Al-Quds University
Faculty of Graduate Studies

Effect of Medica 16 on GTG-Obese Mice

By

Ghadeer Khalil Za'tarah

Supervised By

Dr. Nabeel Nahas

Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Biochemistry and Molecular Biology, Faculty of Graduate Studies, at Al-Quds University, Jerusalem, Palestine.

November 2001

Contents

Abstract

Obesity is characterized by an increase in adipose tissue mass with deranged control of either food intake or energy expenditure or both. It is strongly associated with insulin resistance, manifesting typical symptoms of diabetes type II like, elevated blood glucose, elevated blood insulin and dyslipidemia. Adipose tissue releases several factors that may act as regulators of its own mass as well as of the activity of other tissues; the most intensively studied of these factors are leptin and tumor necrosis factor-α (TNF-α).

TNF-α is a cytokine that is known to be released by macrophages in cases of infection such as bacterial invasion and other traumatic events. Several studies have pointed to an involvement of TNF-α in insulin resistance, and it has been shown that the secretion of TNF-α from adipose tissue is augmented in obesity.

A series of substituted analogs of naturally occurring dicarboxylic long chain fatty acids has been developed and known as MEDICA compounds. One of these compounds, Medica16, has many biological effects, including the ability to increase insulin sensitivity in liver, muscle and adipose tissue in hyperglycemic, hyperinsulinemic animal model for NIDDM, and decrease the expression of apolipoprotein CIII (apoCIII) and Hepatocyte Nuclear Factor-4 (HNF_4) in the liver.

The main goal of this study is to see whether Medica16 exerts its effect on insulin sensitivity through an effect on TNF-α.

In order to approach this question, animals were made obese by injecting them with Gold Thioglucose (GTG) and were then given food containing Medica16. They were sacrificed and blood parameters as well as adipose tissue TNF-α mRNA and liver apolipoprotein CIII and HNF_4 mRNAs were measured.

A remarkable decrease in body weight gain of the GTG+Medica16-treated animals as compared to the GTG-treated ones was observed. There was a decrease in blood cholesterol, triglycerides, insulin and glucose levels in the group of the GTG-obese animals treated with Medica16 as compared to the group not treated with Medica16. TNFα expression in the epididymal fat tissue was decreased by Medica16. However, the biological variation was rather high and the significance of the difference between the adipose tissue mRNA levels of the Medica16-treated animals and the Medica16-non treated animals was rather poor. GTG treatment increased the expression of liver apolipoprotein CIII, and decreased the expression of HNF_4. However, Medica16 treatment lowered the expression of liver apolipoprotein CIII only in the mildly obese mice and increased the expression of HNF_{-4} only in the very obese.

Chapter Four

Discussion

Insulin resistance of adipocytes is reflected by the inability of insulin to control lipolysis, resulting in increased efflux of free fatty acids into the plasma with a concomitant increase in hepatic very low density lipoproteins (VLDL) production and increase in VLDL levels in plasma. It is strongly correlated with obesity and considered to be a component of non insulin-dependent diabetes mellitus (NIDDM).

The adipose tissue is known to release factors into the circulation that may limit its expansion. The most considerable ones are leptin and TNF-α which is implicated in insulin resistance.

Thiazolidinediones are the best known and mostly used drugs for ameliorating insulin resistance. They are peroxisome proliferator-activated receptor-γ (PPARγ) activators (Lehmann *et al*, 1995) and were shown to result in decreasing insulin and glucose levels, with a concomitant decrease in FFA, VLDL and hepatic glucose production (Chicco *et al*, 2000). These drugs have also been shown to have reducing effects on the expression of TNF-α (Sigrist *et al*, 2000).

Medica 16 is the most studied compound of MEDICA Compounds in the process of insulin sensitivity improvement as well as in reducing the expression of apoCIII through an effect on its transcription factor HNF4 *(Hertz et al, 1995)*

The main aim of the present study was to see whether the action of Medica16 as an insulin-sensitizing drug is through decreasing TNF-α.

As mentioned previously, the main tissues expressing TNF-α are the macrophages and adipocytes. In vitro preliminary studies relating Medica16 with TNF-α involved using human monocytes and mouse macrophages treated with phorbol 12-myristate 13-acetate (PMA) and lipopolysaccharide (LPS) respectively, which are known to increase TNF-α production. Medica16 treatment of both types of cells resulted in decreasing the expression of TNF-α mRNA, as well as decreasing the protein level of TNF-α excreted by these cells. Cell culture studies relating Medica16 to TNF-α expression involved the transfection of TNF-α promoter connected to the reporter gene CAT into COS cells. Treatment of these cells with Medica16 decreased the expression of the reporter gene (Brandes *et al*, unpublished data). Further in vivo experiments that involved non-obese mice yielded conflicting results: Medica16 treatment resulted in a nonsignificant decrease in serum TNFα protein levels, while in the macrophages, there was an increase in TNF-α mRNA induced by Medica16 (Brandes *et al*, unpublished data). These confusing preliminary results lead us to look for a possible modulation in adipose tissue, in which the signals were found to be very weak unless the animals were made obese.

Animals in this study were made "chemically obese" by the injection of Gold Thioglucose (GTG) intraperitoneally. GTG is a chemical that causes injury to the hypothalamus, resulting in hyperphagic animals (Ventre et al, 1997). This model of obesity is preferred to the genetically obese model, since this model is closer to the human over-eating obesity. Medica16 was added to the food for about three weeks with

increasing concentrations. During this period, body weights of animals were measured and food consumed by each animal was measured daily. Part of the animals died due to the over consumption of food induced by GTG, resulting in stomach bursts. After three weeks, animals obviously became obese as could be indicated by body weights. Medica16 decreased this weight gain induced by GTG almost to the level of weight gain by non-treated mice. Animals were then sacrificed and blood parameters including glucose, cholesterol, triglycerides, insulin as well as Medica16 were measured. Blood parameters revealed the overt effect of GTG in increasing glucose, triglycerides, cholesterol and insulin in these animals with the effect being more pronounced in the most obese ones. These effects were less obvious in the moderately obese, and no such effects were observed in the mildly obese animals. Medica16 treatment resulted in partial reversal of hyperglycemia, hypercholesterolemia, hypertriglyceridemia and hyperinsulinemia. No effect of Medica16 was observed in mild obesity, probably because this group of mild obesity didn't reach the state of insulin resistance. Degree of obesity and blood parameters in this group didn't reach the state of insulin resistance, so Medica16 could not counteract obesity. This proves the importance of Medica16 in correcting the clinical parameters associated with severe obesity.

Adipose tissue TNF-α mRNA expression revealed considerable variation between the animals. Medica16 resulted in a decrease in TNF-α expression in both perirenal and epididymal tissues. The decrease was non significant in perirenal adipose tissue and of low significance in the

epididymal tissue. The statistical analysis showed a non normalized behaviour of the GTG + M16 group of epididymal fat, composed of a subgroup with high values and another with small values. This implies that M16 does have a sort of an all or none effect on TNF-α; this phenomenon is not well understood and needs further investigation. Here, it could be supposed that if the application of Medica16 would be extended to longer time, more significant effect for Medica16 would be seen.

Some hepatic expression of TNF-α has been reported as well, and thiozolidinediones have been shown to decrease this expression (Sigrist *et al*, 2000).

In an attempt to further explore the effect of Medica16 on obese animals, we have tested its effect on TNF-α in liver. We have prepared RNA from the livers of our obese animals as well. Problems were faced in this part due to the very weak TNF-α signals, and these studies were not continued. However, the expression of two other hepatic genes, apolipoprotein CIII (apoCIII) and hepatic nuclear factor-4 (HNF4) was examined.

Northern blotting for the expression of apoCIII mRNA revealed an increase in mRNA levels in the GTG-treated group relative to controls. Medica16 treatment didn't result in a significant reversal of this effect except for the subgroup of mildly obese. Here, An increase in apoCIII mRNA could result from an increase in its transcription factor activity, HNF4. So, competitive RT-PCR was done to measure the expression of HNF4. Results revealed a decrease in HNF4 mRNA in the GTG-treated

animals relative to controls. This picture was completely opposite to what was expected. Medica16-treated group showed values higher than the GTG group, but still lower than the control group, i.e.: Medica16 did cause partial correction of the GTG effect. The explanation of this complex picture could be aided by results of unpublished data (Hertz *et al*), in which transfection of hepatic cells with exogenous HNF4 resulted in a decrease in endogenous HNF4, a case that is similar to the present study. This could be explained by the fact that an increase in HNF4 activity results in a negative feedback regulation exerted by HNF4 against further production of HNF4. This means that HNF4 undergoes autoregulation and results in the decrease in its own mRNA levels.

On examining the levels of apoCIII expression as a function of obesity, it could be seen that the mildly obese animals showed higher levels than the obese and moderately obese ones. The involvement of PPARα could play a role in this case. It is well known that obesity leads to the augmentation of serum free fatty acids (FFA) which are activators of PPARα. These in turn would reduce the levels of apoCIII mRNA levels. This process is most clear in groups I and II of obese and moderately obese animals, respectively. What occurs in group III of mild obesity is that the degree of obesity was probably not enough to produce high levels of FFA that must exert an obvious effect on PPARα. So, the effect of PPARα was not clear in this group of animals.

Another interesting observation with respect to liver was modulation of liver weights. GTG induced an increase in liver weights mostly in group I of obese animals, and Medica16 slightly reduced this liver weight

increase in this group. The increase in liver weights was much smaller in the other two groups of moderately and mildly obese animals, unless Medica16 was added. This complex pattern is probably due to the peroxisomal proliferation as well as to fat accumulation in these cells, since it is well known that the hypolipidemic effect of M16 is accompanied by an increase in liver size and liver peroxisomes (Hertz *et al*, 1988).

The main question asked in this study, i.e.: does the effect of Medica16 on ameliorating insulin sensitivity involve a decrease in TNF-α expression, is therefore still not conclusively answered. The discrepancy between our in vivo and in vitro studies tells us that the in vivo situation might be complex. This complexity shows itself in human studies as well, since no change in serum TNF-α could be detected in obese individuals, despite the fact that the TNF-α expression in their adipose tissue is elevated (Paquot *et al*, 2000). Experiment including greater number of very obese animals seems to be needed. In vitro studies should be continued as well, mainly exploring the effects of Medica 16 in cell lines expressing TNF-α.

Recent studies suggest a new protein supposed to play a role in mediating insulin resistance. This protein, referred to as resistin, has been shown to be increased in diet-induced obesity. It is secreted by adipocytes, circulates in the blood and recognized by resistin receptors on target cells, and its expression in adipocytes is decreased by thiazolidinediones. Resistin-neutralizing experiments partially reversed insulin resistance (Steppan *et al*, 2001). These studies are very new and

it is too early to evaluate their significance. However, the possibility that Medica 16 acts through resistin should not be ignored.

Conclusion

- The GTG-mouse is a good model for studying the effect of Medica16 on obesity.
- Medica16 remarkably decreases the weight gain induced by GTG.
- Medica16 acts as a hypolipidemic and insulin-sensitizing agent in this model of obesity.
- There is some evidence for the expected decrease of TNF-α expression in the adipose tissue by Medica16.
- The expression of apoCIII in the liver is increased in the GTG-mice, with some reversal by Medica16.
- The expression of HNF4 in the liver is decreased in the GTG-mice, with some reversal by Medica16.

بسم الله الرحمن الرحيم

تأثير مادة (MEDICA 16) على الفئران البدينة التي تم معاملتها بمادة (GTG)

غدير خليل زعترة

اشراف

د. نبيل نحاس

الملخص

تتميز البدانة بازدياد في حجم النسيج الدهني مع عدم القدرة في التحكم في الغذاء أو في صرف الطاقة او كلاهما معا . وترتبط البدانة بعمل الانسولين بحيث يصبح الانسولين غير قادر على القيام بوظيفته . وفي هذه الحالة يعاني المريض من مرض السكري النوع الثاني مع ارتفاع في نسبة السكر في الدم ويصاحبة ارتفاع في نسبة الانسولين وخلل في نسب الدهنيات. يقوم النسيج الدهني بافراز العديد من العوامل التي تقوم بدورها بالتاثير على حجمة بالاضافة إلى التاثير على عمل انسجة اخرى . من هذه العوامل التي تم التركيز على دراستها مادة (ليبتين) (Leptin) ومادة (TNF-α). TNF-α عبارة عن مادة يتم افرازها من خلايا مقاومة تسمى (Macrophages) في حالات المرض مثل تعرض الجسم لهجوم بكتيري وحالات عديدة اخرى , كما يتم افرازة ايضا من قبل النسيج الدهني .

اشارت العديد من الدراسات إلى تدخل (TNF-α) في حالة "مقاومة الانسولين ", وان افراز النسيج الدهني لعامل (TNF-α) يزداد في حالات البدانة .لقد تم التوصل إلى تطوير مجموعة من الاحماض الدهنية تعرف باسم مركبات (MEDICA). تتميز هذه المجموعة بكونها بديلة عن الاحماض الدهنية ذات السلاسل الكربونية الطويلة وتحتوي على مجموعتين من الكربوكسيل . احدى هذه المركبات يعرف باسم (MEDICA 16). وقد عرف هذا المركب بامكانية القيام بالعديد من التاثيرات الحيوية منها قدرتة على ازدياد الاستجابة للانسولين بالاضافة لقدرتة على التقليل من اظهار الصفة ل (apoCIII) و (HNF4) في الكبد . الهدف الرئيسي لهذه الدراسة هو معرفة ما اذا كان مركب (MEDICA 16) يقوم بتاثير ة على عمل الانسولين عن طريق التاثير على (TNF-α). من اجل التوصل لتحقيق هذا الهدف فقد تم تحضير عدد من الفئران للحصول على فئران بدينة, وذلك عن طريق حقنهم بمادة تسمى (GTG) ومن ثم اعطاؤهم طعام يحتوي على مركب (MEDICA 16) وبعد فترة معينة تم التضحية بهم وقياس معليير مختلفة في الدم بالاضافة إلى قياس نسبة (TNF mRNA) في النسيج الدهني وكذلك (mRNA) لكل من (apoCIII) و (HNF4) في الكبد. وقد ادى هذا المركب الى انخفاض ملحوظ في زيادة الوزن في مجموعة الفئران التي تم معاملتها بنفس المركب كذلك كان هناك انخفاض في نسب الكولسترول , الدهنيات , الانسولين والسكر في مجموعة الفئران التي تم معاملتها بمادة (MEDICA 16) بالمقارنة مع المجموعة الاخرى .أما فيما يتعلق باظهار صفة (TNF-α) فقد كان هناك انخفاض في نسبة اظهار الصفة في احد النسيجين اللذين تم دراستهما. من ناحية اخرى فقد كان هناك تباين حيوي كبير بين كلا المجموعتين ومدى الفرق الناتج بينهما كان ضعيفا. بالنسبة للكبد، فان مادة (GTG) أدت إلى ازدياد في اظهار صفة (apoCIII)وانخفاض في اظهار صفة (HNF4). بينما الانخفاض في اظهار صفة (apoCIII) كان ملحوظا فقط في المجموعة الاقل بدانة ، وكان هناك ازدياد في (HNF4) فقط في المجموعة الاكثر بدانة.