Anti-inflammatory and anti diabetic action of Arachidonic acid and its metabolite Lipoxin A4

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I completed the following research work under the supervision of Dr. U. N. Das. It is great experience and excitement to work in the following area.

Obesity and diabetes mellitus (DM) are assuming epidemic proportions throughout the world. The modern urbanized habitat (including lack of adequate exercise) and high fat diet are the leading causes of obesity and diabetes mellitus. Per World Health Organization (WHO) statistics, 1.9 billion people are overweight and 600 million are obese worldwide. About 422 million people are affected with diabetes mellitus. The pervasiveness of obesity and diabetes was observed more exponentially in middle and low income countries. India is the 3rd most effected country after USA and China in the world. About 30 million adults in India are obese. During the past two decades the average level of obesity has raised to 8% in Organization for Economic Cooperation and Development (OECD) countries. WHO projects that diabetes will be the 7th leading cause of death in 2030. Diabetes mellitus can be manifested in two forms: one is type 1 diabetes mellitus (type 1 DM) where complete loss of insulin from pancreas due to autoimmune destruction or genetic disorders; second is the type 2 diabetes mellitus (type 2 DM) in which despite insulin presence targeted cells are resistance to its action and leads to glucose intolerance. Majority of the diabetic patients belong to the second category type 2 DM due to consumption of high energy diet intake and sedentary behavior.

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The metabolic stress due to high calorie intake induces adipose tissue to secret stressful cytokines or adipokines like TNFα, IL-6 and Lipocalin 2 (LPCLN2). These adipokines target the insulin targeted tissues like liver, muscle and adipose tissue and induces insulin resistance. The adipokines target the genes of the metabolic tissues and enhance the expression of inflammatory causing genes like Nf-kB, iNOS and suppress the anti-inflammatory genes like Nrf2 and anti oxidant enzymes. These adipokines also suppress insulin receptor substances (IRS) activation that is needed for the downstream regulation of Insulin receptor. In both the types of DM Type 1 DM and type 2 DM) inflammation occurs. In type 1 DM, the inflammation is localized close to the pancreatic β-cells whereas in type 2 DM it is low-grade systemic inflammation.

Among them, Arachidonic acid (AA, 20:4 n-6) is crucial to regulate inflammation. Studies showed that the Lipoxygenases (LOX) metabolites of AA are important constituents in resolving chronic inflammation. Lipoxin A4 (LXA4) is an endogenous metabolite of AA by the action of LOX enzyme that has a significant role in resolving inflammation. Since major metabolic disorders are due to low grade chronic systemic inflammation, we hypothesized that AA and LXA4 may have an important role in metabolic disorders such as DM (both T1DM and T2DM).

To verify our hypothesis, we studied the effect of AA and its endogenous metabolite LXA4 on the cytotoxic action of alloxan and streptozotocin (STZ) on rat insulinoma cells (RIN5f) in vitro and in vivo. Both AA and LXA4 were tested for their effects on cell viability by using MTT assay of RIN5f cells at various concentrations of these lipids and for various time periods. Our studies showed that optimal doses of alloxan and STZ to induce ~ 50% reduction in the number of cells surviving are 4mM and 21mM at the end of 1 hour and 24 hours of incubation respectively. Depend on the results obtained further studies were carried out using 4 mM of ALX and 21 mM of STZ and the incubation time of 1 hour and 24 hours respectively. Our studies also showed that 5, 10 and 15 μg/ml of AA are optimal doses at which it is likely to show its effects on RIN5f cells in vitro since at these concentrations AA did not show any cytotoxic action by itself. Both AA and LXA4 pre- and simultaneous-treatment schedules were used while testing for their modulator influence on alloxan and STZ- induced cytotoxicity on RIN5f cells in vitro. Preliminary studies revealed that 50 ng/ml of LXA4 is the optimum dose to test its actions on RIN5f cells in the presence of alloxan and STZ. This dose of LXA4 is arrived at after testing 1, 5, 10 and 50 ng/ml of LXA4 in the initial studies. While testing for the mechanisms of actions of AA against the cytotoxic action of alloxan and STZ on RIN5f cells in vitro, we evaluated the affect of cyclo-oxygenase (COX) and lipoxygenase (LOX) inhibitors: indomethacin and nordihydroguaiaretic acid (NDGA) (both were used at 1mM dose) respectively to know whether any metabolites of AA play a role in the actions of AA in our studies.

Our in vitro studies with RIN5f cells revealed that AA prevents the cytotoxic action of alloxan and STZ on RIN5f cells and these cytoprotective actions of AA are not interfered with by both COX and LOX inhibitors suggesting that PGs, LTs and TXs do not have a role in this process. Since AA forms precursor to LXA4, we next tested possible involvement of LXA4 in the cytoprotective actions of AA against alloxan and STZ on RIN5f cells in vitro. These studies showed that (i) both alloxan and STZ inhibit the production of LXA4 by RIN5f cells; (ii) RIN5f cells were protected

by LXA4 from the cytotoxic action of alloxan and STZ; and (iii) addition of AA in RIN5f cells restored the production of LXA4 to normal which was suppressed by alloxan and STZ, These studies are in support of the hypothesis that (i) both AA and LXA4 have cytoprotective actions against alloxan and STZ-induced apoptosis of RIN5f cells; (ii) there is no significant role for PGs, LTs and TXS in the cytoprotective action of AA; (iii) alloxan and STZ suppress production of LXA4 by RIN5f cells; (iv) AA brings about its cytoprotective action by increasing the formation of LXA4 in RIN5f cells; and (v) LXA4 is the mediator of the cytoprotective action of AA seen against alloxan and STZ on RIN5f cells. In a further extension of these studies, we observed that LXA4 can restore the antioxidant status of RIN5f cells to normal that was suppressed by alloxan and STZ. It was also noted that LXA4 s able to prevent both apoptosis and necrosis of RIN5f cells induced by alloxan and STZ in vitro. Furthermore, expression of p65 Nf-kB, IKB, PDX1 and beta actin genes that were altered by alloxan and STZ in RIN5f cells were restored to near normal by LXA4.

In extension to these in vitro results and to assess whether the cell culture studies can be extrapolated to an in vivo situation, we tested the efficacy of AA and LXA4 on STZ-induced type 1 and type 2 DM in Wistar rats. In the present study, we tested AA (oral and intraperitoneal injection) and LXA4 (intraperitoneal injection) against STZ-induced type 1 and type 2 DM.

In STZ-induced type 1 and type 2 DM animal models both AA and LXA were tested and were injected for 7 days. In this instance, AA was administered both orally and intraperitoneally whereas LXA4 was given for 5 days only by intraperitoneal route. In STZ-induced type 1 DM model study, AA was used for 7 days since AA was being tested by oral route and so we wanted to make sure that adequate amounts of AA would reach the target tissues (pancreas after oral administration) and hence, was given for longer time. On the other hand, LXA4 was injected for 5 days while testing against STZ-induced type 1 and type 2 DM.

All experiments were performed for 30 days and at the end of the experiment, animals were sacrificed and plasma, pancreas, liver, kidney, and adipose tissue were collected for further analysis. Blood glucose, body weight and food consumption were measured during the experimental period.

The results of the animal studies revealed that both AA and LXA4 have potent cytoprotective and anti diabetic actions. STZ induced type 1 DM was prevented by AA and both type 1 and type 2 DM induced by STZ were prevented by LXA4. LXA4 reverted to normal STZ-induced hyperglycemia and maintained insulin homeostasis. In STZ-induced type 2 DM animals, LXA4 administration restored to normal anti-oxidant enzymes, nitric oxide and lipid peroxide levels to near normal and so also the relevant gene and protein expression such as NF-kB and IkB, GLUT-2, Pdx1, Nrf2, GLUT-2, COX2 and iNOS and lipocalin 2.

Based on these studies, we conclude that AA and its endogenous metabolite LXA4 are not only potent cytoprotective molecules but also have anti- diabetic actions.