An integrated approach to assessing the bio-activity of nutrients in vitro: The anti-oxidant effects of catechin and chlorogenic acid as an example

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Abstract

To explore how screening for potential nutrient bio-activity could be improved by integrating biological and chemical markers of cellular responses, we assessed the effect of the known anti-oxidants, catechin and chlorogenic acid, on anti-oxidant activity in vitro. We determined an active concentration (25 μM) of each compound and then measured its effects on anti-oxidant gene expression and regulation, enzyme activity, and cellular anti-oxidant activity. We found that only catechin retained biological anti-oxidant effects in vitro,although catechin and chlorogenic acid are chemical anti-oxidants. A limited role for microRNA regulation of anti-oxidant was found, however it cannot be excluded as a possible mechanism because other microRNAs and/or epigenetic factors may be involved. Our approach found that screening for bio-activity of nutrients, in this example anti-oxidant activity, is more appropriate using an integrated approach and highlights the importance of using a range of techniques, such as those used in this study, to properly characterise the effects of dietary ingredients.

Introduction

Our understanding of the effects of nutrients on cellular function requires appropriate *in vitro* models in order to validate hypotheses of interest, or to justify further study in more complex biological models. To assess if using multiple biological measurements of cell function could improve purely chemical measurements of potential bio-activity and/or provide a more relevant biological interpretation, we developed a model to study anti-oxidant activity. Despite the recent EFSA (European Food Safety Authority) ruling that no evidence has been provided that having anti-oxidant activity/content and/or anti-oxidant properties is a beneficial physiological effect, the word anti-oxidant still resonates among consumers [1,2]. This rise in anti-oxidant awareness has enabled anti-oxidant nutrients to become a marketing tool. Although there is still debate as to what is the "gold standard" to test for nutrient benefits (and some may argue that randomised controlled trials are the ultimate test for functionality), *in vitro* studies like the one presented here provide a valuable tool for better mechanistic understanding.

Catechin and chlorogenic acid were used as reference dietary ingredients as they are found in many commonly consumed foods (e.g. coffee, tea, chocolate) and have been shown to act as potent anti-oxidants in a number of studies [3-14]. Epidemiological studies have suggested a link between the consumption of foods rich in these compounds and reduced risk of disease [15-17]. However, most of the preliminary biological studies investigating the capacity of these compounds, or their metabolites, to affect the cellular response to oxidative damage have used highly transformed cancer cell models. How catechin and chlorogenic acid affect the balance of reactive oxygen species (ROS) regulation by the CAT, SOD, and GPX pathways in less diseased or normal cells is not known, nor is the effect of these compounds on the activity of miRNAs regulating anti-oxidant gene expression [18]. ROS

are involved in several physiological processes and an imbalance in their abundance may contribute to the risk of disease [19]. The balance of ROS levels in cells is controlled by the catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPX) anti-oxidant pathways [20-26]. There has been considerable interest in recent years in assessing how phytochemicals from our diet could affect the ROS balance within cells to reduce inappropriate ROS activity [27-32].

However, these studies have largely focused on the chemical anti-oxidant activity of these compounds which often requires a non-physiological pH and temperature; very few of these studies take into account the bioavailability, uptake, and metabolism of the anti-oxidant compounds. Biological systems are much more complex than the simple chemical mixtures employed, and anti-oxidant compounds may operate via multiple mechanisms.An understanding of how potential anti-oxidants from food affect cellular anti-oxidant pathways in normal or early stage disease models is essential in assessing possible health benefits.

The expression and activity of the CAT, SOD1-3, and GPX1-8 genes (and resulting capacity to manage intracellular ROS levels) are influenced by several factors such as mutations in the coding sequence or defective protein folding, both of which lead to inappropriate anti-oxidant

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function [20,22,24,30]. However, the role of epigenetic factors such as microRNA in these pathways, and how phytochemicals may influence these epigenetic factors, is poorly understood [33,34]. MicroRNAs (miRNAs) are post-transcriptional regulators that profoundly co-ordinate cell physiology by influencing mRNA stability leading to translational repression of protein-coding genes. The ability of miRNAs to regulate many distinct mRNA transcripts and the evidence for inappropriate miRNA regulation in disease suggests a fundamental role for miRNAs in how cell biology changes during disease [35]. Furthermore, dietary polyphenols, for example catechin, have been shown to influence the expression of miRNAs and affect markers of cell function and health [36-53].

The aim of the study was to develop a model to study how genetic, epigenetic, enzymatic, and phenotypic markers of the cellular response to oxidative damage may be altered by dietary factors. An immortalised (human with papillomavirus-18) prostate epithelial cell line (RWPE-1) and an early-stage prostate cancer cell line (WPE1-NA22) provided a cell model for approximately "normal" and abnormal cell health, respectively. The effects of catechin and chlorogenic acid on free radical scavenging capacity, viability, and oxidative defence, as well as expression of anti-oxidant genes and miRNAs, and anti-oxidant enzyme activity, were assessed in the RWPE-1 and WPE1-NA22 cells lines. The concentration of each compound for study was based on that which was more effective at scavenging free radicals than cell lysates alone.

Materials and methods

Cell culture

Authenticated RWPE-1 and WPE1-NA22 cells were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). All cell culture reagents were obtained from Gibco (Life Technologies, Auckland, NZ) unless otherwise stated. Both cell lines were cultured in keratinocyte serum-free media supplemented with 25 mg of bovine pituitary extract, 2.5 µg of recombinant epidermal growth factor and 1% penicillin/streptomycin (Pen Strep). All cell lines were maintained at 37°C and 5% CO_2 in a humidified atmosphere according to their individual ATCC guidelines and all experiments were completed within 12 sub-cultures from the original ATCC stock.

Chemicals

All chemicals were purchased from Sigma-Aldrich (Auckland, NZ) unless otherwise stated. Stock solutions of 2.29 mM (+)-Catechin hydrate (CAS Number 225937-10-0, ≥98%, C1251), and 3.12 mM chlorogenic acid (CAS Number 327-97-9, ≥95%, C3878) were prepared in DMSO. The negative control was culture medium supplemented with DMSO matched to the DMSO concentration in the treatment medium (0.02% (v/v)).

Free radical scavenging capacity assay

The capacity of catechin, chlorogenic acid, and untreated cell lysates to scavenge the free radical formed from the reduction of 2,2-Di(4-*tert*-octylphenyl)-1-picrylhydrazyl (DPPH, Sigma-Aldrich, Auckland, NZ) was measured based on changes in the absorbance (at 515 nm) of the free radical [54-56].

For the first experiment, twelve replicates of catechin or chlorogenic acid at 0, 0.1, 1, 10 and 25 µM in methanol (Sigma-Aldrich, Auckland, NZ, HPLC grade >99.9%) were prepared and mixed with 60 µM of DPPH (in methanol) in 96-well micro-titre plates (Corning, Corning,

NY, USA). After 30 minutes the absorbance of the DPPH radical was measured at 515 nm using a SpectraMax 250 spectrophotometer (Molecular Devices, Downington, PA, USA). For the second experiment, twelve lysates (using DMSO) of healthy untreated RWPE-1 or WPE1-NA22 cells (5×10^5 cells per replicate) were assayed as described above.

Cell viability assay

The effect of catechin or chlorogenic acid on the metabolic activity (a marker of cell viability) of the RWPE-1 and WPE1-NA22 cell lines after 24 hours was measured using the water-soluble tetrazolium (WST-1) cytotoxicity assay (Clontech, Mountain View, CA, USA). This assay is based on the cleavage of the tetrazolium salt to a formazan dye by mitochondrial succinate-tetrazolium reductase in viable cells and is quantified by measuring the formazan absorbance at 450 nm.

For each cell line, 25 µM catechin or chlorogenic acid were tested on twenty-four replicates of 9×10^3 cells per well in 96-well tissue-culture plates (Corning, Corning, NY, USA). The cells were seeded and cultured for 24 hours to facilitate attachment after which the medium was replaced with 90 µl of the appropriate supplemented (or control) medium and cultured for a further 24 hours. After this, 10 µl of WST-1 was added to each well and the plates incubated for 2 hours before the absorbance of the formazan produced was measured at 450 nm (reference 650 nm) using a SpectraMax 250 spectrophotometer (Molecular Devices, Downington, PA, USA).

Cellular oxidative defence assay

The effect of 25 µM catechin or chlorogenic acid on the cellular oxidative defence capacity of the RWPE-1 and WPE1-NA22 cell lines was measured. This assay is based on the capacity of samples to prevent the formation of dichlorofluorescein (DCF, emission at 538 nm) from dichlorofluoresceindiacetate (DCF-DA) by 2,2'-azobis(2-amidinopropane) dihydrochloride (ABAP)-generated peroxyl radicals in human cells [57].

For each cell line, 25 µM catechin or chlorogenic acid were tested on twelve replicates of 6×10^4 cells per well in opaque 96-well tissue-culture plates (Corning, Corning, NY, USA). The cells were seeded and cultured for 24 hours to facilitate attachment after which the medium was replaced with 50 µl of the appropriate supplemented (or control) medium and 50 µl of DCF-DA (Sigma-Aldrich, Auckland, NZ). After incubation (1 hour at 37°C and 5% CO_2), the cells were washed with 100 µl of PBS and 100 µl of 600 µM ABAP (in Hank's balanced salt solution, Sigma-Aldrich, Auckland, NZ) was added. The fluorescence emission at 538 nm (excitation at 485 nm) was measured every five minutes for one hour using a SpectraMax 250 spectrophotometer (Molecular Devices, Downington, PA, USA).

Quantification of oxidative defence gene expression

The expression of genes associated with oxidative defence: catalase (CAT), superoxide dismutase 2 (SOD2) and glutathione peroxidase 1 (GPX1), were quantified using TaqManreal-time PCR relative to the hypoxanthine phosphoribosyltransferase 1 (HPRT1) reference gene. RWPE-1 and WPE1-NA22 cells were exposed to 25 µM catechin or chlorogenic acid and gene expression measured at 24 hours.

For each cell line, 5×10^5 cells were seeded into sufficient wells of a 6-well tissue-culture plate (Corning, Corning, NY, USA) and cultured for 24 hours to facilitate attachment. The medium was replaced with the appropriate supplemented (or control) media

and cultured for 24 hours. The medium was removed and 2.5 ml of TRI reagent (Life Technologies, Auckland, NZ) was added for five minutes to facilitate cell lysis. After mixing the lysates were stored in sterile nucleic acid-free tubes at -80°C. The total RNA from each cell lysate was isolated using the RiboPure RNA isolation kit (Life Technologies, Auckland, NZ) according to the manufacturer's instructions, with the following modifications; 1-bromo-3-chloropropane, 100 μl, (Sigma-Aldrich, Auckland, NZ) was used in place of chloroform, two wash steps were completed and the total RNA eluted in two volumes of 30 μl. The total RNA was stored at -80°C overnight and quantified from its A260:280 nm and A260:230 nm ratios using a NanoDrop 1000 spectrophotometer (ThermoFisher Scientific, Scoresby, Vic, AU).

For real-time PCR analysis, 900 ng of total RNA was reverse transcribed into cDNA using random octomers and a high capacity RNA-to-cDNA kit (Life Technologies, Auckland, NZ) according to the manufacturer's instructions. The cDNA was stored at -20°C until required. The TaqMan assays (Life Technologies, Auckland, NZ) used were; CAT (Hs00156308_m1), SOD2 (Hs00167309_m1), GPX1 (Hs00829989_gH) and HPRT1 (333768F). All PCRs (no-template controls, untreated and treated samples) were prepared as triplicate 10 μl reactions comprising a 9.0 μl aliquot of master mix (5.0 μl of 2x GE master mix (Life Technologies, Auckland, NZ), 0.5 μl of 20x gene expression assay (Life Technologies, Auckland, NZ), 3.5 μl of nuclease-free water (Life Technologies, Auckland, NZ)), and 1 μl of cDNA (at a 1 in 18 dilution in nuclease-free water). The thermal profile used was; 50°C for 2 minutes, 95°C for 10 minutes and 40 cycles of 95°C for 15 seconds and 60°C for 60 seconds. The experiment was completed using a RotorGene 6000 qPCR instrument (Qiagen, Hilden, DE). The data were normalised to the reference gene and analysed for expression level changes using the ΔΔCt method. The experiment was completed as three biological replicates each with three technical replicates for all samples.

Quantification of microRNA gene expression

The expression of miRNAs predicted to target CAT, SOD2, or GPX1 mRNA translation were quantified using TaqManreal-time PCR in RWPE-1 and WPE1-NA22 cells treated with 25 μM catechin or chlorogenic acid for 24 hours The expression of the MIR107, MIR30E, and MIRLET7G genes, relative to the small nucleolar RNA, H/ACA box 74A (SNORA74A) reference gene were measured.

An enriched small RNA fraction was isolated using the mirVana miRNA isolation kit (Life Technologies, Auckland, NZ) according to the manufacturer's instructions. For real-time PCR analysis, 10 ng of the small RNA fraction was reverse transcribed into cDNA using the TaqMan small RNA assay kit (Life Technologies, Auckland, NZ) according to the manufacturer's instructions. The cDNA was stored at -20°C until required. The TaqMan assays used were; MIR107 (000443), MIR30E (002223), MIRLET7G (002282) and SNORA74A (001003). All PCRs were completed and analysed as described in the quantification of oxidative defence gene expression experiment.

Quantification of oxidative defence enzyme activity

The activity of the catalase, glutathione peroxidase, and superoxide dismutase oxidative defence enzymes in the RWPE-1 and WPE1-NA22 cell lines in response to 25 μM catechin or chlorogenic acid was measured using the absorbance-based CAT, SOD and GPx assay kits (707002, 706002 and 703102, Cayman Chemicals, Ann Arbor, MI, USA) according to the manufacturer's instructions.

For each cell line, 5×10^5 cells were seeded into sufficient wells of a 6-well tissue-culture plate (Corning, Corning, NY, USA) flask and cultured for 24 hours to facilitate attachment. The medium was replaced with the appropriate supplemented (or control) media and cultured for 24 hours. CAT activity was measured at 540 nm, SOD activity was measured at 450 nm and GPx activity was measured at 340 nm (at 25°C every minute for 10 minutes) using a SpectraMax 250 spectrophotometer (Molecular Devices, Downington, PA, USA). The experiment was completed with six biological replicates each with thirty technical replicates for all samples.

Statistical analyses

All data were analysed for normality and statistical significance determined using ANOVA. Following analysis, significantly different means were identified using the least significant difference (LSD) post-hoc test. A P-value of less than 0.05 was considered to show a significant difference. All analyses were completed using GenStat v16 software (VSN International, Hertfordshire, UK).

Results

Free radical scavenging capacity assay

The effects of catechin, chlorogenic acid, and RWPE-1 and WPE1-NA22 cell lysates on free radical scavenging, as measured by DPPH radical formation, are shown in Figure 1. Catechin and chlorogenic acid significantly reduced the formation of the DPPH radical compared to the untreated sample (Figure 1a). Catechin was more effective than chlorogenic acid because it reduced DPPH radical formation at 1, 10, and 25 μM, whereas chlorogenic acid was only effective at 10 and 25 μM. Both the cell lysates are effective at reducing DPPH radical formation compared to the untreated sample (Figure 1b). When compared to the RWPE-1 cell lysate, only 10 and 25 μM catechin and 25 μM chlorogenic acid were more effective than the cell lysate alone Figure 1c). None of the chlorogenic acid concentrations tested were more effective that RWPE-1 or WPE1-NA22 cell lysates (Figure 1d).

Cell viability assay

Catechin or chlorogenic acid, at 25 μM, did not substantially or significantly alter the metabolic activity of RWPE-1 or WPE1-NA22 cells after 24 hours, indicating that this concentration of either compound does not alter the viability of these cell lines.

Cellular oxidative defence assay

The effect of 25 μM catechin or chlorogenic acid on the cellular oxidative defence of RWPE-1 and WPE1-NA22 cells, as measured by DCF formation, is shown in Figure 2. These data indicate that only catechin (at 25 μM), and not chlorogenic acid, reduces DCF formation in either cell line. There was no significant difference in the magnitude of catechin-mediated inhibition of DCF formation between the two cell lines.

The effect of catechin and chlorogenic acid on biological markers of the oxidative defence response and regulation by microRNA

The effect of 25 μM catechin or chlorogenic acid on the expression of the CAT, GPX1, and SOD2 anti-oxidant genes by RWPE-1 and WPE1-NA22 cells after 24 hours, as measured by probe-based real-time PCR, is shown in Figures 3a and 3d. These data indicate that CAT expression in the WPE1-NA22 cell line was affected by catechin or chlorogenic acid (reduced by 54 and 56%, respectively). Although not
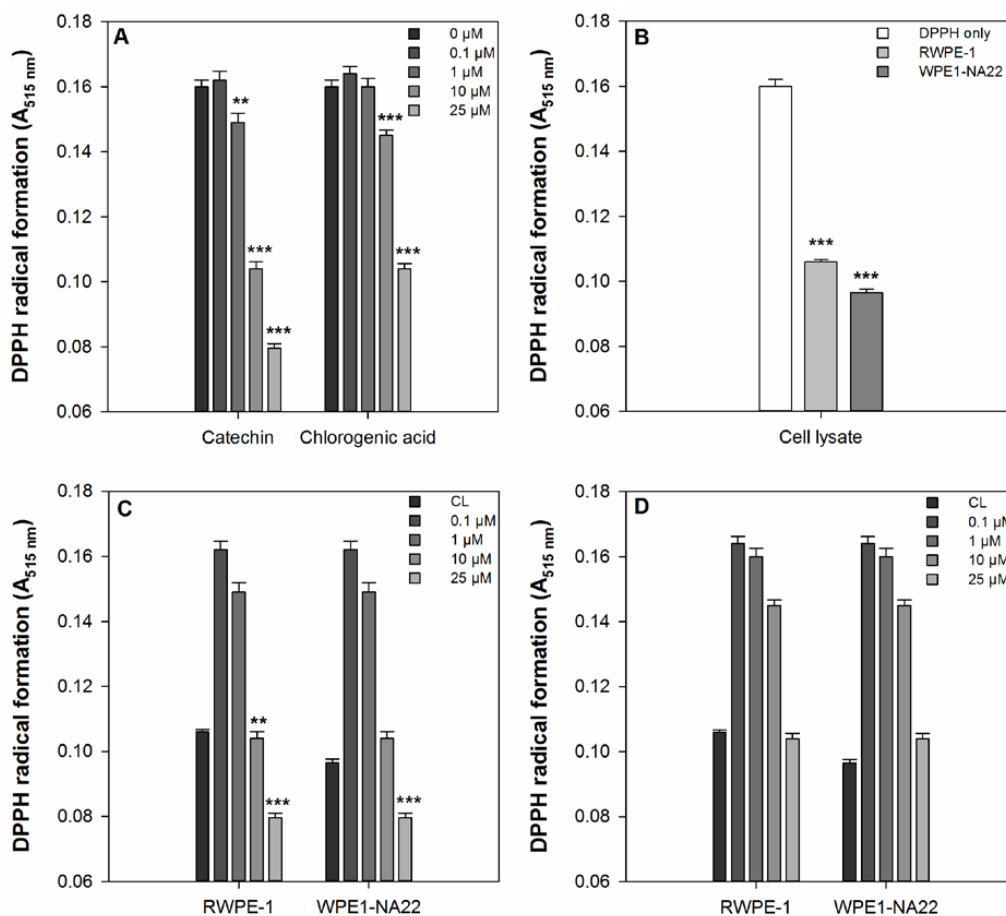
Figure 1. (a) The effect of catechin and chlorogenic acid compared to DPPH alone. A significant difference between treated and untreated samples is indicated by **(P<0.01) or ***(P=0.001). (b) The effect of RWPE1- and WPE1-NA22 cell lysates compared to DPPH alone. A significant difference between treated and untreated samples is indicated by ***(P=0.001). (c) The effect of catechin on DPPH radical formation compared to RWPE-1 and WPE1-NA22 cell lysates. A significant difference between treated and untreated samples is indicated by ***(P=0.001). (d) The effect of chlorogenic acid on DPPH radical formation compared to RWPE-1 and WPE1-NA22 cell lysates. A significant difference between treated and untreated samples is indicated by ***(P=0.001).

significant, the expression of the three genes was generally increased in the RWPE-1, and decreased in the WPE1-NA22 cell lines.

The effect of 25 µM catechin or chlorogenic acid on the expression of the microRNAs MIRLET7G, MIR30E, and MIR107 (predicted to regulate the translation of the CAT, GPX1, and SOD2 anti-oxidant genes) by RWPE-1 and WPE1-NA22 cells after 24 hours, as measured by probe-based real-time PCR, is shown in Figures 3b and 3e. MIRLET7G is predicted to regulate CAT translation, MIR30E is predicted to regulate CAT and SOD2 translation, and MIR107 is predicted to regulate GPX1 and SOD2 translation. These data indicate that only the expression of MIR107 was affected by any treatment (reduced by 51% in chlorogenic acid treated WPE1-NA22 cells).

The effect of 25 µM catechin or chlorogenic acid on the activity of the CAT, GPx, and SOD enzymes in the RWPE-1 and WPE1-NA22 cell lines after 24 hours, is shown in Figures 3c and 3f. These data indicate that only the activity of the CAT enzyme was affected by any treatment (chlorogenic acid-treated WPE1-NA22 cells).

Discussion

Given the consumer interest in the phrase "anti-oxidant" [1,2] and that the primary method of assessing anti-oxidant activity is based on
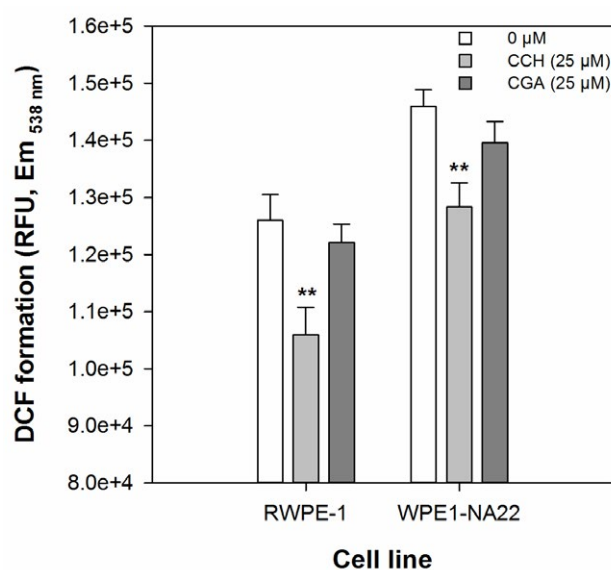

Figure 2. The effect of 25 µM catechin and chlorogenic acid on the cellular oxidative defence of RWPE1- and WPE1-NA22 cells, as measured by DCF formation. All data are expressed as the mean (±SEM) of twelve independent replicates. A significant difference between treated and untreated samples is indicated by **(P<0.01).

purely chemical assays [56], we developed a model as a tool to study how genetic, epigenetic, enzymatic, and phenotypic markers of the cellular response to oxidative damage may be altered by dietary factors. Catechin and chlorogenic acid were used as reference dietary factors because they are known anti-oxidants and can be readily consumed

in our diet, e.g. coffee, tea, and chocolate. In order for our integrated method to be of value it is essential that it provides a more coherent and relevant interpretation of bio-activity than purely chemical screening alone.
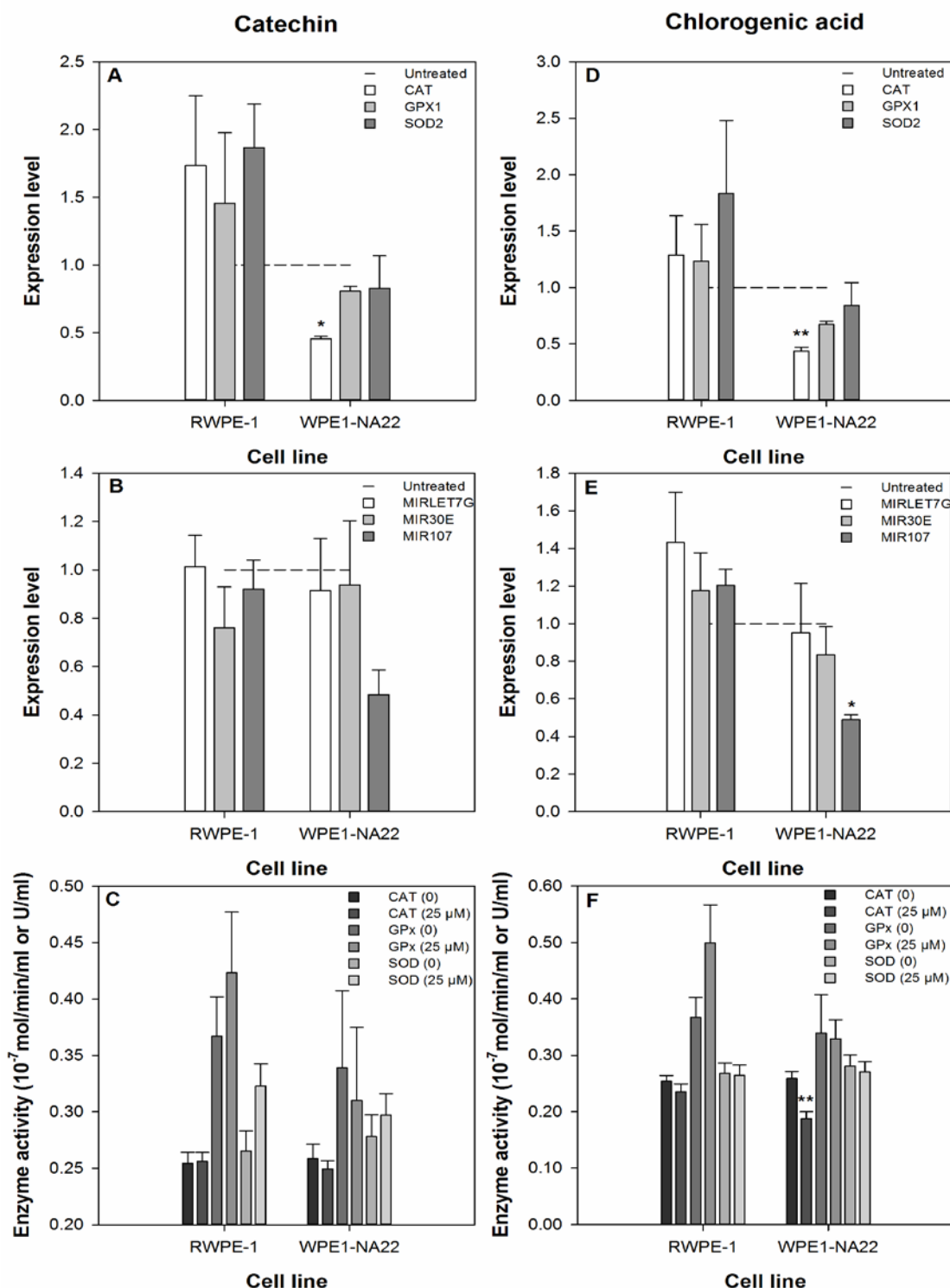

Figure 3. The effect of 25 μM catechin (a-c) and chlorogenic acid (d-f) on the expression and activity of biological markers of the oxidative defence response and expression of selected microRNAs. The expression level data are expressed as the mean (±SEM) of three independent replicates. A significant difference between treated and untreated samples is indicated by *(P<0.05). The enzyme activity data are expressed as the mean (±SEM) of six independent replicates. A significant difference between treated and untreated samples is indicated by **(P<0.01).

The data show that although both catechin and chlorogenic acid act as anti-oxidants in a purely chemical *in vitro* assay (Figure 1); this is not the case in a biological *in vitro* anti-oxidant assay (Figure 2). These data indicate that using a cellular anti-oxidant assay provides more meaningful biological information than a chemical assay alone. This cellular anti-oxidant assay, developed by Wolfe *et al.* [57], is straightforward and can be completed in any standard cell culture facility. In our study we used the RWPE-1 cell line as a 'normal' cell line; however, it is not truly 'normal' because it is an immortalised cell line and is likely a mixture of cancer stem cells and non-cancer stem cells. The other cell line, WPE1-NA22, is derived from the RWPE-1 cell line and has a higher growth rate which is indicative of carcinogenic transformation [58,59]. We used these cell lines, not strictly for their carcinogenic state, but as relatively crude models of a healthy/less diseased and a more diseased cellular state. It is essential that the appropriate cell type, given the inherent limitations of cell culture, is used for screening nutrients for any bio-activity.

Our data show that chemical anti-oxidant activity is not necessarily predictive of biological activity and because of this we examined aspects of the cellular anti-oxidant systems to determine if measuring these added further value to the interpretation of biological response. At the transcriptional level, both catechin and chlorogenic acid substantially reduced the expression (~50%) of CAT in the WPE1-NA22 cell line (Figures 3a and 3d). The pattern of the response was similar for both cell lines, i.e. a tendency for increased expression in the RWPE-1 cell line and a decrease in the WPE1-NA22 cell line. This indicates that at the transcriptional level these cell lines appear not to differ sufficiently to function as appropriate models of 'normal' and diseased cellular states. Give the interest in the literature in the regulation of mRNA translation by microRNA, we assessed if the mRNA response we measured might be due to altered miRNA activity (Figures 3b and e). However, only the expression of MIR107 was affected by our treatments (chlorogenic acid reduced its expression in the WPE1-NA22 cell line). This is inconsistent with the mRNA data as MIR107 is predicted to target GPX1 and we measured no significant change in the expression of GPX1. Although microRNA may be involved in the regulation of the anti-oxidant response, we did not find sufficient evidence to justify its further inclusion in our integrated approach.

Logically, a change in mRNA abundance should lead to a change in protein abundance or activity so we measured the activity of the anti-oxidant enzymes encoded by the genes we studied (Figures 3c and 3f). We chose to measure activity rather than abundance because from a screening perspective, it is easier to do and it is the activity of the enzyme rather than just its abundance (although the two are clearly linked) that is more biologically relevant. We found that the activity of the catalase enzyme was reduced by chlorogenic acid in the WPE1-NA22 cell line. This is consistent with the reduction in CAT gene expression measured and indicates that when transcriptional data and enzyme activity data are considered together, it may provide a clearer interpretation of a biological response.

Although we have shown that chemical anti-oxidant activity does not necessarily predict biological activity, it is unclear from our data if measuring other biological activity, such as transcription, epigenetics, or enzyme activity substantially improves the interpretation of the cellular response to anti-oxidants. There is a disparity in our data in how the compounds tested respond biologically at the mechanistic level. Only catechin was an effective anti-oxidant in the cellular assay, but had no substantial effects on the mechanistic markers measured.

Conversely, chlorogenic acid was ineffective in the cellular assay, but had some effects on the mechanistic markers measured.

However, the chemical and biological effects shown in Figures 1 to 3 occur at concentrations that may not be physiologically relevant. Both catechin and chlorogenic acid are extensively metabolised during digestion and absorption of foods containing them. The selection of a likely non-physiological concentration for study was based on the most effective concentration of both compounds in the chemical *in vitro* assay to maximise the assessment of our integrated approach.

These data show that despite both compounds having potent free radical scavenging capacity, this does not necessarily occur in biological systems. However, it is not clear how the amount of cellular uptake of these compounds or extracellular effects contribute to the reported effects, or absence of them. Several polyphenols have been shown to alter miRNA abundance [33,34,36-38,40-45,47,48,51,53] and the selection of dose in this study may not reflect physiological concentrations and exposure or the effect of metabolites. However, the primary aim of this study was to identify a concentration that was more effective chemically than cell lysates alone and to assess if using cellular and mechanistic tools improve the biological interpretation of anti-oxidant activity. We found that no single method (chemical, biological, or mechanistic) is satisfactory when assessing the bio-activity of nutrients. Furthermore, our data highlight the importance of selecting appropriate cell models and not relying on one screening method, chemical, cellular, or mechanistic, but rather an integrated approach consisting of at least a chemical and biological activity screening method.

Conclusions

In this study, a suite of tools was used to investigate the cellular aspects of the anti-oxidant activity of dietary ingredients, and provide exploratory mechanistic evidence for regulatory and marketing purposes. The effects of two well-characterised anti-oxidants, catechin and chlorogenic acid, were assessed in two cell lines, representing a model for normal and abnormal cell health. The application of assays to measure free radical scavenging and cellular oxidative defence enabled the anti-oxidant capacity of catechin and chlorogenic acid within the context of a biological system. Measurement of the expression and activity of key anti-oxidant genes, and miRNA molecules known to regulate these genes, were used to interrogate the potential mechanisms by which these compounds act as anti-oxidants.

These data show that although catechin and chlorogenic acid are potent anti-oxidants in a chemical assay, this activity does not necessarily occur in the cell models used. Although we found a limited role for epigenetic (miRNA) regulation of cellular response to oxidative stress, it cannot be excluded as a possible mechanism as other miRNAs and/or epigenetic factors may be involved. This study highlights the importance of using a range of techniques to properly characterise the effects of dietary ingredients.

Acknowledgments

Author contributions

M.M. completed data collection, sample analysis and data analysis. All authors contributed to the study design, review of the data, and preparation of the manuscript.

Conflicts of interest

The authors declare no conflict of interest. The authors alone are responsible for the content and writing of this article.

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