Chapter 5

To investigate the role of indirubin 3'-monoxime (I3M)-induced $A_{2A}AR$ signalling in alleviating lipid-induced adipocyte insulin resistance

5.1 Introduction

Insulin resistance is an impairment of insulin-stimulated glucose disposal in insulin-responsive cells which is a major defect and early sign for future development and progression of type 2 diabetes (T2D) pathogenesis [1,2]. Over the last decade, mounting evidence has emerged demonstrating a close link between a state of chronic low-level inflammation in adipose tissue and obesity-induced insulin resistance and T2D [2–9]. Increasing accumulation of intra-abdominal adipose tissue in obese subjects is frequently associated with the enhanced rate of free fatty acids (FFAs) mobilization and higher levels of circulating FFAs [10,11] which trigger inflammatory pathways that compromise insulin sensitivity [2–9]. Consequently, targeting adipose tissue inflammation by genetic knockdown of inflammatory receptors or mediators has a beneficial effect on insulin sensitivity and glucose homeostasis [8–14]. Accumulating evidence highlights a critical role of adenosine signaling in the regulation of insulin synthesis from the pancreatic beta cells and also modulate the insulin responsiveness in adipose tissue, muscle, and liver that governs glucose homeostasis [15,16]. The purine nucleoside adenosine is an endogenous signaling molecule, and its physiological level is very low in the extracellular microenvironment. In response to cellular insult by metabolic stress, tissue injury and inflammation, increased accumulation of extracellular adenosine exerts a range of responses by binding with adenosine receptors (ARs) to succumb cellular homeostasis [15–17]. Adenosine mediate its effects by binding to specific G-protein–coupled ARs which are widely distributed in metabolically active sites such as adipose tissue, liver, pancreas and various immune cells. Among the four different subtypes of AR, A_1, A_{2A}, A_{2B}, and A_3 [16], adenosine orchestrates its anti-inflammatory effect through the activation of $A_{2A}AR$ and $A_{2B}AR$ [18–22] and therefore the signaling pathway of these receptors subtypes are more intensely studied to counter the pathophysiology of various inflammatory diseases including T2D. Natural products, particularly phytochemicals, have been traditionally utilized for the management of

various human diseases [23] and to develop various derivatives with reduced toxic side effects, improved pharmacokinetics and enhanced efficacy [24]. Indirubin, an active component of Indigo naturalis and 3,2' bis-indole isomer of indigo, has been shown to be the main ingredient of the traditional Chinese herbal medicine, Danggui Longhui Wan, which is used to treat various leukemias and inflammatory diseases [25–28]. Indirubin-3'-monoxime (I3M) is one of the most studied synthetic indirubin derivatives exhibiting higher potency and bioavailability in comparison to its parent compound [29]. Indirubin and its analogs act as potent inhibitors of cyclin-dependent kinases, glycogen synthase kinase-3β, JAKS/Src family kinase and nuclear factorkappa B [29–32] which confers their potential therapeutic efficacies as anti-cancer, anti-angiogenic, anti-viral, anti-aging, anti-inflammatory and anti-diabetic functions. However, the underlying mechanisms of many of these effects remain largely unexplained and that considerably delays their integration into the modern healthcare system. In searching for an effective A_{2A}AR agonist, investigators primarily relied on the modification of adenosine [33] considering a time long notion that preserving sugar moiety is critical for receptor activation [34]. We found that I3M, a non-adenosine chemotype, is capable of binding and activating A_{2A}AR signaling which effectively attenuates lipid-induced adipocyte inflammation and insulin resistance. Thus, our study revealed that I3M could be an effective therapeutic alternative to alleviate lipid-induced adipocyte inflammation and insulin resistance.

5.2 Results:

5.2.1 I3M directly binds with and stimulates A_{2A}AR signaling

To test the cytotoxicity of I3M, we performed the LDH release assay that affirms nontoxic nature of I3M (Fig. 5.1A). To determine the concentration of I3M that gives a half-maximal response (EC_{50}), we investigated cAMP activity at varying concentrations of I3M in the CHO cells stably over expressing A_{2A}AR (Fig 5.1B,C) and found the EC_{50} value of I3M is 0.12 μM (Fig 5.1D). To further confirm the A_{2A}AR ligand nature of I3M, a radioligand binding assay was performed. The radioligand competition curve of I3M competing for [^{3}H]NECA (10 nM) to bind with A_{2A}AR showed the Ki value of 0.83 μM where nonspecific binding amounts ∼10% of total binding (Fig. 5.1E).

Radioligand binding assay displayed a potent high-affinity binding of I3M with $A_{2A}AR$ (Ki: 0.52 µM). These results support the notion that I3M is a potent agonist of $A_{2A}AR$.

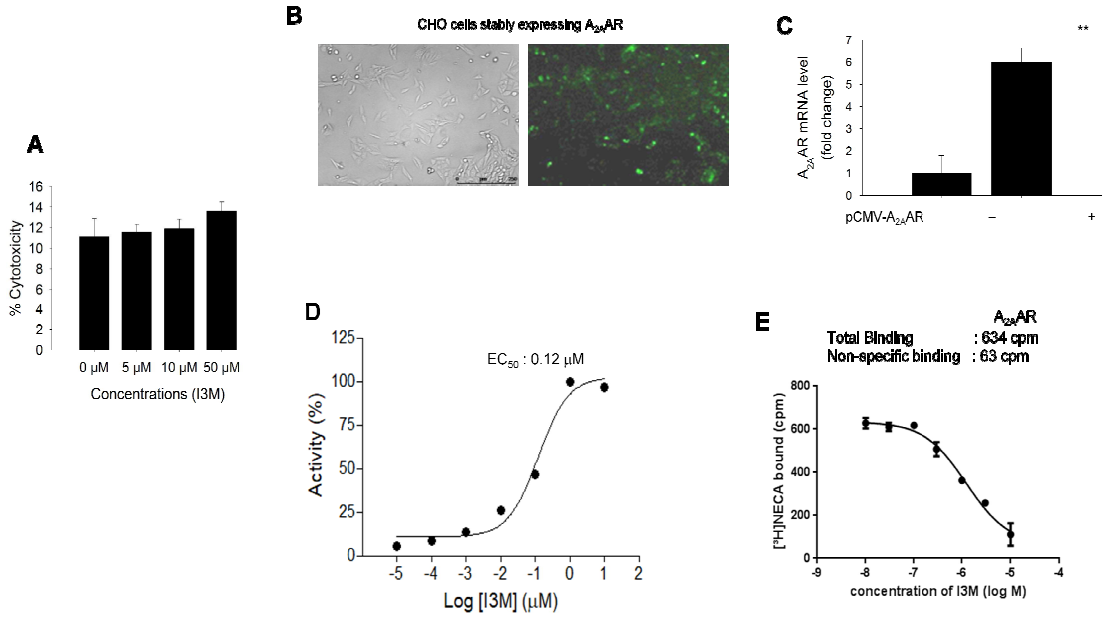


Fig 5.1 (A) LDH release assay indicates % cytotoxicity in response to indicated concentrations of I3M treated 3T3-L1 adipocytes. **(B)** Cellular image of $A_{2A}AR$ stable clone of CHO cells. Scale bar, 250 mm. **(C)** RT-qPCR analysis of $A_{2A}AR$ gene expression in CHO cells stably transfected with or without $A_{2A}AR$ plasmid. All experiments were performed in triplicate. Each value is the mean ± SEM of three independent experiments. **p < 0.01; *p < 0.05. **(D)** $A_{2A}AR$ stably expressed CHO cells were incubated with the indicated concentrations of I3M followed by the determination of EC_{50} value of I3M by measuring percentage activity of cAMP assay. % activity = 100 × [(A − Abasal)/(Amax − Abasal)], where A is the sample absorbance, Amax is the absorbance at maximum stimulation, and A basal is the absorbance at basal level (without I3M). **(E)** Representative curve with I3M competing for [^{3}H]NECA (10 nM) binding to $A_{2A}AR$. The Ki value is 0.83 µM. The total binding is 634 cpm and non-specific binding is 63 cpm. Nonspecific binding amounts to about 10% of total binding.

To substantiate the $A_{2A}AR$ agonistic nature of I3M, $A_{2A}AR$ stably expressed CHO cells were incubated with I3M followed by the treatment of increasing concentrations of SCH 58261. Stimulation of CREB phosphorylation in response to I3M was significantly attenuated with increasing concentrations of SCH 58261 (Figure 5.2A). These results support the notion that I3M is a potent agonist of $A_{2A}AR$. Furthermore, to investigate whether a selective $A_{2A}AR$ agonist could able to mimic the effects of I3M, we observed that treatment with $A_{2A}AR$ selective agonist CGS 21680 noticeably up-regulated CREB phosphorylation (Fig. 5.2B) and insulin-stimulated glucose uptake (Fig. 5.2C) in 3T3-L1 adipocytes.

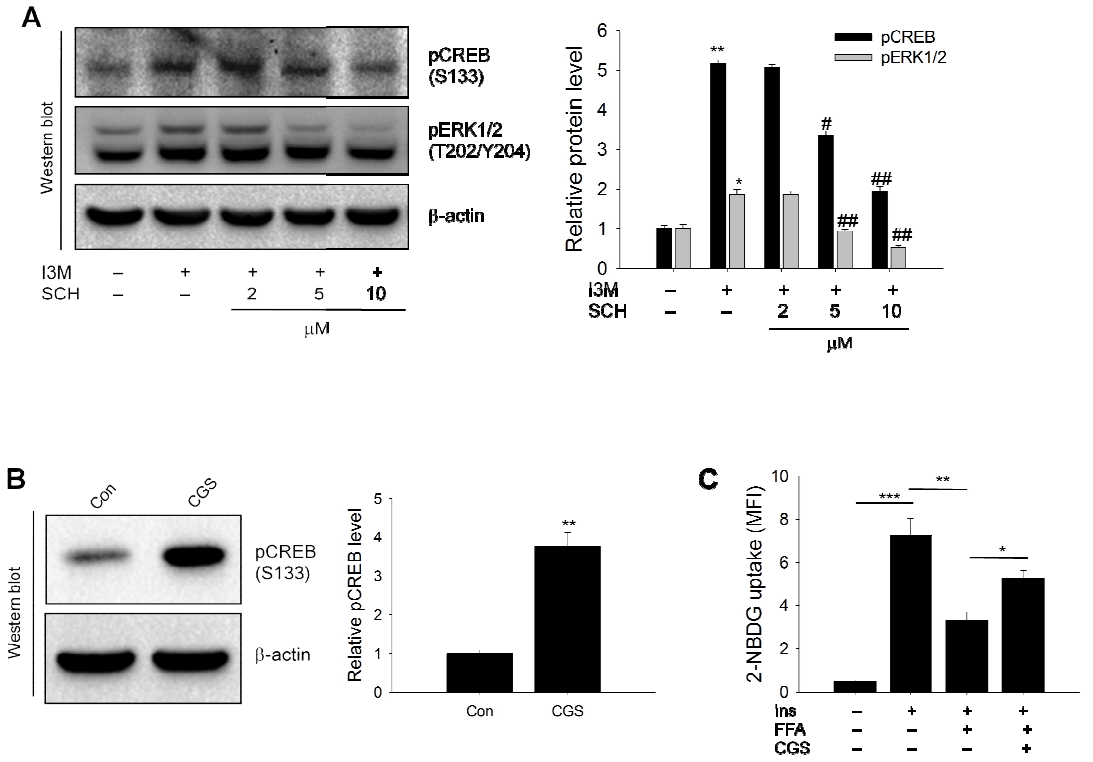
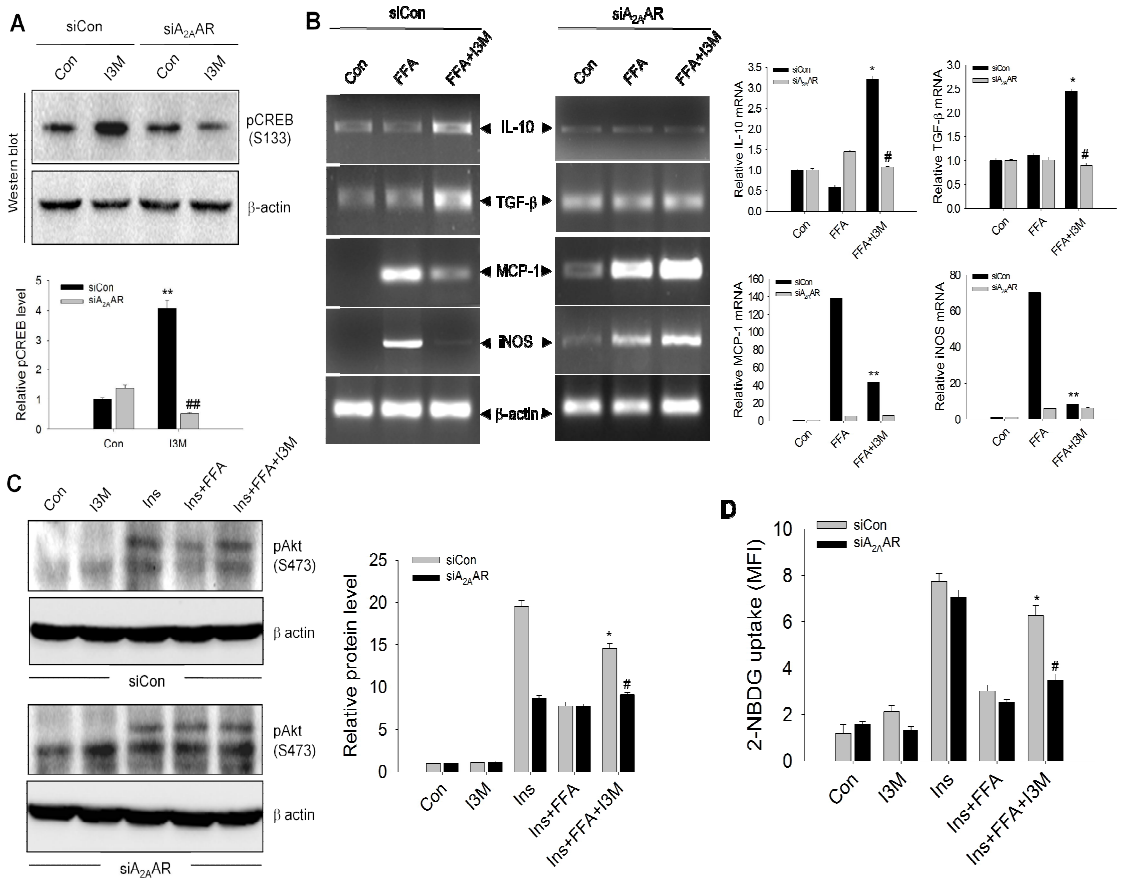


Fig. 5.2(A) Western blots (left) and its quantification (right) showing pCREB (S133) abundance in 3T3-L1 adipocytes incubated without or with A_{2A}AR specific agonist CGS 21680 (CGS, 1 mM). b-actin was serves as loading control. (B)Analysis of 2-NBDG uptake by 3T3-L1 adipocytes in response to insulin (Ins, 100 nM) or Ins + FFA (palmitate, 0.75 mM) in absence or presence of CGS 21680 (CGS, 1 mM). All experiments were performed in triplicate. Each value is the mean ± SEM of three independent experiments. **p < 0.01; *p < 0.05.

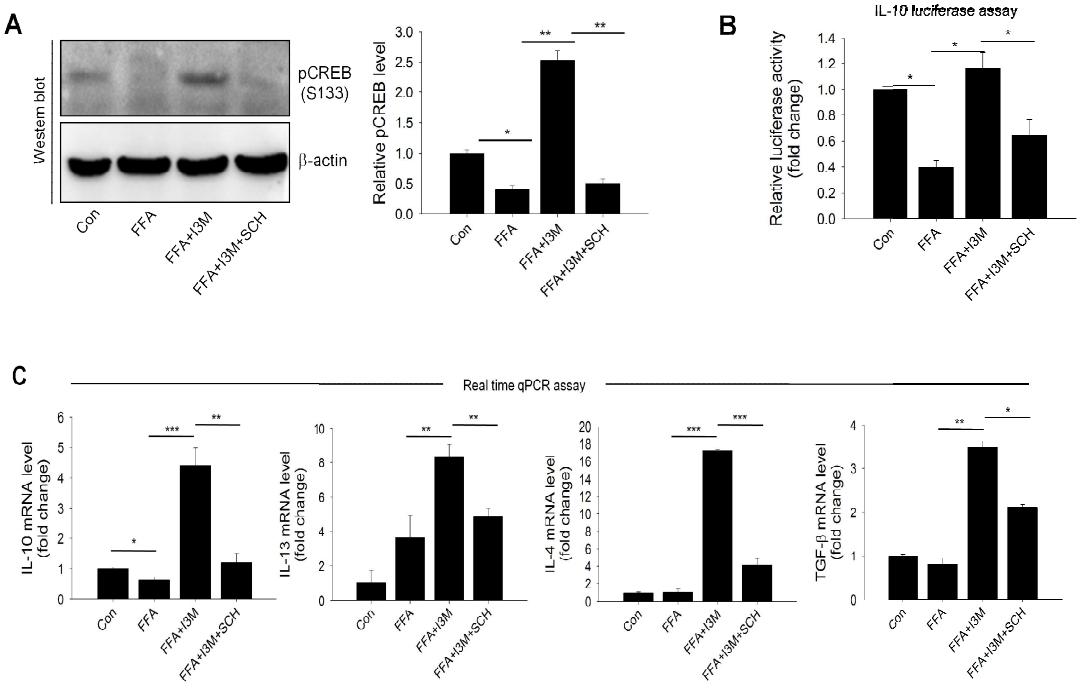
To secure direct evidence of A_{2A}AR involvement in I3M effect, we examined the efficacy of I3M on CREB activation, inflammatory mediators' expressions and insulin signaling in A_{2A}AR silenced 3T3-L1 cells. I3M-induced CREB phosphorylation was considerably attenuated in A_{2A}AR knockdown cells (Fig 5.3A). I3M incubation markedly up-regulates the anti-inflammatory (IL-10 and TGF-β) gene expressions and prevents FFA-induced pro-inflammatory mediators (MCP-1 and iNOS) gene expressions in 3T3-L1 adipocytes, however, such effects were significantly waived in A_{2A}AR silenced cells (Fig 5.3B). We also observed that I3M notably attenuates FFA-induced impairment of Akt activation and insulin-stimulated glucose uptake which were significantly compromised in A_{2A}AR silenced cells (Figure 5.3C,D). All these results indicate the participation of A_{2A}AR in I3M mediated effect, however, it would be interesting to note that I3M alone was unable to activate Akt phosphorylation and glucose uptake (Figure 5.3C,D) that void the possibility of the direct effect of I3M on insulin signaling cascade.



5.3 (A) Western blots (left) and its quantification (right) showing pCREB (S133) abundance in control siRNA (siCon) and $A_{2A}AR$ siRNA ($siA_{2A}AR$) transfected 3T3-L1 adipocytes incubated without or with I3M (10 μM). β-actin was used as a loading control. Each value is the mean ± SEM of three independent experiments, ** $P < 0.01$ vs control siRNA transfected cells control cells, ## $P < 0.01$ vs control siRNA transfected cells treated with I3M. (B) RT-PCR analysis (left) and its quantification (right) showing pro-inflammatory (MCP-1 and iNOS) and anti-inflammatory (IL-10 and TGF-β) markers gene expressions in 3T3-L1 adipocytes treated without (Con) or FFA (0.75 mM) in absence or presence of I3M (10 μM). β-actin was used as a loading control. Each value is the mean ± SEM of three independent experiments, ** $P < 0.01$, * $P < 0.05$ vs control siRNA transfected cells treated with FFA, # $P < 0.05$ vs control siRNA transfected cells treated with FFA + I3M. (C) Western blots (left) and its quantification (right) showing pAkt (S473) abundance in control siRNA (siCon) and $A_{2A}AR$ siRNA ($siA_{2A}AR$) transfected 3T3-L1 adipocytes incubated without (Con) or with I3M (10 mM) or Insulin (Ins, 100 nM) or Ins + FFA (palmitate, 0.75 mM) or Ins + FFA + I3M. β-actin was used as a loading control. (D) Analysis of 2-NBDG uptake by 3T3-L1 adipocytes transfected with control siRNA (siCon) and $A_{2A}AR$ siRNA ($siA_{2A}AR$) in response to I3M (10 μM) or Insulin (Ins, 100 nM) or Ins + FFA (palmitate, 0.75 mM) or Ins + FFA + I3M. Each value is the mean ± SEM of three independent experiments, * $P < 0.05$ vs control siRNA transfected cells treated with Ins + FFA, # $P < 0.05$ vs control siRNA transfected cells treated with Ins + FFA + I3M.

5.2.2 I3M promotes anti-inflammatory state in adipocytes through the activation of A_{2A}AR signalling.

We next explored the induction of A_{2A}AR signaling by I3M on the stimulation of anti-inflammatory state of adipocytes in the *in-vitro* model of lipid-induced insulin resistance. I3M incubation markedly prevents palmitate induced suppression of CREB phosphorylation in adipocytes which was strikingly attenuated by SCH 58261 (Fig. 5.4A). Investigating the I3M-mediated induction of A_{2A}AR signaling in the transactivation potential of CREB, we found that incubation of palmitate significantly attenuates IL-10 luciferase reporter activity in 3T3-L1 adipocytes which was considerably rescued by I3M. However, the I3M effect was diminished when cells were pre-treated with SCH 58261 (Fig. 5.4B). We then explored the efficacy of I3M-mediated A_{2A}AR signaling on gene expression of various anti-inflammatory cytokines. I3M distinctly up regulated IL-10, IL-13, IL-4 and TGF-β gene expression, which were considerably, attenuated by SCH 58261 incubation (Fig. 5.4C). Moreover, I3M-induced IL-10 protein secretion from 3T3-L1 adipocytes was significantly inhibited by SCH 58261 (Fig. 5.4D). To examine the direct effect of I3M, we observed dose dependent CREB activation and anti-inflammatory (IL-10 and TGF-β) gene expressions in 3T3-L1 adipocytes in response to I3M incubations (Fig. 5.4E,F).



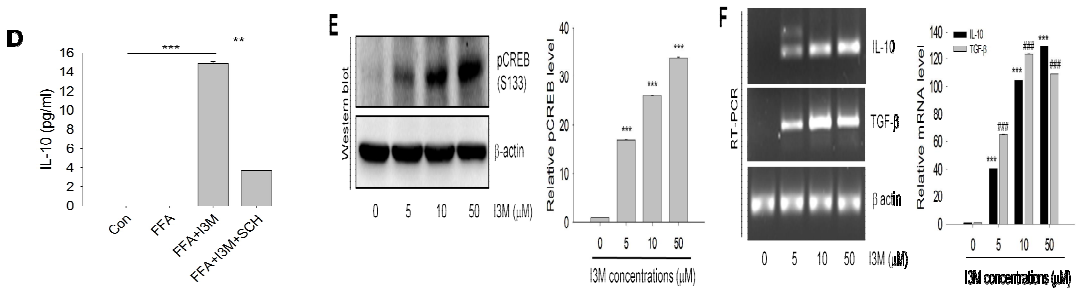


Figure 5.4 (A) Western blots (left) and its quantification (right) showing abundance of pCREB (S133) level in 3T3-L1 adipocytes incubated without or with FFA (palmitate, 0.75 mM) in absence or presence of I3M (10 mM) without or with SCH 58261 (SCH, 300 nM). β-actin was used as loading control. (B) 3T3-L1 adipocytes transfected with IL-10 promoter-luciferase plasmid (pGL2B −1538/+64) were incubated without or with FFA (palmitate, 0.75 mM) in absence or presence of I3M (10 mM) or I3M (10 mM) + SCH 58261 (SCH, 300 nM). On termination of incubations, cells were lysed and luciferase activity was measured by the multimode reader. (C) Real-time quantitative PCR analysis showing fold change of IL-10, IL-13, IL-4 and TGF-β mRNA level in 3T3-L1 adipocytes incubated without or with FFA (palmitate, 0.75 mM) in absence or presence of I3M (10 mM) or I3M (10 mM) + SCH 58261 (SCH, 300 nM). GAPDH served as an internal control for normalization. All experiments were performed in triplicate. Each value is the mean ± SEM of three independent experiments, *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$. (D and E) Western blots (left) and its quantifications (right) showing pCREB (S133) level (D) and RT-PCR analysis (left) and its quantification (right) showing anti-inflammatory cytokines (IL-10 and TGF-β) gene expressions (E) in 3T3-L1 adipocytes in response to different concentrations of I3M. β-actin was serves as loading controls. All experiments were performed in triplicate. Each value is the mean ± SEM of three independent experiments, *** $P < 0.001$ Vs Con, ### $P < 0.001$ Vs Con.

Further, to clarify the role of CREB as a mediator of I3M anti-inflammatory actions, we suppressed CREB activity in adipocytes by transfecting A-CREB, a dominant negative mutant of CREB [35], and investigated its role on anti-inflammatory gene expressions. Interestingly, I3M effected induction of IL-10 and TGF-β gene expressions were markedly attenuated in A-CREB transfected adipocytes (Fig. 5.5A). It is now well established that adipocytes inflammatory state play a critical role in regulating systemic insulin sensitivity [2-9]. We therefore envisioned that I3M mediated alteration of inflammatory factors expressions in adipocytes could able to prevent the FFA-induced insulin resistance. To address this issue, we incubated L6 myotubes with the conditional media isolated from I3M incubated adipocytes and investigated the insulin-stimulated muscle glucose uptake. Intriguingly, I3M incubated adipocytes conditional media notably mitigate the FFA-induced impairment of insulin stimulated glucose uptake in L6 myotubes (Fig. 5.5B). Collectively, all these results suggest that I3M strongly promotes an anti-inflammatory state in adipocytes through the activation of the A_{2A}AR-

cAMP-CREB pathway which could be sufficient to improve FFA-induced insulin resistance.

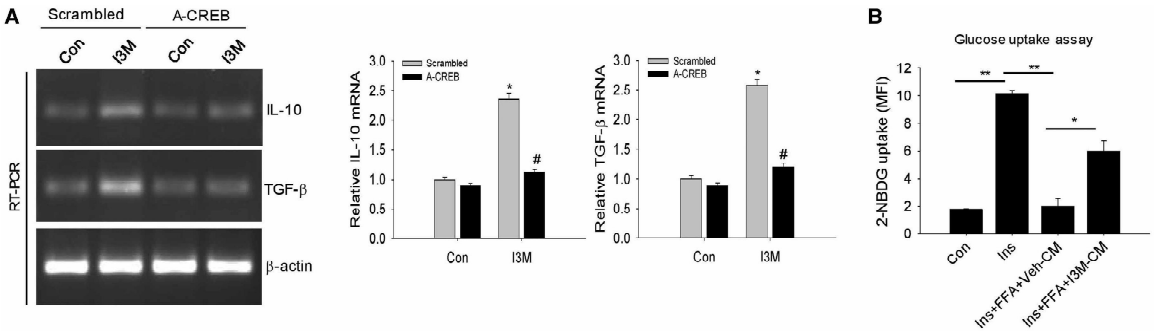
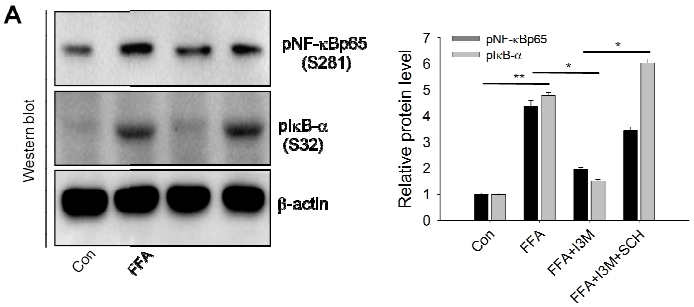


FigurE 5.5 (A) 3T3-L1 adipocytes transfected with scrambled plasmid or CMV500 A-CREB (dominant-negative inhibitor of CREB) followed by the incubations without (Con) or with I3M (10 μM). On termination of incubations, cells were subjected for RT-PCR analysis of IL-10 and TGF-β gene expressions. β-actin was used as a loading control. * $P < 0.05$ vs scrambled transfected control cells, # $P < 0.05$ vs scrambled transfected I3M treated cells. (B) Effect of conditional media, isolated from the control or I3M (10 μM) treated 3T3-L1 adipocytes, on 2-NBDG uptake in FFA-induced insulin-resistant L6 myotubes. All experiments were performed in triplicate. Each value is the mean ± SEM of three independent experiments, ** $P < 0.01$, * $P < 0.05$.

5.2.3 Activation of A_{2A}AR by I3M attenuates lipid induced adipocyte inflammation.

We and several other investigators have shown that FFA-induced activation of nuclear factor-κB (NF-κB) stimulates chronic low-grade inflammation in adipose tissue causing obesity induced insulin resistance [2-9]. Interestingly, palmitate induced NF-κBp65 and IκBα phosphorylation in 3T3-L1 adipocytes was substantially impeded by I3M. However, the beneficial effect of I3M was compromised when cells were treated with SCH 58261 (Fig. 5.6A). Examining the direct effect of I3M on the inflammatory markers in 3T3-L1 adipocytes, we did not notice any significant change of NF-κB activation and its target genes (MCP-1 and iNOS) expression (Fig. 5.6B,C) in response to I3M incubation.



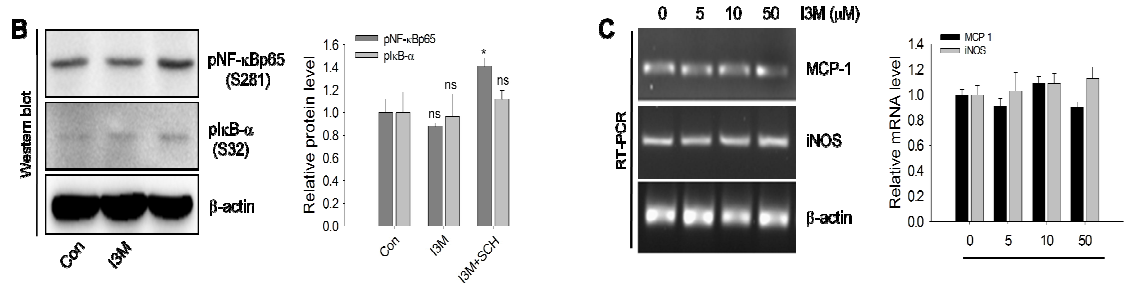


Figure 5.6.(A) Western blots (left) and its quantification (right) showing pNF-κB (S281) and pIκB-α (S32) abundance in 3T3-L1 adipocytes incubated without or with FFA (palmitate, 0.75 mM) in absence or presence of I3M (10 μM) or I3M (10 μM) + SCH 58261 (SCH, 300 nM). β-actin was used as loading control. (B) Western blots (left) and its quantification (right) showing pNF-kB (S281) and pIkB-a (S32) abundance in 3T3-L1 adipocytes treated without (Con) or with indirubin -3'-monoxime (I3M, 10 mM) in absence or presence of SCH 58261 (SCH, 300 nM). b-actin was serves as loading control. (C) RT-PCR analysis (left) and its quantification (right) of MCP-1 and iNOS gene expressions in 3T3-L1 adipocytes treated with different concentrations of I3M (0, 5, 10, and 50 μM). b-actin was serves as loading control. All experiments were performed in triplicate. Each value is the mean ± SEM of three independent experiments. *p < 0.05. ns = non significant.

To have a better insight of I3M effect and the contribution of A_{2A}AR in attenuating palmitate stimulation of NF-κB transactivation potential, we investigated κB luciferase reporter activity in 3T3-L1 adipocytes. Palmitate-enhanced κB luciferase reporter activity was significantly reduced in I3M treated cells, however, I3M effect was reversed when cells were pre-treated with SCH 58261 (Fig. 5.7A) indicating that I3M downregulates NF-κB transactivation competence by activation of A_{2A}AR. Since FFA-induced activation of PKCs, particularly PKCθ and PKCε, are known to be involved in the negative action on insulin signalling [36], we examined the effect of I3M on PKC activation and observed that FFA-induced stimulation of PKC phosphorylation was strikingly prevented by I3M, however, such effect was considerably hindered by SCH 58261 (Fig. 5.7B).

We then examined the expression of NF-κB-regulated genes and found I3M incubation remarkably prevents palmitate-induced upregulation of MCP-1, IL-6, IL-1β and TNF-α cytokines gene expression which was significantly inhibited by SCH 58261 (Fig. 5.7C). Moreover, a significant inhibition of FFA-induced IL-6 protein secretion was observed when 3T3-L1 adipocytes were pre-treated with I3M, however, I3M effect was relinquished in response to SCH 58261 (Fig. 5.7D). All these results suggest I3M via A_{2A}AR activation alleviate lipid-induced adipocyte inflammation.

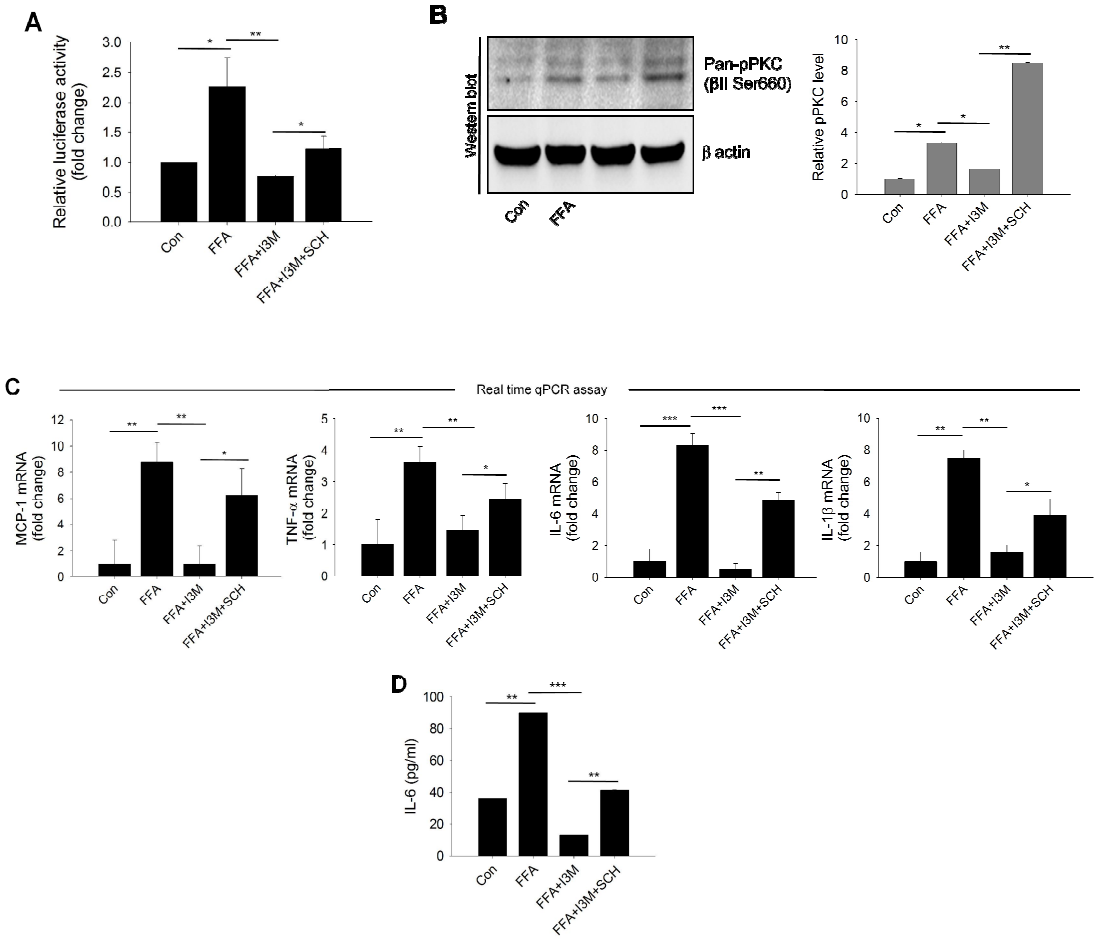


Figure 5.7(A) 3T3-L1 adipocytes transfected with κB luciferase plasmid were incubated without or with FFA (palmitate, 0.75 mM) in absence or presence of I3M (10 μM) or I3M (10 mM) + SCH 58261 (SCH, 300 nM). On termination of incubations, cells were lysed and luciferase activity was measured by the multimode reader. Western blot (left) and its quantification (right) showing abundance of pan-phospho-PKC (βII Ser660) level in 3T3-L1 adipocytes incubated without (Con) or with FFA (palmitate, 0.75 mM) in absence or presence of I3M (10 μM) without or with SCH 58261 (SCH, 300 nM). β-actin was used as loading control. (C) Real-time quantitative PCR analysis showing fold change of MCP-1, TNF-α, IL-6 and IL-1β mRNA level in 3T3-L1 adipocytes incubated without or with FFA (palmitate, 0.75 mM) in absence or presence of I3M (10 μM) or I3M (10 μM) + SCH 58261 (300 nM). GAPDH served as an internal control for normalization. All experiments were performed in triplicate. Each value is the mean ± SEM of three independent experiments, *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$. (D) ELISA detecting IL-6 secretion level in cell culture supernatant of 3T3-L1 adipocytes treated without (Con) or with palmitate (FFA) in absence or presence of indirubin -3'-monoxime (I3M, 10 μM) or SCH 58261 (SCH, 300 nM). Experiments were performed in triplicate. Each value is the mean ± SEM of three independent experiments. ***$p < 0.001$, **$p < 0.01$.

5.3 Discussion:

After almost a century of intense research, adenosine receptors have now become the centre of attention and are considered a potential therapeutic target for cancer, diabetes, cardiovascular disease and immune, inflammatory and neurodegenerative disorders. In

normal physiological conditions, the extracellular level of the purine nucleoside adenosine is rather low which can rapidly increase in various pathological conditions such as metabolic stress, tissue injury and inflammation [15-19]. Extracellular adenosine mediates a range of responses to restore tissue homeostasis through ligation of G-protein coupled cell-surface adenosine receptors (ARs). These receptors are widely distributed in metabolically active organs such as liver, pancreas and adipose tissue as well as in the immune system and categorised into four subtypes that include A_1, A_{2A}, A_{2B} and A_3 [15-17]. Several studies have indicated that AR, particularly A_{2A}AR signaling stimulates anti-inflammatory effect in adipose tissue and its resident immune cells that promote cellular insulin responsiveness [15-22]. A recent study showed that activation of A_{2A}AR increased brown adipocytes and 'browning' of white adipocytes which augmented thermogenesis and inhibited insulin resistance [37].

Experiments on mouse islets indicate that, beside insulin responsiveness, adenosine also stimulates insulin secretion through activation of A_{2A}AR receptors which can be reversed by A_{2A}AR antagonist SCH 58261 [38]. Several studies have documented the association of adipose tissue inflammation for the promotion of insulin resistance and type 2 diabetes (T2D) [2-9]. Despite considerable progress in research and development of various anti-diabetic agents, currently, no anti-diabetic drugs have been proven clinically effective for T2D therapy. All these studies attest A_{2A}AR could be an imperative target for the development of therapeutics against T2D which raises the demand for selective A_{2A}AR agonist. The main approach to develop novel A_{2A}AR agonists lies on the modification of adenosine itself. Many attempts have been made to develop various adenosine derivatives with most useful modifications in position 2 or N^6 of the adenine ring and 3', 4' and 5'-position of the ribose that provides better binding affinity and activity in comparison to adenosine as selective agonists for four different ARs [39].

The recent breakthrough in crystallography of the human A_{2A}AR bound to its agonist adenosine, revealed that the major interactive regions of the adenine scaffold are the hydrogen bonding with both Glu169 and Asn253, and the π-stacking and hydrophobic interactions with Phe168 and Ile274 [40]. The ribose moiety of adenosine plays a critical role in receptor activation, by forming the hydrogen bonds with Ser277 and His278 of A_{2A}AR along with conformational changes of receptor due to the positional shift of Val84 and Trp246 [41-42]. However, non-adenosine compounds with different

chemotypes exhibited selective and potent agonistic activity against ARs [43-44]. All these studies open an opportunity to discover new ligand chemotypes for selective AR agonist. Ligplot analysis of the docked compounds has depicted the presence of hydrogen bonds and hydrophobic interactions with the active site residues. It is known that two important residues Phe168 and Glu169 of A_{2A}AR play a critical role in ligand binding and His278 residue is critical for receptor activation [41]. This is corroborated from our results as indirubin-3'monoxime (I3M) form H-bonds with these important residues. It has been observed that a novel ligand chemotype I3M has better interaction pattern with the A_{2A}AR. I3M effectively up regulates the activation of ERK1/2 and CREB, however, such effect was compromised in A_{2A}AR knockdown cells indicating possible involvement of A_{2A}AR signaling in mediating I3M effect.

The radioligand binding assay confirms direct high affinity binding of I3M to A_{2A}AR. Investigating the cAMP level in response to increasing concentrations of I3M and the effect of increasing concentrations of A_{2A}AR antagonist on I3M mediated induction of CREB activation in the A_{2A}AR stable clone of CHO cells revealed the A_{2A}AR agonist nature of I3M. Utilizing various antagonists of AR subtypes, we have shown the selective involvement of A_{2A}AR in mediating I3M effect. Using A_{2A}AR deficient mice combined with a pharmacological approach, it has been shown that adenosine through A_{2A}AR activation inhibits LPS induced macrophage inflammation by suppressing TNF-α, IL-6 and IL-12 expression and augmenting IL-10 production [42]. In this regard, it is of note that our results demonstrated that I3M strongly attenuates palmitate-induced NF-κB activation, κB-promoter-reporter induction, and gene expression of various pro-inflammatory cytokines; however, such effects were compromised by pharmacological inhibition of A_{2A}AR. Further, I3M induced A_{2A}AR-cAMP-CREB signaling also leads to the upregulation of IL-10 and other anti-inflammatory cytokines gene expression which were attenuated in presence of A_{2A}AR selective antagonist. All these results support the agonistic nature of I3M adding a novel scaffold of selective non-adenosine based A_{2A}AR agonists. Taken together, our results demonstrate that I3M could be a selective novel agonist of A_{2A}AR which efficiently attenuates lipid-induced adipocyte inflammation and insulin resistance. Thus, I3M has therapeutic potential for the prevention and/or treatment of type 2 diabetes.

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