell proliferation assay were as expected (Fig. 1). Increasing levels of ecdysteroid caused an

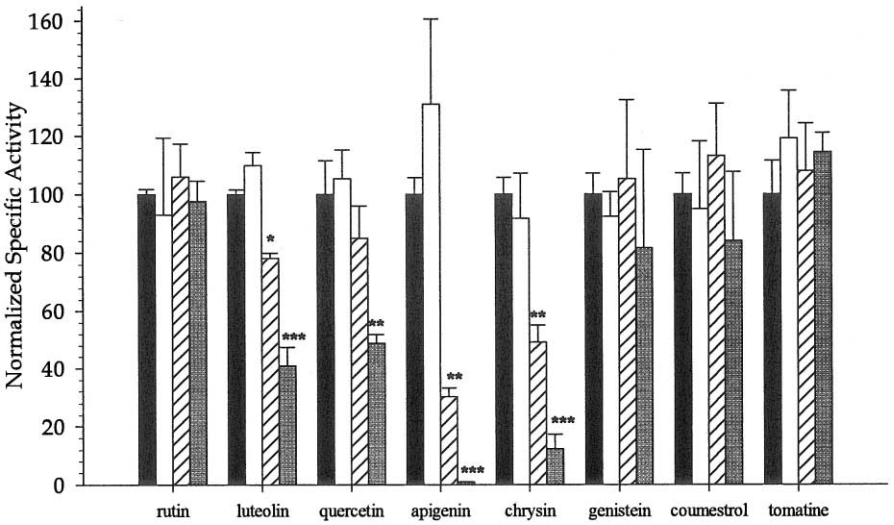


Fig. 2. Anti-ecdysone activity by phytochemicals in reporter gene assay. The flavones, luteolin, quercetin, apigenin and chrysin, were the only phytochemicals to significantly inhibit ecdysone-dependent gene transcription. Tomatine was tested at 10 and 100 nM and 1 μM (≠), all other phytochemicals were tested at 1, 10 and 25 μM. Each bar represents the mean of three experiments, done in triplicate. ■, Muristerone A only; □, 1 μM phytochemical (or 10 nM tomatine) + muristerone A; ▨, 10 μM phytochemical (or 100 nM tomatine) + muristerone A; ▩, 25 μM phytochemical (or 1 μM tomatine) + muristerone A. Error bars are S.E.M. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

increase in reporter gene transcription in transfected CHO cells, and a decrease in cell counts in Cl.8 + cells.

3.1. Reporter gene assay

None of the eight phytochemicals tested were able to activate the EcR-dependent reporter-gene expression when given alone, in other words they were not ecdysone agonists in this assay (data not shown). However, when given in conjunction with muristerone A, several phytochemicals were able to act antagonistically and significantly decrease EcR-dependent reporter gene activation (Fig. 2). Rutin, the flavone-glycoside, which disrupts molting in caterpillars, had no effect. The rutin metabolites, luteolin, quercetin, apigenin and chrysin, were each able to significantly inhibit reporter-gene expression at doses as low as 10 μM. The potent anti-estrogens, coumestrol and genistein, had no effect on reporter gene expression. Tomatine (an alkaloid) was extremely cytotoxic and was, therefore, tested at lower concentrations, where it did not affect reporter-gene expression.

3.2. Differentiation of Cl.8 + cells

In Cl.8 + cells, morphological changes observed due to ecdysteroid response included tightening of aggregates and appearance of cells with cuticle casing in the aggregates; more cells with pronounced processes; and a more rounded appearance to remaining cells which seemed larger. For cytotoxicity, morphological changes included cell debris due to cell death and cell lysis and the detachment of cells from the substrate. These morphological changes have been documented before [49] and can be viewed on the Internet at http://www.sbms.st-and.ac.uk/sites/flycell/

Several of the phytoestrogens caused reduced cell proliferation or proved toxic, with only rutin, tomatine, coumestrol and apigenin tested at the maximum doses of up to 25 μM. Luteolin proved especially toxic. Cells treated with test compounds alone did not show any of the morphological changes associated with 20 HE, with the single exception of apigenin (Table 2).

Both rutin and luteolin showed slight but inconclusive evidence of interaction with 20 HE; these results were not sufficient to claim agonistic or antagonistic effects on Cl.8 + cells, reflecting the results from the reporter gene assay. In each case one assay showed evidence of interaction; also in the case of rutin in the combined overall percentages.

Table 2
Summary of ecdysteroid effects in either reporter-gene assays or cell differentiation assays

Phytochemical	Reporter gene assay	Cell differentiation assay
Rutin	No effect	No effect
Luteolin	Antagonist	No effect, highly toxic
Quercetin	Antagonist	Mixed agonist/antagonist, slightly toxic
Apigenin	Antagonist	Synergistic effect
Chrysin	Antagonist	No effect, slightly toxic
Genistein	No effect	Synergistic effect, slightly toxic
Coumestrol	No effect	Mixed agonist/antagonist
Tomatine	No effect, slightly toxic	Synergistic effect

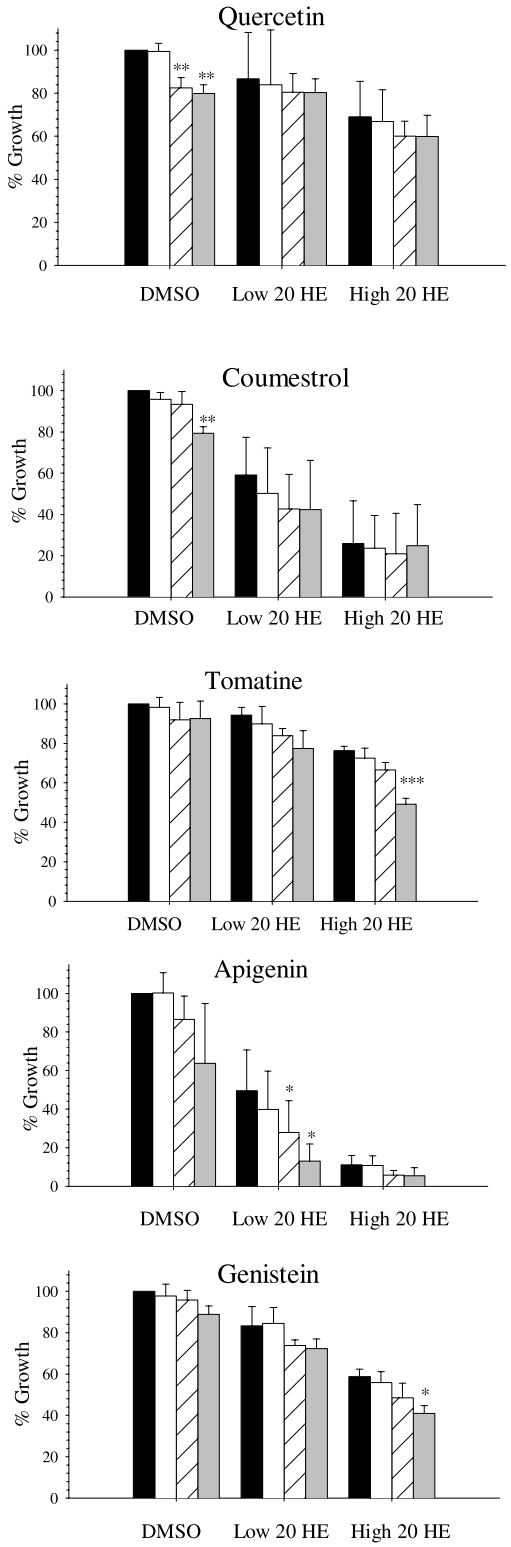


Fig. 3. Activity of several phytoestrogens in the Cl8 + cell proliferation assay. Both quercetin and coumestrol decrease cell proliferation when given alone, but not when given in conjunction with either low or high 20 HE doses, indicating mixed agonist/antagonist activity. Apigenin, tomatine and genistein showed a combined effect with 20 HE to decrease cell proliferation. Each bar represents the mean of three experiments, done in triplicate. ■, 0.1% DMSO only; □, low dose phytochemical; ▨, medium dose phytochemical; ▩, high dose phytochemical. Error bars are S.D. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

Quercetin caused some decrease in cell proliferation at 5–10 μM. The three assays showed variable results. In one case the presence of quercetin seemed to boost the effect of 20 HE on cell numbers at both 20 HE dose levels, in a second experiment to counteract the effect, in a third the interaction counteracted the lower 20 HE dose level but boosted the higher 20 HE dose level. Overall, quercetin appears to have a mixed antagonist/agonistic effect on the ecdysteroid response. Combining the results, cell counts were reduced in quercetin only treatments (Fig. 3), but this reduction in cell count was not seen when quercetin was given in combination with either low or high 20 HE. What should have been additive effects—decrease due to ecdysteroid and quercetin beyond that of either compound alone—were not seen at 5 or 10 μM co-exposures.

It has not yet been established whether Cl.8 + cells, or the culture conditions, cause the conversion of rutin into its metabolites luteolin and quercetin; if so, these may be responsible for some of the effects seen in the rutin assays.

The phytoestrogen coumestrol showed similar results to quercetin, with a mixed agonist/antagonist effect at 25 μM.

In contrast to the CHO reporter gene assay, tomatine showed no particular evidence of toxicity up to the maximum dose of 25 μM in Cl.8 + cells. Both tomatine and genistein showed clear evidence of a combined effect with 20 HE in reducing cell proliferation more than was the case for either compound alone (Fig. 3).

Both chrysin (tested to the slightly toxic level of 10 μM) and coumestrol (tested to 25 μM) showed some evidence of a synergistic effect with 20 HE in one assay but no effect overall (data not shown).

Apigenin also showed clear, statistically significant, evidence of synergistic action with 20 HE in the reduction of cell proliferation (Fig. 3). In addition, observations of cell morphology prior to harvest showed a more marked expression of 20 HE's effects, of inducing cells to produce long processes and to form very tight aggregates, in those cultures also treated with a high dose of apigenin.

4. Discussion

It is not unusual for phytochemicals to have mixed agonist/antagonist activities on steroid hormone systems. Mixed agonists/antagonists have been reported for phytochemicals interacting with other steroid hormone receptors via several different mechanisms [50–54]. Resveratol, found in grapes, is a partial estrogen receptor (ER) agonist. In MCF-7 cell culture, resveratol is found to be an agonist at doses below 1 μM, and an antagonist at doses higher than 1 μM [50]. One mechanism which has been proposed for the mixed estrogen

agonist/antagonist phenomenon is the up- or down-regulation of ER or progesterone receptor (PR). Genistein is such an example, where it is an agonist at low doses (below 5 μM) and an antagonist at high doses (greater than 5 μM) [53]. Genistein downregulates the ER, but upregulates the PR [53]. Cross-talk between the two receptors may have led to the mixed estrogen agonist/antagonist activity of genistein [53,55]. Differences in steroid receptor isoforms can lead to either agonist or antagonist activity. For example, the methoxychlor metabolite HPTE is an agonist of the ERα, but an antagonist of the ERβ, making it a mixed estrogen agonist/antagonist [51]. Similarly in our study, coumestrol had no effect on ecdysteroid-dependent reporter-gene activity but was an agonist/antagonist in cell culture. It is possible that coumestrol may have acted via a non-EcR receptor pathway in cell culture, including cross-talk with USP or other receptors; or by differentially interacting with EcR isoforms. In the reporter gene assay, the EcR A isoform was used while the Cl.8 + cells are most likely capable of displaying several EcR isoforms.

Of the phytochemicals tested, only the flavones inhibited EcR-dependent gene-expression. Rutin, which is potent at inhibiting molt in insect caterpillars, had no effect, but it's metabolites, luteolin and quercetin, were both potent anti-ecdysones at the EcR. This is extremely interesting since many studies with rutin fed caterpillars have shown a profound effect on both molting and growth. Caterpillars spend more time in each instar stage, and molt is initiated late [56]. Caterpillars are also not able to fully molt the head-capsule, leading to death. This scenario is possible if there is an anti-ecdysone present which can decrease the effect of molting hormone and halt the molting process before it is completed. Some plants have as much as 8% rutin in their leaves [33]. *Pseudomonas* bacteria, which are widely distributed not only on plants but also in insect guts, specifically cleave the sugar molecule from rutin to form luteolin or quercetin [33]. From these data, it is highly likely that the active molt-inhibiting agent is not the parent compound rutin, but rather one of the metabolites. Since quercetin was found to interact with both the reporter-gene assay and cell differentiation assay, this is a likely candidate for the active metabolite which leads to the molting effects in caterpillars.

Of the remaining flavones tested, no effects were seen from chrysin, but apigenin showed some intriguing effects on the 20 HE-induced changes in the morphology of Cl.8 + cells, in addition to boosting the effect on cell proliferation.

Baker [1] raised the question of whether there is a connection between the action of flavones in lower animals and vertebrates. Two examples of parallel systems may provide clues to this connection. The first example is the similarity between NodD protein and ER. In plants, flavones are key signals to cause nodulation of the rhizobia (nitrogen-fixing bacteria) [57,58]. Low concentrations of quercetin and luteolin induce the rhizobia to form nodules. This very early evolutionary system is mediated by the bacterial regulatory protein, NodD, which binds the flavones and causes gene transcription. The two ligand-binding domains of NodD are highly conserved, and show 45 and 36% homology to the human ERα [59]. Gyorgypal and Kondorosi [59] argue that the NodD and ER may have evolved from a common ancestor protein because of several similarities, including ligand binding of flavones, analogous signal transduction mechanisms, and homologous polypeptide modules in the ligand-binding domain. Evolutionarily, insects and EcR occurred much earlier than vertebrates and ER [60]. The interaction of quercetin and coumestrol with the EcR and ER could help delineate the evolutionary relationship between NodD proteins and steroid hormone receptors.

Another system in which there are plant/mammalian parallels involves metabolizing enzymes. Flavones also serve as precursors for flower pigments, the anthocyanins [61]. According to Baker et al. [62], the plant enzymes which convert flavonoids to anthocyanins (dihydroflavonol 4-reductases) may share a common ancestor with vertebrate 3β-hydroxysteroid dehydrogenase, which converts pregnenolone to progesterone. These two examples show that plant signaling pathways, used either as chemical or visual signals, may be ancestral to steroid hormone signaling pathways. The phytochemical could be a potential tool for identifying the evolution of the steroid molecule.

The question of whether the flavone interaction with these diverse systems is convergent evolution or divergent evolution is difficult to answer. Baker [1] argued convincingly that estrogen binding to proteins has evolved at least three different times: with ER, rat α-fetoprotein, and sex steroid binding globulin. Interestingly, these three proteins also preferentially bind flavones over other phytochemicals. On the other hand, the steroid, thyroid and retinoic acid receptors are descended from a common ancestor, but have very different ligand structures [60]. The flavones again overlap in their ability to interact with these different classes.

Another phytochemical that has been reported to affect caterpillar molting, the alkaloid tomatine, has no anti-ecdysone activity in the reporter-gene system. Although tomatine is extremely cytotoxic in this system, it may have a different mechanism of action that is not mediated by EcR. The results from the Cl.8 + differentiation assay would seem to support this view, as tomatine clearly boosted the effects of 20 HE at the highest dose levels. Similar results were seen for the well-known phytoestrogen genistein, an isoflavone.

As previously mentioned, non-genomic pathways of steroid hormone action have been reported [8,9]. Although the aim of this study was to investigate the effects at the ecdysteroid receptor of a number of potential agonists or antagonists, the non-genomic effects should be borne in mind when interpreting the results.

Non-genomic effects of ecdysteroids on adenylylcyclase activity [63] and on protein phosphorylation [64] have been reported. Brassinosteroids, structurally related to ecdysteroid and known to interact with the EcR [32], may act through a receptor kinase in plants, leading to the speculation that animal steroids may also have a signal transduction chain involving receptor kinases [65]. Several of the phytochemicals we tested have well-documented effects as inhibitors of various protein kinases, including receptors [66–68], and as inhibitors of various stages of inositol phosphate and MAPK pathways [66,69,70]. Thus these phytochemicals may have effects on key elements of pathways needed for cell proliferation, boosting 20 HE's effects. This leaves aside the speculation that the non-genomic effects may directly modulate classical steroid effects [9] which may also be a factor.

The multiple effects of various phytochemicals with the capacity to influence estrogenic action have been the basis of many studies, but the parallel effects in invertebrate steroid hormone systems have been relatively under-researched. The various and intriguing results presented in this report indicate several areas which should be pursued in signal transduction and the mixed genomic/non-genomic actions of ecdysteroids.

In conclusion, the four flavones (luteolin, quercetin, apigenin and chrysin) are able to inhibit ecdysone-mediated gene transcription, while genistein, coumestrol and tomatine have no effect. Quercetin and coumestrol are mixed agonists/antagonists in the Cl.8 + cells, while apigenin, genistein and tomatine show an effect in combination with 20 HE only on reduction of cell proliferation. It is likely that the metabolites of rutin are the active forms which elicit the inhibitory effect on molting. Most interestingly, the flavones are able to modulate not only invertebrate ecdysone, but also the vertebrate estrogen system, bacterial nodule formation, and serve as precursors for visual signals between plants and insects. Further studies on ligand-binding similarities between EcR and ER, especially with respect to overlap in responses to flavones, would be useful to explore the evolutionary relationships of steroid molecules.

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