The Effect of the Green Tea Component Epigallocatechingallate (EGCG) on CD36 Receptor Cell Surface Expression in Human Monocytic Cell Line, THP-1
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Abstract

Atherosclerosis is the major source of morbidity and mortality in cardiovascular disease (CVD). The signalling cascade induced by the presence of oxidised LDLs involving the nuclear receptor PPAR-γ subsequently inducing the expression of cell surface scavenger receptor CD36, is essential for the formation of lipid-laden, monocyte-derived macrophages that penetrate the intimal layer of the blood vessel. These intimal monocyte-derived macrophages, or foam cells, are pivotal in the pathogenesis and exacerbation of atherosclerosis. In this study, two test groups of pre-differentiated THP-1 monocytes were treated in vitro with two concentrations of epigallocatechingallate (EGCG), the most abundant and bioactive polyphenol component of green tea that has previously been reported to attenuate atherosclerosis. In one test group, THP-1s were treated with the PPAR-γ ligand rosiglitazone prior to introduction of EGCG to act as a positive control for the induction of cell surface CD36 via the PPAR-γ pathway. The purpose of these were to determine the effect EGCG has on the nuclear receptor PPAR-γ by measuring the surrogate marker CD36, and whether a synergistic effect occurs between EGCG and rosiglitazone. Indirect flow cytometry of 20,000 cells was used to quantify relative CD36 cell surface expression in each sample for both test groups. EGCG was found to significantly affect cell membrane permeability and thus be cytotoxic to THP-1s in vitro at 200µM within 24hrs. Contrary to prior evidence, aspirin was found to have no significant effect on THP-1 CD36 cell surface expression at 24hrs incubation. Rosiglitazone was found to be a dose-dependent, significant agonist for cell surface expression of CD36 in THP-1s. 50µM and 100µM EGCG appeared to lower cell surface CD36 expression with and without prior treatment with rosiglitazone, however, statistical analysis rendered these differences insignificant. These findings demonstrate that EGCG does not up-regulate PPAR-γ and is unlikely to induce macrophage foam cell differentiation from monocytes in a diabetic, atherosclerotic model; and that EGCG neither exacerbates nor reverses the agonistic effects rosiglitazone has on PPAR-γ. Therefore, EGCG would not be suitable for treatment in conjunction with rosiglitazone as it does not significantly reduce the atherosclerotic potential, and subsequent cardiovascular risk. However, EGCG may be considered an appropriate alternative to rosiglitazone anti-diabetic treatment as it is unlikely to be accompanied by high cardiovascular risk. Further research is needed into the anti-diabetic mechanisms of EGCG.
**Introduction**

Diabetes is now recognised as a global epidemic. Incidence rates have almost doubled since 1980 with an estimated 422 million adult cases worldwide in 2014, accounting for around 8.5% of the population\(^1\); this has been partially attributed to the rapidly increasing prevalence of Type 2 diabetes which is now estimated to account for up to 95% of all diagnosed diabetes cases\(^2\). These incidences, along with the subsequent economic burden, are predicted to rise\(^3\).

Cardiovascular disease (CVD) is the most prevalent complication associated with diabetic morbidity and mortality in both adult male and female patients\(^4\). NICE guidelines dictate that primary prevention for adults with cardiovascular disease should be lifestyle and dietary changes. However, for those with Type 2 diabetes with ≥ 10% 10-year risk of CVD development it is recommended to offer a high-intensity statin dose (atorvastatin 20 mg) as the primary preventative\(^5\) rather than dietary/lifestyle changes alone: further highlighting the increase in risk and need for early, effective intervention in patients with Type 2 diabetes.

Not only do cardiovascular implications arise due to risk factors associated with diabetes, such as obesity, but they have more recently been shown to be a direct consequence of Type 2 diabetes: hyperglycaemia, hyperlipidaemia and hyperinsulinaemia having been shown to play a role in the pathogenesis of diabetic CVD; more specifically, in the development and acceleration of atherosclerosis\(^6\)\(^7\)\(^8\). Atherosclerosis has historically been shown to single-handedly account for the majority of the pathology of cardiovascular disease\(^9\) and as such, the potential to treat, prevent or interrupt atherogenesis has become a lucrative area of research and has particular significance for those at higher risk, such as patients with Type 2 diabetes.

Monocyte-derived macrophages play a central role in the atherogenic process: their uncontrolled lipid accumulation and internalisation of retained lipoproteins in the intima layer of blood vessels results in the formation of lipid-laden ‘foam cells’\(^10\), which in turn exacerbate the inflammatory effect in the local area. The scavenger receptor CD36 has a regulatory role in the lipid homeostasis of the monocyte-derived macrophages\(^11\), and the consequent uncontrolled uptake of lipids (the majority being modified or oxidised low-density lipoproteins (LDLs)) in foam cells. CD36 (in conjunction with another scavenger receptor, SR-A) has been shown to account for up to 90% of all
oxidised-LDL that occurs in the macrophage\textsuperscript{[12]}. Thus, CD36 may be considered as one of the most important receptors in the atherogenic production of foam cells.

Peroxisome proliferator-activated receptor $\gamma$ (PPAR-$\gamma$) has long been known to play a role in foam cell gene expression\textsuperscript{[13]} and has also been shown to be a regulator of CD36 expression\textsuperscript{[14]}. Transcriptional regulation of CD36 by the ligand-activated receptor PPAR-$\gamma$ occurs due to the presence of a PPAR-$\gamma$-responsive element in the proximal area of the promotor for CD36 gene expression\textsuperscript{[15]}. The drug rosiglitazone, a member of the class thiazolidinedione, is a selective ligand and agonist for PPAR-$\gamma$ and was used in the treatment of type 2 diabetes as an insulin sensitising agent and anti-hyperglycaemic agent by the subsequent regulation of PPAR-$\gamma$-responsive genes\textsuperscript{[16]}.

Green tea derived from the plant \textit{Camellia sinensis} is one of the most popular beverages worldwide; it is increasingly the topic of investigation for the potential preventative health benefits habitual consumption may induce including, but not limited to, cardiovascular health\textsuperscript{[17]}. There is evidence that the catechin components of green tea (of which epigallocatechingallate (EGCG) is the most abundant and bioactive) have beneficial effects in atherosclerosis through several different mechanisms\textsuperscript{[18]}. Studies of the effects of green tea on the PPAR-$\gamma$ pathway have been reported with conflicting results: some studies report that EGCG reverses high-fat diet-induced alterations of CD36 expression\textsuperscript{[19]} and down-regulates PPAR-$\gamma$\textsuperscript{[20][21]}; while others report an increase in PPAR-$\gamma$ (via alleviation of phosphorylation of the receptor)\textsuperscript{[22]} and/or CD36 expression\textsuperscript{[23]}.

The aim of this study was to determine whether the green tea component, EGCG, activates PPAR-$\gamma$ in undifferentiated THP-1 monocytes by measuring the cell surface levels of the surrogate marker CD36, using a pre-established PPAR-$\gamma$ ligand for comparison.

**Methods & Materials**

**Reagents**

THP-1 cells were supplied by the European Collection of Authenticated Cell Cultures (ECACC), Public Health England. Epigallocatechingallate (EGCG), rosiglitazone, resveratrol, genistein, luteolin, aspirin, propidium iodide and tissue culture grade
dimethyl sulfoxide (DMSO) were all purchased from Sigma-Aldrich, Dorset, UK. 50ml 15mM aspirin stock solution was made using 0.5ml DMSO in RPMI media (Life Technologies, ThermoFisher Scientific UK). Primary (mouse anti-human CD36) and secondary (goat anti mouse IgG/IgM) antibodies were purchased from BD Biosciences. Phosphate buffered saline (PBS) (Dulbecco, no calcium/magnesium) was purchased from Life Technologies, ThermoFisher Scientific UK. FACS buffer was made in-house containing 1% foetal bovine serum, 99% PBS, 0.1% sodium azide.

**Cell Culture**

RPMI 1640 medium was used, supplemented with: 100nM non-essential amino acids; 1mM Sodium pyruvate; 2 mM L-glutamine (all sourced from Life Technologies, ThermoFisher Scientific UK); 10% (v/v) heat-inactivated, low endotoxin FBS. THP-1s were maintained as suspension cultures in 75cm² flasks incubated at 37°C in 5% CO₂ and subcultured once they reached a cell density of approximately 1 – 1.2 x10⁶ cells/mL. At each passage cells were counted using an Improved Neubauer haemocytometer and seeded into new vessels at a minimum density of 3 x 10⁵ cells/mL.

**Propidium Iodide Cell Viability with EGCG Flow Cytometry**

Cells were seeded at 0.5million per ml. 3.5ml aliquots were spun at 150G for 5 minutes; supernatant was removed and replaced with 3.5ml of media containing either: 25µM, 50µM, 100µM or 200µM total concentration of EGCG, and the cells were resuspended. 1ml of resuspended cells and media were transferred to a 24-well culture plate in triplicate, and incubated under previously described culture conditions. A cells only negative control was also used. 0.5ml aliquots were taken from each well and spun at 190G for 5 minutes. Cell pellets were washed with FACS buffer (dPBS containing 3% FBS, 1% sodium azide), centrifuged at 190G for 5 minutes and resuspended in 110µl PI (final concentration of 10µg/ml) solution per sample. Samples were incubated at room temperature in the absence of light for 20 minutes, briefly vortexing samples at 10 minutes and 20 minutes incubation. 500µl FACS buffer (dPBS containing 3% FBS, 1% sodium azide) was added after incubation, and samples were analysed via the single colour, FL-2 setting on the BD AccuriTM C6 flow cytometer in accordance with the manufacturer’s recommended settings[24]. Viability readings were taken at 24 hours and 48 hours of incubation.
Response of monocyte CD36 cell surface expression induced by aspirin via indirect flow cytometry

5 x 1ml of cells at 1 million cells/ml were spun down at 100G for 5 mins. The supernatant was replaced with 1ml media containing final concentrations of aspirin at 1mM, 5mM and 10Mm (from stock solution previously outlined) and resuspended, in triplicate. A vehicle control of DMSO 0.6% v/v and a negative, cells only, control were also included, in triplicate. These were incubated for 24hrs under previously outlined culture conditions in a 24-well culture plate. After incubation period, cells were spun down at 200G for 5 minutes. Supernatant was replaced with FACS buffer containing 0.5µg primary antibody mouse anti-human CD36 in all samples except for cells only, and incubated on ice for 30 minutes gently vortexing every 10 minutes; followed by a washing step by centrifugation with FACS buffer. Cells were then incubated in the absence of light for 30 mins with 1µg per test secondary antibody goat anti-mouse IgG/IgM conjugated with FITC in all samples except for cells only, and gently vortexed every 10 minutes. Samples were then washed twice and resuspended in 500µl FACS buffer. Samples were kept in the absence of light and on ice until analysis. Analysis was carried out using FL-1 setting on the BD Accuri™ C6 flow cytometer, as per the manufacturer’s recommendations[24].

Response of monocyte CD36 cell surface expression induced by rosiglitazone, resveratrol, luteolin and genistein via indirect flow cytometry

0.5ml of cells at 1million/ml per test were spun down. The supernatant was replaced with media containing potential CD36/PPAR-γ agonist in two different concentrations: resveratrol 5µM and 10µM; genistein 10µM and 20µM; rosiglitazone 5µM and 10µM and luteolin 5µM and 10µM. A cells only negative control and no antibody negative control were also included. The same protocol as in the aspirin induction of CD36 indirect flow cytometry was adhered to.

Response of monocyte CD36 cell surface levels in the presence of EGCG and rosiglitazone via indirect flow cytometry

With cells at initial density of 1 million/ml, 22 x 1ml of cells were seeded in a 24-well culture plate and incubated for 1 hour, half with (rosi +) and half without (rosi -) the presence of 10µM rosiglitazone under previously described cell culture conditions. EGCG was then introduced in two concentrations previously identified as safe to use
(50µM and 100µM) in triplicate. For each test condition, rosi + and rosi -, a cells only negative control, a no antibody control and a secondary antibody only control were included. These were incubated under previously described conditions overnight, around 21hrs. Cells were then centrifuged at 200G for 5 minutes, supernatant was removed and replaced with 0.5ml FACS buffer containing 1µg primary antibody, mouse anti-human CD36 and resuspended. Samples were then incubated on ice for 40 minutes, gently vortexing every 10 minutes. Washing step: cells were spun down at 200G for 5 mins and supernatant discarded; 500ml FACS was added and cells were resuspended, then spun down again and supernatant discarded. 1µg secondary antibody per test was introduced to cells and incubated on ice in the dark for 40 mins, gently vortexing every 10 mins. Analysis was carried out using the FL-1 setting on the BD Accuri™ C6 flow cytometer, as per the manufacturer’s recommendations\[24\].

**Statistical Analyses**

All graphical data are displayed as the mean average of raw data readings, with ± SEM indicated for each. Statistical analyses were carried out using multiple 2-sample t-tests, with the significance level for all hypotheses taken as <0.05.

**Ethical Approval**

Ethical approval was granted by Cardiff School of Health Sciences at Cardiff Metropolitan University (project reference number: 9381).

**Results**

**EGCG is cytotoxic to THP-1s in vitro within 24hrs**

Given the known adverse effects of REDOX activity on cells in high concentrations, we investigated the viability of THP-1 cells in the presence of several concentrations of EGCG. The membrane-impermeable dye propidium iodide (PI) was used to evaluate membrane integrity on a single cell level analysis\[25\] due to its proven performance when used in the presence of anti-oxidative agents\[26\]. By intercalating with extraneous genetic material, the absorbance of PI at FL-2 is proportional to the number of cells present with a compromised membrane and/or those that are dead. As shown in Figure
PI absorbance was only markedly increased when THP-1s had been exposed to 200µM EGCG, with ~60% increase in absorbance at 24hrs and over a four-fold increase at 48hrs (p=0.029 and p=0.003, respectively). There was a 13% increase in absorption observed at 24hrs in the presence of 100 µM EGCG, the significance of this result was just above our previously determined threshold of significance (p=0.05). Despite a 79% increase in absorption at 48hrs with 100 µM EGCG, the difference between the two means was found not to be statistically sound (p=0.061), and therefore 100 µM EGCG was deemed the highest appropriate concentration of EGCG to use for extended incubations with THP-1s.

Figure 1

Figure 1: The effect of increasing concentrations on THP-1 cell viability at 24hrs and 48hrs via PI flow cytometry. Data shown are percentage viability relative to that for the respective test group negative control (one each for 24hrs and 48hrs). Visually, an increase in PI absorbance (and therefore a decrease in viable cell count) was seen from 24hrs to 48hrs incubation. The increase in PI absorbance at 200µM EGCG was the only concentration found to induce a statistically significant increase in both the 24hrs and 48hrs test groups (p=0.029 and p=0.03, respectively). SEM error bars are shown for each data set.
Aspirin is not a significant agonist for CD36 cell surface expression on THP-1s

Prior literature research identified aspirin as an up-regulator of CD36 expression in THP-1 cells\[^{27}\]. With the aim to titrate the aspirin concentration to maximise CD36 cell surface expression, we investigated 3 concentrations within the range reported to be effective in previous literature (1 µM, 5 µM and 10 µM). All tests were carried out in triplicate and with 24hrs incubation. As aspirin has a reportedly low solubility in water we used the organic solvent DMSO to assist in dissolving aspirin into water. As a result of this we included a DMSO vehicle control. We found no evidence of any significant up-regulation of CD36 as a result of treatment with any of the concentrations of aspirin used, as can be seen in Figure 2. The possibility that DMSO negatively affected the efficacy of aspirin in this context was ruled out by the lack of a significant down-regulation of CD36 in the presence of the DMSO vehicle control (9.2% decrease compared to cells only control \((p=0.342)\). The secondary antibody only control confirmed that little to no non-specific binding of the conjugated secondary antibody to the cell surface was occurring (Mean FL-1 Absorbance was around 8% of that for control containing both primary and secondary antibody).

Figure 2

![Figure 2: The relative effect of increasing concentrations of aspirin on THP-1 cell surface CD36 level via indirect flow cytometry. Data shown are relative to that for cells only 0mM aspirin control and have been adjusted to account for natural fluorescence of](image)
THP-1s. Although visually the DMSO vehicle control appears to lower cell surface CD36 level, this decrease was not statistically significant \((p=0.342)\). Statistical analysis showed that the +5.2%, +4.9% and +4.5% increases for 1 mM, 5 mM and 10mM aspirin respectively were not significant \((p=0.501, p=0.546\) and \(p=0.553\), respectively). SEM error bars are shown for each data set.

**Rosiglitazone is an effective, dose-dependent agonist for CD36 cell surface expression on THP-1s**

Due to a lack of significant increase of CD36 expression induced by aspirin (see Figure 2), further testing to identify an efficacious positive control was required. Subsequent literature research indicated that rosiglitazone, resveratrol, luteolin and genistein were alternative potential agonists of PPAR-\(\gamma\) and/or CD36 expression\(^{28-31}\). Substances were tested at two concentrations, previously determined as safe in literature, each to gauge the potency of the potential agonists. The no antibody control confirmed THP-1 natural fluorescence at FL-1 was insignificant. Resveratrol appeared to have an inverse effect on CD36 expression; inducing a large (~44% at 5\(\mu\)M, \(p=0.021\)) increase in CD36 cell surface expression at the lower concentration, compared to a smaller increase of ~31% at higher concentration (10 \(\mu\)M), which was found to be statistically insignificant \((p=0.136)\). Luteolin proved to be ineffective at both concentrations tested (~15.8% at 5\(\mu\)M \((p=0.688)\) and ~15.7% at 10\(\mu\)M \((p=0.579)\), respectively). Genistein produced a significant agonistic (~51% \((p=0.044)\) effect at the higher concentration (20\(\mu\)M) tested, however at the lower concentration (10\(\mu\)M) the observed differences were deemed statistically insignificant. Rosiglitazone produced the highest increase in CD36 of all potential agonists tested, and was significantly agonistic at both concentrations used: ~37% at 5\(\mu\)M \((p=0.04)\) and ~59% at 10\(\mu\)M \((p=0.028)\). See Figure 3 for representation of results. Having shown to be a potent exogenous agonist for CD36 expression in THP-1s at both concentrations, rosiglitazone was chosen to be the appropriate agonist for comparison of CD36 EGCG-induced cell surface levels.
Figure 3: The effect of rosiglitazone, resveratrol, luteolin and genistein on THP-1 cell surface CD36 levels via indirect flow cytometry. Data shown are relative to the cells only negative control and have been adjusted to account for natural fluorescence of THP-1s. All four chemicals tested appeared to increase the relative level of cell surface CD36, however only rosiglitazone induced a significant increase at both concentrations tested (+37% at 5µM \((p=0.04)\) and +59% at 10µM \((p=0.028)\)). The only other significant increase was observed in genistein at 20µM (+51% \((p=0.044)\)). All other variances in cell surface CD36 observed were found to not be statistically significant \((p>0.05)\). SEM error bars are shown for each data set.

EGCG is not a significant antagonist for CD36 cell surface receptor expression in THP-1s in vitro

Test cells were first treated with rosiglitazone (10mM) to mimic higher, disease state cell surface levels of CD36. In both the test cells (rosi +) and the negative control cells (rosi -) EGCG treatment induced no significant change, either positive or negative, in CD36 cell surface levels at either of the concentrations of EGCG tested (50µM and 100µM): there was no significant difference in the mean average readings compared to their relative controls (rosi -, 50 µM EGCG: \(P=0.141\); rosi -, 100 µM EGCG \(P=0.450\); rosi +, 50 µM EGCG \(P=0.931\); rosi +, 100 µM EGCG \(P=0.937\)): see Figure 4. In an attempt to ensure representative results, the same number of cells (20,000) were analysed per test where possible. However, it should be noted that for three rosi +
readings less than 20,000 (two for 0µM EGCG and one for 100µM EGCG) cells were analysed, due to the lack of cells present in the sample and the resulting extended length of test reading by the flow cytometer. Moreover, in these samples a markedly lower proportion of healthy cells were present: proportion of healthy cells present ranged from 70.4% - 86.4% and 58.8% - 86.1% for rosi – and rosi + respectively. The readings for the secondary antibody only negative control confirmed that there was no significant non-specific binding of the secondary, conjugated antibody to the THP-1 cell surface (16% and 14% of rosi-/rosi + cells only, respectively).

Figure 4

![Figure 4: The effect on THP-1 CD36 cell surface level in the presence of both EGCG and rosiglitazone](image)

Figure 4: The effect of both EGCG and rosiglitazone on THP-1 cell surface CD36 levels via indirect flow cytometry. Data shown are relative to each test group cells only negative control (rosi +/-rosi -) and have been adjusted to account for natural fluorescence of THP-1s. On average, all data showed a lower CD36 cell surface level than the negative cells only controls, with 50µM EGCG (rosi -) inducing the largest decrease (~31%). However, no data were found to be significantly different to that of their respective cells only controls (p>0.05). SEM error bars are shown for each data set.
**Discussion**

In 2010, the marketing authorisation for the use of rosiglitazone as an anti-diabetic drug was suspended across Europe by The European Committee on Medicinal Products for Human Use; the cardiovascular risks associated with the drug were found to outweigh the benefits in all patient groups\(^{[32]}\). Our investigation of the effect EGCG has on PPAR-\(\gamma\), via surrogate marker CD36, had the potential to determine whether EGCG could either be an efficacious alternative to, or have synergistic properties with, rosiglitazone.

Previously, EGCG has not only been shown to affect cell mobility and adhesion in THP-1s, but to also induce apoptosis in several cell types\(^{[33]}-[35]\). In light of this, we chose to examine THP-1 cell viability with EGCG using a similar methodology to that which would be applied throughout the remainder of our investigation: via a PI flow cytometry assay. Although this method allowed us to assess cellular morphology and membrane permeability/cytolysis on a single cell basis, a major shortfall of this technique is that it is unable to highlight any internal metabolic changes that have yet to translate to physical changes. To account for this, an MTT assay could be used in conjunction with a PI flow cytometry assay to give a truer representation of the effect EGCG has on cell viability.

Our intention for the indirect aspirin assay was to titrate for the most appropriate concentration to stimulate cell surface CD36 based on aforementioned research\(^{[27]}\), however, our findings were contradictory to what this paper reported. Although we tested concentrations within the range this paper had stated significantly induced CD36 cell surface expression, there was one major discrepancy between our methodologies: the previous research incubated with aspirin for 45 hours whilst we incubated for only 24hrs; this may account for the inconsistency between our findings. It is possible that what changes aspirin may induce in CD36 expression, as we know aspirin is an agonist of PPAR-\(\gamma\) expression at mRNA level\(^{[36]}\), did not have time to translate to the cell surface, or cross-talk within the cell interrupted this. Alternatively, it is possible that the acetylating mechanism of action of aspirin\(^{[37]}\), on serine residues in particular, had an effect on the conformation of CD36 on the cell surface: subsequently affecting the binding of the primary antibody to the antigen, reducing the sensitivity by inducing a potentially false negative result.
Following the inadequate level of CD36 induction via aspirin, we determined that rosiglitazone was an effectual, dose-dependent agonist of CD36 cell surface expression at 5µM and 10µM. This result was somewhat unsurprising as rosiglitazone, a thiazolidinedione, has a well-documented history of PPAR-γ stimulation: indeed, the basis of its former use as an anti-diabetic drug was largely based on this mechanism. Arguably, the induced increase of CD36 as a result of rosiglitazone treatment may have been a contributor to the increase in cardiovascular risk observed with this drug: there is a marked increase in CD36 expression when differentiation of macrophages from monocytes occurs in the formation of foam cells in atherosclerosis[38]. The clinical significance of rosiglitazone in this capacity was our rationale for using it over resveratrol 5µM or genistein 20µM, the only test conditions to induce a significant increase in CD36 other than rosiglitazone, as the positive control moving forward. An argument can be made for the use of oxidised-LDLs (Cu²⁺ ox-LDL) as the PPAR-γ agonist, as a prototype of LDL modification, and thus a truer representation of the processes occurring in the diabetic atherosclerotic model[39]. There is also the possibility that rosiglitazone is a superagonist of PPAR-γ in comparison with the endogenous ox-LDL ligand; in order to make accurate conclusions about the potential effects our research would have in the diabetic model, further investigation would be needed as to the pharmacodynamics of rosiglitazone in comparison with ox-LDL on PPAR-γ binding and stimulation.

Our work found no significant change, either with or without prior rosiglitazone treatment, in THP-1 cell surface CD36 levels after treatment with EGCG. The lack of a significant change in CD36 level from the test group with no rosiglitazone pre-treatment would indicate that EGCG does not stimulate PPAR-γ, if it binds to it at all: this has potential clinical significance in that EGCG treatment does not appear to have atherosclerotic potential via the mechanism of monocyte lipid internalisation and foam cell formation. Further research into the mechanisms by which rosiglitazone had anti-diabetic effects is required for comparison: for example, in the diabetic model rosiglitazone upregulates GLUT 4 expression[40] to effectively lower blood glucose level; if EGCG proves to be efficacious in this same pathway, without the associated cardiovascular risk, EGCG could be deemed an appropriate alternative to rosiglitazone for anti-diabetic treatment. Having said that, the lack of any significant decrease of cell surface CD36 after EGCG treatment excludes the PPAR-γ pathway as the mechanism
by which EGCG attenuates atherosclerosis. Ligand binding analysis assays are needed to elucidate as to the interaction, if there is any, between EGCG and PPAR-γ. The lack of any significant change observed in the test group that was exposed to prior treatment with rosiglitazone shows that EGCG neither exacerbates nor reverses the atherogenic effects of rosiglitazone. As such, EGCG is not an appropriate co-treatment with the objective of mitigating the associated cardiovascular risk of rosiglitazone. However, it should be noted that prior rosiglitazone treatment duration was only 1 hour before EGCG was introduced, whereas our previous assay analysing the effect rosiglitazone had on cell surface CD36 expression involved a 24-hour incubation period; as such the reliability of our rosiglitazone test group is questionable. To rectify this, I would suggest either further investigating the effect induced on cell surface CD36 expression after just 1 hour incubation with rosiglitazone; or, to extend the initial rosiglitazone treatment period to 24hrs before introducing EGCG. It is possible that what changes rosiglitazone treatment induced at mRNA level did not have sufficient time to translate to the cell surface before it was interrupted by crosstalk stemming from the introduction of EGCG. Further investigation to analyse and quantify mRNA expression levels of PPAR-γ would provide only a limited further insight: mRNA-level variations may be considered irrelevant if they don’t result in a physical, phenotypic change.

Interestingly in the test group that had prior rosiglitazone treatment, we observed three samples that did not reach the desired cell count of 20,000. This could indicate that cell viability was affected when THP-1s were exposed to both rosiglitazone and EGCG and highlights a major flaw in our methodology: on reflection, cell viability in the presence of both substances should have been evaluated, and the subsequent reduction in viable cells may have been the result of a stress response[41].

Further research using oxidised low-density lipoproteins (ox-LDLs) as the agonist for PPAR-γ, introducing EGCG, could potentially affect this pathway: EGCG is known to interact with LDLs by inhibiting their oxidation both in vitro and in vivo[42], and ox-LDLs, with EGCG exhibiting significant lipoprotein bound antioxidant activity[43]. If EGCG can significantly affect and alter the ox-LDL ligand for PPAR-γ it is possible that the atherogenic potential may be mitigated with intravenous EGCG.

It is important to note that CD36 is not specific to THP-1s and is involved in other pathways. For example, hepatocyte CD36 has been shown to have a direct contribution
to fatty liver development; furthermore, the disruption of the internalisation of fatty acids in hepatocytes by CD36 has been shown to protect against systemic inflammation and insulin resistance[44]. Investigation of the effect EGCG has on the PPAR-γ pathway in human hepatocytes could have clinical significance in the treatment of diabetes.

In conclusion, EGCG has no significant effect on THP-1 cell surface expression of CD36, and does not attenuate the atherogenic effect induced by rosiglitazone; as such it is not an appropriate co-treatment. Whilst EGCG has no adverse cardiovascular effects via the PPAR-γ pathway, it has no treatment potential for the suppression of the formation of monocyte-derived macrophage foam cells. Further research into the alternative mechanisms by which EGCG may attenuate atherosclerosis is needed.

References


