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The molecular mechanisms underpinning the therapeutic properties of oleanolic acid, its isomer and derivatives for type 2 diabetes and associated complications

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Abstract

Recent research has uncovered the molecular mechanisms responsible for the therapeutic properties of oleanolic acid (OA), its isomer ursolic acid (UA), and derivatives. In particular, recent reports have highlighted the benefits of these compounds in the prevention and treatment of type 2 diabetes and associated life-threatening complications, such as nonalcoholic fatty liver disease, nephropathy, retinopathy, and atherosclerosis. The prevalence of type 2 diabetes is of major concern since it is reaching global epidemic levels. Treatments targeting the signaling pathways altered in type 2 diabetes are being actively investigated, and OA and UA in natural and derivative forms are potential candidates to modulate these pathways. We will explore the findings from in vitro and in vivo studies showing that these compounds: (i) improve insulin signaling and reduce hyperglycemia; (ii) reduce oxidative stress by upregulating anti-oxidants and; (iii) reduce inflammation by inhibiting proinflammatory signaling. We will discuss the molecular mechanisms underpinning these therapeutic properties in this review in order to provide a rationale for the future use of OA, UA, and their derivatives for the prevention and treatment of type 2 diabetes and associated comorbidities.

Keywords

Cell signaling, Inflammation, Oleanolic acid derivatives, Oxidative stress

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The molecular mechanisms underpinning the therapeutic properties of oleanolic acid, its isomer and derivatives for type 2 diabetes and associated complications

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Keywords: Oleanolic acid derivatives; cell signalling; inflammation; oxidative stress

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Abbreviations: oleanolic acid (OA), ursolic acid (UA), high fat diet (HFD), insulin receptor (IR), insulin receptor substrate (IRS), phosphoinositide 3 kinase (PI3K), Protein kinase B (Akt), glucose transporter 4 (GLUT4), protein tyrosine phosphatase 1B (PTP1B), glucose 6 phosphate (G6P), forkhead box protein O1 (FOXO1), aldose reductase (AR), sorbitol dehydrogenase (SDH), advanced glycation end product (AGE), glycated haemoglobin ($HbAl_c$), N^{ε}-(carboxymethyl) lysine (CML), chronic kidney disease (CKD), fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), nuclear factor kappa B (NF-κB), tumour necrosis factor alpha (TNFα), suppressor of cytokine signalling 3 (SOCS3), nuclear factor kappa b inhibitor alpha (IκB), phosphorylated IκB kinase (IKK), TNF receptor (TNFR), interleukin 1 (IL-1), nuclear factor like 2 (Nrf2), reactive oxygen species (ROS), aspartate aminotransferase (AST), alkaline phosphatase (ALP), carbon tetrachloride (CCl4), glutathione peroxidase (GSHpx), superoxide dismutase (SOD), kelch-like ECH-associated protein 1 (Keap1), anti-oxidant response element (ARE), inducible nitric oxide synthase (iNOS), CDDO-Me (Bardoxolone Methyl), NADPH dehydrogenase quinone 1 (Nqo1), glutamate cysteine ligase catalytic subunit (GCLC), glomerular filtration rate (GFR).

Abstract: Recent research has uncovered the molecular mechanisms responsible for the therapeutic properties of oleanolic acid (OA), its isomer ursolic acid (UA) and derivatives. In particular, recent reports have highlighted the benefits of these compounds in the prevention and treatment of type 2 diabetes and associated life-threatening complications, such as non-alcoholic fatty liver disease, nephropathy, retinopathy and atherosclerosis. The prevalence of type 2 diabetes is of major concern since it is reaching global epidemic levels. Treatments targeting the signalling pathways altered in type 2 diabetes are being actively investigated, and OA and UA in natural and derivative forms are potential candidates to modulate these pathways. We will explore the findings from in vitro and in vivo studies showing that these compounds: 1) Improve insulin signalling and reduce hyperglycaemia; 2) Reduce oxidative stress by upregulating anti-oxidants and; 3) Reduce inflammation by inhibiting proinflammatory signalling. We will discuss the molecular mechanisms underpinning these therapeutic properties in this review in order to provide a rationale for the future use of OA, UA and their derivatives for the prevention and treatment of type 2 diabetes and associated comorbidities.

1. Introduction

Type 2 diabetes has reached epidemic proportions worldwide. Recent predictions indicate that the prevalence of diabetes globally will increase from 285 million in 2010 to 439 million in 2030 [1]. Along with hyperglycaemia and reduced insulin sensitivity, other characteristics featured in type 2 diabetes include proinflammation and oxidative stress which contributes to damage to tissue in the liver, kidney, adipose tissue, pancreas and vasculature. Type 2 diabetes can lead to the progression of a number of potentially life-threatening macrovascular and microvascular complications, including non-alcoholic fatty liver disease, nephropathy, retinopathy and atherosclerosis [2]. Currently available anti-diabetic drugs have limited

efficacy and/or safety concerns. Therefore, identifying new medicinal agents, especially extracted from natural products, offers exciting possibilities for future development of successful anti-diabetic therapies.

2. Oleanolic Acid, its isomer and derivatives

Both oleanolic acid (OA) and its isomer, ursolic acid (UA) exist widely in nature and can be extracted from fruits, herbs and vegetables. OA can be found in olive leaves, olive pomace, mistletoe sprouts and clove flowers, whilst UA can be found in apple pomace. A mixture of these two triterpenes can also be found in rosemary leaves [3]. Both OA and UA are pentacyclic triterpenes, which is a group of widespread natural compounds containing six isoprene units, the basic molecular formula C30H48 and with five rings in their skeleton [3, 4]. Recently, OA and UA have received great attention because of their benefits including anti-hyperglycaemic, anti-hyperlipidemic, anti-inflammatory and anti-oxidative properties and potential application for the treatment of type 2 diabetes and associated complications [5-12]. Although they differ only in the position of a side chain in their structure, a number of in vitro and animal studies have demonstrated that OA and UA exhibit different degrees of potency in particular functions including their direct binding to insulin signalling molecules such as PTP1B [13-16].

Triterpenoids are structurally similar to steroids and may, like steroids, diffuse freely through cell membranes to interact with intracellular molecular targets. The semisynthetic triterpenoid 2-cyano- 3,12-dioxooleana-1,9(11)-dien-28-oic acid (CDDO) has been developed along with chemically modified derivatives containing various functional groups on rings A and C [17, 18]. These novel compounds are far more potent than natural triterpenoids and can affect signalling pathways in mammalian cells that are associated with detoxification [19], inflammation, and apoptosis [20, 21]. These sites can be accentuated and manipulated

through chemical modification of the natural compound into derivative form. Examples of highly potent synthetic OA derivatives are the CDDO derivatives, which are strong anti-oxidant compounds. In particular, the OA CDDO derivative, CDDO-Me (Bardoxolone Methyl) highlights the promising potential of these compounds as it has successfully completed phases I and II of human clinical trials [5, 22].

3. OA and UA lower blood glucose levels by improving insulin Akt signalling

Several studies have demonstrated the ability of OA and UA in normalising blood glucose levels in rodents with diet-induced obesity or diabetes [6-9, 15, 23]. In particular, in two preventative studies where mice were administered OA or UA at a dosage of 10mg/kg in conjunction with being fed a high fat diet (HFD) for 15 weeks, blood glucose levels were significantly lower compared to non-triterpene HFD controls by 37% and 42% respectively [7, 8]. This demonstrates the strong hypoglycaemic effects of UA and OA and implicates that their effects are caused by targeting insulin signalling and/or glucose producing molecules.

Recent evidence suggests that OA, UA and a number of their derivatives can improve insulin signalling by enhancing IR β subunit phosphorylation and Akt *in vitro* [24-26]. Insulin regulates glucose homeostasis through binding of its receptor to initiate a signalling cascade; activation and phosphorylation of the insulin receptor substrate proteins (IRS), and mediation of the phosphatidylinositol 3-kinase-dependent/the protein kinase Akt (PI3K/Akt) pathway (Fig 1). The activation of the Akt pathway can 1) mediate the translocation of glucose transporter 4 (GLUT4) to the plasma membrane, thereby facilitating glucose uptake into adipose tissue, cardiac muscle and skeletal muscle [27-32], and 2) inhibit glucose production via gluconeogenesis by glucose 6 phosphate (G6P) and forkhead box protein O1 (FOXO1) in liver.

OA and UA promote glucose uptake from the bloodstream into peripheral tissues through upregulation of GLUT4 [27-32]**.** In an *in vitro* study, UA promoted glucose uptake by enhancing the translocation of GLUT4 to the plasma membrane in 3T3-L1 adipocytes [24]. 3T3-L1 adipocytes treated with an OA derivative, NPLC441, had increased GLUT4 mRNA and protein expression compared to untreated cells indicating increased glucose uptake into the cells [26, 33]. A glucose uptake assay in L6 myotubes revealed that a 1μM dose of an OA derivative increases basal glucose uptake by 40% [26, 33].

Another mechanism of OA and UA in lowering blood glucose is by the reduction of endogenous glucose production via inhibition of gluconeogenesis in the liver. Glucose production via gluconeogenesis can exacerbate hyperglycaemic states, and favours the development and progression of type 2 diabetes. Key molecules in the gluconeogenic pathway are glucose 6 phosphate (G6P) and forkhead box protein O1 (FOXO1) [34, 35]. 0.05% UA supplement in diet decreased glucose-6-phosphatase activity in the livers and significantly elevated the hepatic glycogen content in STZ and high-fat diet-induced diabetic mice [34, 35]. Adding 0.05% OA extracted from dietary wine pomace in HF diet significantly downregulated the mRNA expression of G6P (49%) and FOXO1 (52%) in liver of rats [34, 35].

4. OA and UA inhibit PTP1B resulting in improved insulin signalling

Protein tyrosine phosphatase 1B (PTP1B) has been proposed as a novel target whose inhibition would specifically address insulin resistance. Protein tyrosine phosphatase 1B (PTP1B) is a molecule that negatively regulates insulin signalling [15, 26, 36]. Several *in vitro* studies have provided evidence that OA, UA and a number of their derivatives can directly inhibiting PTP1B and improve insulin sensitivity [15, 26, 36] (Fig 1). In particular, a PTP1B inhibition assay concluded that OA and UA adhered to the linear mixed type

inhibition model in their interaction with PTP1B [15]. Interestingly, the binding site of PTP1B targeted by OA and UA was uncovered to be a secondary region, known as site B rather than the typical catalytic binding site A [15]. This suggests that compounds that have high specificity for this region should be developed, such as through modifying OA and UA, to derivative forms in order to achieve strong PTP1B inhibition and subsequent maximal improvement to insulin signal transduction. In addition, the OA and UA derivatives (C-28 addition) were more potent than their natural structures by 22 and 10 fold in inhibition of PTP1B activity respectively [25, 33]. PTP1B can inhibit the PI3K/Akt signalling pathway to induce insulin resistance by inhibiton of the translocation of GLUT4 to the plasma membrane [37] and causes disinhibition of FOXO1, thereby promoting reduction of glucose reuptake and gluconeogenesis. Therefore, the direct inhibition of PTP1B by OA, UA and their derivatives enables signal transduction of insulin and thus improves insulin sensitivity.

In addition to insulin sensitisation, inhibition of PTP1B also has the potential to promote weight loss, which is a benefit since obesity largely contributes to the type 2 diabetic pathology. Oral administration of OA, UA and their derivatives decreased body weight gain in high-fat diet induced obese rodents [6-9, 15, 23]. PTP1B-deficient mice were resistant to weight gain and remained insulin-sensitive when subjected to a high-fat diet, while the amount of food consumed was not different [38]. The increased insulin sensitivity of PTP1B knockout mice cannot explain the reduced weight gain on a high-fat diet [38]. Several *in vitro* studies demonstrated that PTP1B is a negative regulator of the leptin-JAK2-STAT3 signalling pathway. The development of small molecule competitive inhibitors of PTP1B with modifying the UA and OA might provide novel therapeutic agents for obesity and type 2 diabetes.

5. OA and UA inhibit keap1 causing Nrf2 activation and subsequent reduced oxidative stress and tissue damage

In type 2 diabetes, hyperglycaemia promotes an increase in free radicals and decrease in anti-oxidants causing increased lipid peroxidation. Free radicals such as reactive oxygen species (ROS) can be detrimental since they can diffuse into cells causing damage to the mitochondrial enzymes and DNA, which subsequently leads to cellular dysfunction [9]. ROS are generated by oxidative stress such as the conversion of sorbitol to fructose in the polyl pathway [35]. In particular, ROS have been found to play a role in kidney fibrosis [39, 40]. A study has shown that damaged tubular cells in kidneys exacerbate ROS leading to apoptosis following unilateral ureteral obstruction [41].

One of the complications of type 2 diabetes is hepatocellular enzyme leakage, indicated by an increase in plasma enzyme activity of aspartate aminotransferase (AST) and alkaline phosphatase (ALP), which eventually results in severe liver damage [10]. A hepatoprotective effect of OA has been observed in diabetic mice through a reduction in the activity of ALP and AST, suggesting reduced hepatoxicity [10]. In animal studies, OA and UA treatment decreased liver damage induced by oxidative stress inducing chemicals such as carbon tetrachloride (CCl4) [42]. OA and UA also increased the activities of the anti-oxidant enzymes superoxide dismutase (SOD) and glutathione peroxidase (GSHpx) [10, 42, 43]. Increased levels of these anti-oxidant enzymes reduced the levels of free radicals lipid peroxidation [10]. The anti-oxidative effects of these compounds appear to be beneficial for the treatment and prevention of type 2 diabetes and associated complications, including oxidative stress induced liver damage.

Excess glucose in the blood promotes renal and hepatic tissue damage, and the polyl pathway is a major contributor to this damage. The function of this pathway is to metabolise unused

glucose and it is activated during hyperglycaemic states. The key enzymes in this pathway are aldose reductase (AR) and sorbitol dehydrogenase (SDH), which facilitate the production of sorbitol and fructose. The elevated sorbitol and fructose levels that occur due to the polyl pathway results in an increase in advanced glycation end product (AGE) formation and glycative injury [44-46]. AGEs such as glycated haemoglobin ($HbAl_c$), N^{ε}-(carboxymethyl) lysine (CML) and glycated albumin are thought to be involved in the development of diabetic nephropathy, with CML and glycated albumin shown to contribute to its progression [47-50]. OA and UA administration in diabetic mice has been found to reduce the renal and liver activity of AR and SDH and mRNA expression of AR causing suppression of the polyl pathway via decreased sorbitol and fructose production and AGE formation [13, 35]. OA treatment can also upregulate mRNA expression of glyoxalase I, an enzyme that metabolises the AGE precursor methylglyoxal [13, 51]. The suppression of these molecules integral to the polyl pathway and inhibition of AGEs, including precursors, by OA and UA ameliorates liver and kidney injury [35]. This may hinder the progression of type 2 diabetes related complications of the liver and kidneys, including diabetic nephropathy, chronic kidney disease (CKD) and non-alcoholic fatty liver disease (NAFLD).

Nuclear factor like 2 (Nrf2) promotes the transcription of many anti-oxidant genes [52-54], and its intracellular interactions are summarised in figure 2. Nrf2 is usually bound to its inhibitor kelch-like ECH-associated protein 1 (Keap1) in the cytoplasm. An increase in oxidative or electrophillic stress inducing agents such as ROS causes Keap1 to lose its ability to inhibit Nrf2 which results in the disassociation of Nrf2 from Keap1[55]. Nrf2 can then translocate into the nucleus where it binds to the anti-oxidant response element (ARE) to stimulate the transcription of anti-oxidant genes [56-58]. The activation of Nrf2 and concurrent inactivation of Keap1 results in a reduction of oxidative stress and inflammation in a variety of tissues including the kidneys, liver and retina. Nrf2 activation results in a

reduction of blood urea nitrogen levels and the amelioration of glomerular and tubular injury in the kidneys [59]. In the livers of knockout and knockdown Keap1 mice, Nrf2 activation causes reduced hepatic inflammatory genes including IL-1β, IL-6 and TNFα [60]. Following induced retinal ischemia reperfusion (IR), Nrf2 knockout mice have been found to have increased inflammatory cells, increased inducible nitric oxide synthase (iNOS) and oxidative stress compared to wild type mice [57]. Nrf2 up-regulates the antiapoptotic protein B-cell lymphoma (Bcl-2) and prevents cellular apoptosis [61]. In addition, Keap1 has been shown to promote a proinflammatory response through binding with the p65 subunit of NF-κB in HepG2 and HEK293 cells [62]. This suggests that Nrf2 activation influences both inflammation and oxidative stress. OA, UA and derivatives have been found to have anti-inflammatory and anti-oxidative effects, which may be credited to Nrf2 activation. Briefly, the mechanisms behind this effect include inhibition of pro-inflammatory signalling and increasing the transcription of anti-oxidants; both of which are associated with Nrf2 activation. Therefore, further studies are warranted to determine if this effect is due to activation of Nrf2, which may be the optimum drug target for decreasing inflammation and oxidative stress.

OA and a number of synthetic derivatives of OA including CDDO-Me (Bardoxolone Methyl), CDDO-TFEA, CDDO-Im and CDDO-Ea have been found to activate Nrf2 signalling [15,72, 74]. OA has been found to increase Nrf2 activation and heme oxygenase 1 (Hmox1) expression causing a reduction in fibrosis and apoptosis in mice with unilateral ureteral obstruction [41]. Bardoxolone Methyl and CDDO-TFEA attenuate retinal damage, such as in diabetic retinopathy, via Nrf2 activation and subsequent transcription of several anti-oxidant genes including Hmox1, NADPH dehydrogenase quinone 1 (Nqo1) and glutamate cysteine ligase catalytic subunit (GCLC) [57, 63]. The treatment of retinal IR induced mice with bardoxolone methyl increased retinal superoxide levels and reduced

capillary degeneration by 60%. In addition to decreasing retinal damage, CDDO-Im has been found to induce the phosphorylation of Akt in retinal epithelial cells. On inhibition of the PI3K/Akt pathway, CDDO-Im treatment had no effect in inducing Hmox1 transcription, reiterating the relationship between Akt activation and Hmox1 expression [63]. This demonstrates a link between Nrf2 and Akt signalling pathways, and supports the previously described effect of these compounds on insulin signalling. This also suggests that the anti-oxidative and anti-inflammatory effects of Nrf2 activation may also be influenced by the activity of the PI3K/Akt pathway, perhaps through inhibition of PTP1B. Therefore, Nrf2 activation by OA derivatives appears to be a promising target for reducing oxidative stress in type 2 diabetes and associated complications, such as diabetic retinopathy.

Clinical application of OA and an OA derivative: Bardoxolone methyl

OA is currently used as a dietary supplement in traditional Chinese medicine for treating liver injuries, inflammatory diseases, various types of cancers and diabetes [10, 64-67]. However, investigation of highly potent OA derivatives, including the OA CDDO derivative, bardoxolone methyl is still currently undergoing several human clinical trials to test its potential future use in a clinical setting. Bardoxolone methyl has successfully completed phase I and II of human clinical trials for treating CKD in type 2 diabetics, and phase I clinical trials for the treatment of leukaemia and solid tumours [68], indicating its potential in treating multiple diseases. The phase IIb human clinical trial study in 227 patients with type 2 diabetes and CKD showed that bardoxolone methyl improved kidney function with minimal side effects and no sign of hepatic injury [5]. The therapeutic effects of bardoxolone methyl were through upregulation of Nrf2 and Hmox1 expression in various regions of the kidneys [59]. Since bardoxolone methyl has successfully completed phase II of human clinical trials with positive benefits in patients with CKD and type 2 diabetes, this compound has potential

clinical applications in the treatment of kidney disease in type 2 diabetes. The ability of bardoxolone methyl to activate Nrf2 may reduce oxidative stress and inflammation in other tissues such as the liver and retina, thereby ameliorating tissue damage in individuals with type 2 diabetes and prevent the development of associated microvascular and macrovascular complications. Further scientific investigation of the effect of bardoxolone methyl is needed in the future to determine if this drug has a similar effect in promoting Nrf2 activation in other tissues and organs and whether another molecule, such as PTP1B is responsible for its therapeutic effects.

Despite the number of benefits of OA and the OA derivative bardoxolone methyl, caution for specific populations should be taken when applied to patients with severe chronic kidney, hepatic and/or heart diseases. In a clinical setting, the dose of OA can be as high as 80mg three times per day for months in humans [69]. However, caution must be taken since it has been reported that OA can cause hepatotoxicity in long term use or if the dose is too high (>500μmol/kg per day) in mice [69]. . Phase III of human clinical trials testing bardoxolone methyl in patients with end stage chronic kidney disease (stage 4 and up) was terminated due to a higher incidence of cardiovascular events compared to the placebo group [70]. Therefore, future human clinical trials using bardoxolone methyl should monitor blood pressure and heart function of participants, and overall caution should be taken in patients with a higher risk of cardiovascular events.

Recruitment for a human clinical trial in patients with pulmonary arterial hypertension (NCT02036970) is currently being undertaken in order to determine the efficacy and safety of bardoxolone methyl in this population, which is proposed to be completed by June 2015. This will aim to address safety issues in the phase III human clinical trials in advanced chronic kidney disease patients.

6. OA and UA inhibit NF-κB and inflammatory cytokines resulting in reduced inflammation

Overnutrition leads to an accentuated proinflammatory state in several tissues including adipose tissue, liver, skeletal muscle, pancreas and the hypothalamic region of the brain [71]. Inflammation of these tissues contributes to hyperglycaemia, insulin resistance and type 2 diabetes [71]. On a molecular level, proinflammatory signalling is mediated by nuclear factor kappa B (NF-κB) activation. The proinflammatory NF-κB signalling pathway in the target cell is summarised in figure 3. Briefly, NF-κB remains inactive when bound to and inhibited by nuclear factor kappa b inhibitor alpha (IκB) in the cytoplasmic region of the cell. NF-κB is activated by phosphorylated IκB kinase (IKK), which stimulates its translocation into the nucleus. Once NF-κB is in the nucleus, it regulates the expression of a variety of molecules such as the cytokine tumour necrosis factor alpha (TNFα). TNFα secreted due to the activation of the NF-κB can also increase the production of ROS, contributing to the development and progression of co-morbidities associated with type 2 diabetes such as cardiovascular disease [72]. This proinflammatory signalling pathway is a positive feedback loop since TNFα can bind to TNF receptor (TNFR) resulting in the phosphorylation and activation of IKK then subsequent NF-κB interaction. NF-κB activation can also promote the expression of the negative regulators of insulin signalling, PTP1B and suppressor of cytokine signalling 3 (SOCS3), thereby reducing insulin sensitivity and subsequent glucose regulation [61-64].

OA and UA have been found to reduce proinflammation by inhibiting proinflammatory signalling molecules and cytokines [73-75]. OA reduced NF-κB signalling by inhibiting lipopolysaccharide (LPS) induced phosphorylation of IkB, and subsequently the expression of the cytokines TNFα and interleukin 1 (IL-1) [34, 76]. UA administration in mice fed a

HFD also inhibited signalling through the NF-κB pathway [77]. The OA derivative, CDDO-Me has been found to directly influence proinflammatory signalling in Human U-937 myeloud leukemia cells by inhibiting IKK which causes blocking of the NF-κB pathway [78]. This OA derivative has also been found to suppress LPS induced inflammation in normal human PBMC cells by reducing the expression of the cytokines IL-6 and TNFα [79]. However, a very high concentration of CDDO-Me was required to suppress NF-κB in macrophages, suggesting that NF-κB signalling is not the only target by this compound and that its effects may occur through another pathway, possibly through Nrf2 [78, 80].

7. Nrf2, NF-κB and PI3K/Akt signalling: molecular pathways inextricably linked that contribute to the therapeutic effects of OA, UA and derivatives

It has been found that Nrf2, NF-κB and PI3K/Akt signalling pathways crosstalk. Several studies have demonstrated that an increase in PI3K/Akt activity has been linked to Nrf2 activation [81-85]. For example, recently, it was found that 3,4-dihydroxybenzalacetone administration enhanced Nrf2 activation, which was abolished with the treatment of a PI3K or Akt inhibitor, suggesting a role of the PI3K/Akt pathway in Nrf2 activation [85]. Furthermore, Nrf2 has been found to be activated as a result of NF-κB-induced inflammation and ROS production as a defensive response [86, 87]. The ability of OA, UA and/or its derivatives to influence the activity of Nrf2, NF-κB and the PI3k/Akt signalling suggests potential targets of these compounds in these molecular signalling pathways. Further studies are required to elucidate the exact mechanisms linking Nrf2, NF-κB and PI3K/Akt pathways to induce the therapeutic benefits of OA, UA and derivatives in type 2 diabetes and associated complications.

Conclusions, commentaries and future directions

In summary, OA and its isomer, UA target signalling molecules that increase insulin signal transduction, and reduce inflammatory and oxidative stress signalling. OA and UA's promotion of insulin signalling has been demonstrated to occur through enhancement of IRβ subunit phosphorylation, upregulated Akt, and increased glucose uptake via GLUT4. In addition to improved Akt signalling, OA and UA also reduce glucose production by targeting FOXO1 and G6P. OA and UA directly inhibit the negative regulator of insulin signalling, PTP1B. In addition, OA and UA reduce inflammation through reduction of NF-κB signalling and inhibition of cytokines such as IL-6 and TNFα, and increase antioxidant production via promotion of Nrf2 signalling.

Furthermore, modification of these triterpenes such as at C-28 leads to a higher potency in their interactions, such as in the anti-inflammatory and anti-oxidative properties of bardoxolone methyl. OA CDDO derivatives including bardoxolone methyl, have been found to reduce oxidative stress through activating Nrf2 signalling to stimulate antioxidant production, and reduce inflammation by reducing proinflammatory cytokine expression and NF-κB signalling. The potential of OA, UA and their derivatives for clinical applications has been highlighted by bardoxolone methyl, which has been effective in phase II of human clinical trials for treating chronic kidney disease in patients with type 2 diabetes where activation of Nrf2 reduced tissue damage. However, caution should be taken in higher doses and particular populations such as patients with advanced stages of chronic kidney disease. With further research and human clinical trials, the possibility of using OA, UA and their derivatives for the treatment and prevention of type 2 diabetes and their complications appears promising.

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Figure legends

Figure 1

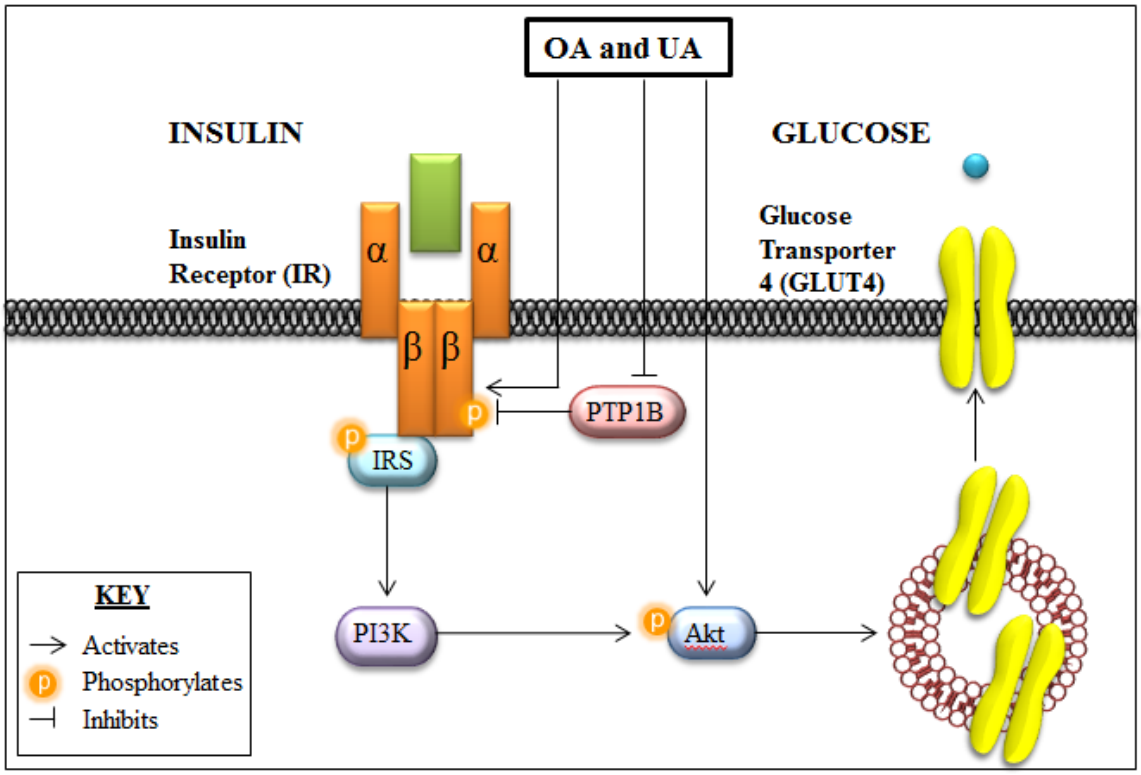

Figure 1. Ursolic acid (UA) and oleanolic acid (OA) effects on PTP1B inhibition of PI3K/Akt insulin signalling and glucose uptake in the target cell. Insulin binds to the insulin receptor (IR) at the α subunits resulting in a conformational change. Tyrosine residues on the β subunits phosphorylate resulting in downstream insulin signalling. PTP1B inhibits insulin signalling. Insulin receptor substrate (IRS) proteins are activated resulting in subsequent activation of phosphoinositide 3 kinase (PI3K) and Protein kinase B (Akt). In peripheral tissues including cardiac muscle, skeletal muscle and adipose tissue, Akt activates the translocation of glucose transporter 4 (GLUT4), which is sequestered in vesicles before activation, into the plasma membrane. GLUT4 facilitates glucose uptake into the cell. Oleanolic acid (OA) and ursolic acid (UA) treatment inhibits PTP1B by directly binding to site B. This causes improved insulin signal transduction.

Figure 2

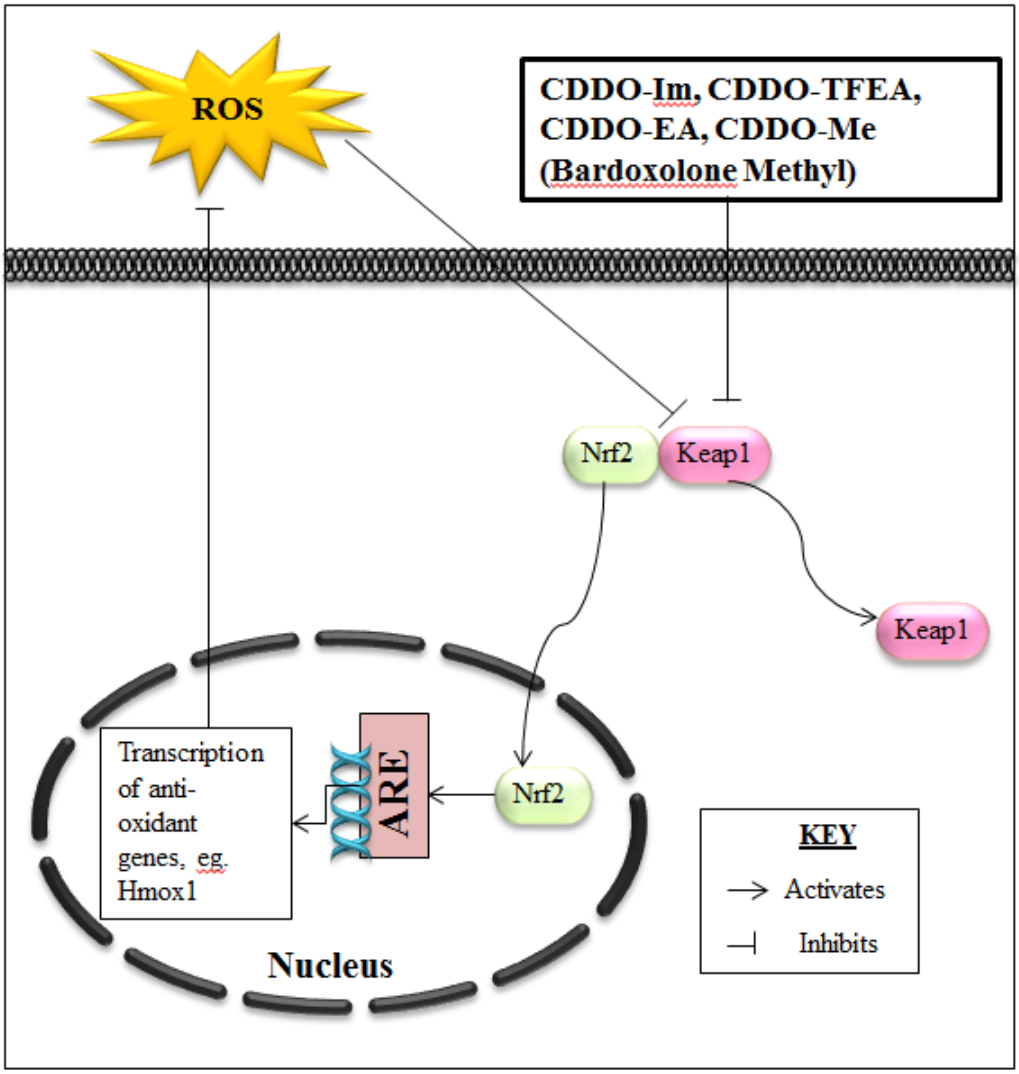

Figure 2. The effects of oleanolic acid (OA) CDDO derivatives on nuclear factor like 2 (Nrf2) activation. Oxidative stress such as reactive oxygen species (ROS) reduces the inhibitory activity of Kelch-like ECH-associated protein 1 (Keap1) on Nrf2. Nrf2 disassociates from Keap1 resulting in subsequent translocation of Nrf2 into the nucleus. Nrf2 binds to the anti-oxidant response element (ARE) to promote the transcription of a number of anti-oxidant genes such as Hmox1. Anti-oxidant enzymes transcribed by Nrf2 can inhibit ROS. The OA CDDO derivatives, CDDO-Im, CDDO-TFEA, CDDO-EA and CDDO-Me (Bardoxolone methyl) activate Nrf2 by reducing Keap1 inhibition of Nrf2.

Figure 3

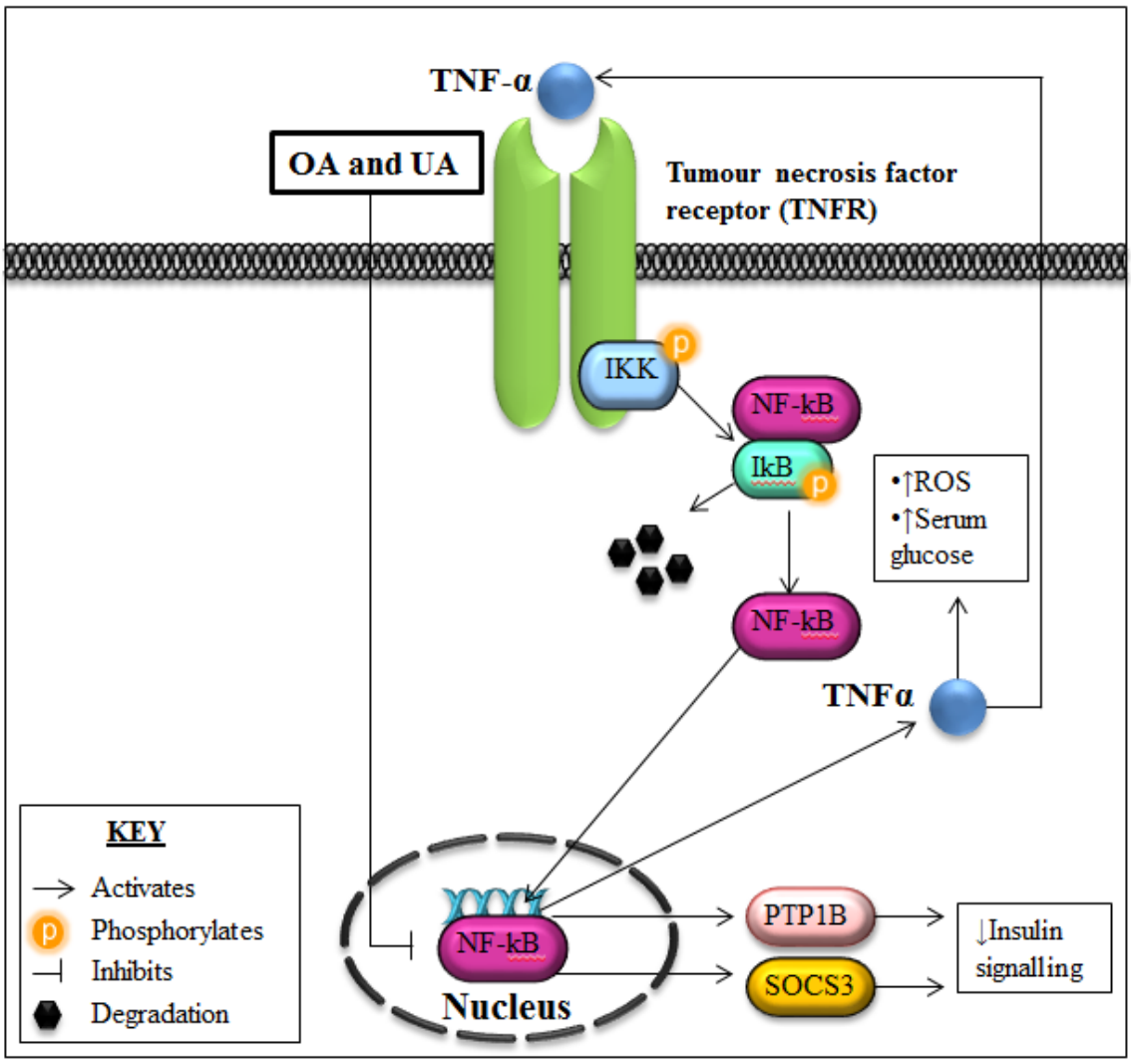

Figure 3. Ursolic acid (UA) and oleanolic acid (OA) effects on proinflammatory signalling in the target cell. Tumour necrosis factor alpha (TNFα) binds to the TNF receptor (TNFR). IκB kinase (IKK) is phosphorylated causing nuclear factor kappa b inhibitor alpha (IκB) phosphorylation. Disassociation of IκB from nuclear factor kappa B (NF-κB) and subsequent degradation. NF-κB is translocated into the nucleus where it functions to activate the transcription of a variety of molecules. NF-kB regulates the expression of TNFα, protein tyrosine phosphatase 1B (PTP1B) and suppressor of cytokine signalling 3 (SOCS3) , which are negative regulators of insulin signalling,. TNFα can then bind to the TNFR causing a detrimental feedback loop. UA has been found to inhibit NF-κB activation in mice whilst OA has been found to reduce NF-κB translocation into the nucleus by inhibiting IκB phosphorylation.