

EFFECT OF ERYTHROMYCIN ON INSULIN RESISTANCE AND TUMOUR NECROSIS FACTOR-ALPHA LEVELS IN FRUCTOSE FED INSULIN RESISTANT RATS

BY

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ABSTRACT

Tumor necrosis factor-alpha (TNF- α) has been suggested to play a key role in insulin resistance (IR), in obesity and may contribute to the development of type 2 diabetes mellitus. Erythromycin, one of the macrolide antibiotics was found to exert various pharmacological effects including suppression of TNF- α and other cytokines levels as well as antioxidant activity in various studies in human and animals. The present study was designed to investigate the beneficial effect of erythromycin on insulin sensitivity in rats fed high fructose diet. Albino rats of both sexes were divided into four groups containing 10 rats each. Control rats received the standard diet, while fructose fed rats received high fructose diet. Fructose and erythromycin treated rats received high fructose diet and erythromycin 40 mg/kg/day orally, while control and erythromycin treated rats received standard diet and erythromycin. After the treatment period of 30 days insulin resistance index, by homeostasis model assessment (HOMA) was determined. The levels of lipid per-oxidation, TNF- α and lipogram were measured. Rats feeding high fructose diet developed a significant insulin resistance associated with increased TNF- α , lipid per-oxidation products, TG-LDL and decrease in HDL and no change in total cholesterol level. Administration of erythromycin to high fructose fed rats produced beneficial effect on insulin sensitivity, dyslipidemia, TNF- α and lipid per-oxidation. A fall in TNF- α concentration and lipid per-oxidation products by erythromycin signify the potential utility of erythromycin as an adjunct in treatment of insulin resistance.

INTRODUCTION

Feeding rats with high fructose diet affects both glucose and lipid metabolism which result in cluster of metabolic abnormalities such as glucose intolerance, hypertension, dyslipidemia and reduced insulin action (Dai et al., 1994) thus fructose-

fed rats form a useful experimental model for insulin resistance.

Tumor necrosis factor-alpha (TNF- α) is a peptide constitutively expressed and secreted by adipose tissue (Erosy et al., 2004). It has been demonstrated that TNF- α may be a mediator of insulin resistance

in obese subjects by inhibiting tyrosine kinase activity at the insulin receptor. Phosphorylation of the insulin receptor by tyrosine kinase is critical for the binding of insulin to its receptor (Hotamisligil et al., 1994).

Fructose feeding is also reported to facilitate oxidative damage and has deleterious effects both due to reduction in antioxidant defense and enhanced free radical production (Busserolles et al., 2002).

Macrolide antibiotics are active against gram-positive bacteria, *Mycoplasma* spp., *Legionella* spp., *Chlamydia* spp., and *Haemophilus influenza* (Barry et al., 1987). Apart from their anti-bacterial activity, these agents exhibit a broad spectrum of pharmacological effects including anti-inflammatory activity in humans and animals (Tarayre et al., 1987; Mikasa et al., 1992; Sanz et al., 2005).

Macrolides have been shown to affect several pathways of the inflammatory process, such as the migration of neutrophils. The oxidative burst in phagocytes and the production of pro-inflammatory cytokines such as TNF- α production (Takeshita et al., 1989; Hand et al., 1990; Mikasa et al., 1992; Konno et al., 1994).

Furthermore, erythromycin mimics the

effect of the gastrointestinal hormone motilin by binding to its receptor and acting as motilin agonist which recently was found to stimulate insulin secretion at lower doses than that required to stimulate gastric contractile activity (Ueno et al., 2000).

This study was carried out to determine whether erythromycin could improve insulin resistance and the related abnormalities induced by high fructose feeding in rats.

MATERIAL AND METHODS

Drugs:

Erythromycin: (Eromycin tab. Julphar Co.).

Animals grouping:

Forty Albino rats of both sexes were used throughout this study. Their weight ranging from 170 gm to 190 gm. They were kept under the same housing condition and were divided into the following groups consisting of 10 rats each.

1) Group I: "Control group": Each rat in this group received the control diet and tap water ad libitum.

2) Group II: "High fructose fed-rats": Each rat received a diet in which fructose composed 33.64% of total carbohydrate (Faure et al., 1999).

3) **Group III:** Each rat received control diet and erythromycin 40 mg/kg/day orally in water (Ianaro et al., 2000).

4) **Group IV:** Each rat received high fructose diet and erythromycin in the same dose. The diet composition was prescribed in table (1). Data are given in grams per 100 grams of dry weight.

The animals were maintained in their respective groups for 30 days, food intake and fluid intake were measured regularly.

An oral glucose tolerance test was carried out two days before the sacrifice of the animals, for this the rats were fasted overnight and glucose (2g/kg B.w) was given from 30% solution orally. Blood samples were collected before glucose load and, sequentially for every half an hour after glucose load up to 90 min and were immediately analysed for glucose. At the end of the experimental period, the rats were sacrificed by cervical decapitation, blood samples were allowed to clot and were centrifuged, serum was separated and frozen at -70°C until time of the assay of the following parameters :

Serum glucose: according to the enzymatic glucose oxidase method of Trinder, (1969).

Fasting serum insulin: by enzyme linked immunosorbent assay kit, using hu-

man insulin as standard.

Homeostasis model assessment (HOMA) was used as an index to measure the degree of insulin resistance and was calculated by the formula: [insulin (um/ml) glucose in mmol/l)/22.5] (Pickavance et al., 1999).

Total serum cholesterol : Using Kits of Biomerieux Company according to Richmond (1973); HDL and LDL according to Burstein (1970) using Kits of Biomerieux Company and TG according to Fassati and Prencipe (1982) .

Serum TNF- α concentration was measured by TNF- α enzyme immune assay kit (Aderka et al., 1992).

Thiobarbituric acid reactive substance (TBARs) was determined as described earlier (Nandhini et al., 2002). In brief, the concentration of TBARs was estimated by measuring the pink- coloured chromophore upon reaction with thiobarbituric acid at 535 nm a standard curve was prepared using tetra methoxy propane as the standard.

Statistical Analysis:

All the values were expressed as means \pm SE and statistically evaluated by two-way analysis of variance considering diet and treatment as two factors. The means were tested for significance by Tukey's

test (Zar, 1984) for multiple comparison. A value of $p < 0.05$ was considered significant.

RESULTS

The level of plasma glucose, insulin and insulin resistance index of control and experimental animals are listed in table (2). The levels are significantly elevated in fructose-fed rats as compared to control rats. Erythromycin treatment to fructose fed rats prevented such increase. The degree of insulin resistance as measured by HOMA is higher in fructose fed rats while in erythromycin treated rats the values are normal.

Table (3) shows the influence of high fructose diet and erythromycin on the levels of serum TG, LDL, HDL and cholesterol. Fructose fed rats show significant increase in serum TG and LDL associated with significant decrease in HDL with no change in total serum cholesterol. Erythromycin treatment shows significant decrease in serum TG and LDL associated with increase in serum HDL and no change in total serum cholesterol level.

Table (4) shows the influence of high fructose-diet and erythromycin on the levels of TBARs and serum TNF- α . Fructose fed rats show significantly higher peroxidation as compared to control rats, furthermore there is significant increase in

serum TNF- α compared to control rats.

Erythromycin treatment significantly reduced the levels of lipid per-oxidation and TNF- α .

The result of oral glucose tolerance test in experimental animals are depicted in (Fig 1). The fasting glucose level is higher in fructose-fed rats as compared to control rats and the level is significantly lower in fructose fed rats treated with erythromycin. Significant elevations are observed in the glucose levels after the oral glucose load at all the time points in fructose fed rats. The response is similar to control in erythromycin treated rats.

DISCUSSION

In the present study high fructose-fed rats showed significant increase in serum insulin, glucose and increase in insulin resistance. Insulin resistant has been attributed to low level of insulin-stimulated glucose oxidation due to modification in the post receptor cascade of insulin action (Catena et al., 2003). High levels of dietary fructose and severe hyperglycemia may have interactive effects which contribute to the progression and development of pathology. Furthermore, high fructose feeding showed significant increase in TNF- α which may mediate insulin resistance by inhibiting tyrosine kinase activity at the insulin receptor (Dan Dona et al., 1998).

Phosphorylation of the insulin receptor by tyrosine kinase is critical for the binding of insulin to its receptor (Hotamisligil et al., 1994). In addition TNF- α may cause insulin resistance in vivo by raising free fatty acid concentration which in turn impairs muscle glucose metabolism (Garg, 1996). High fructose feeding showed significant increase in LDL and TG associated with significant decrease in HDL and no change in total cholesterol. The increased TG level is derived from the increased production of VLDL and decrease its catabolism via decrease in lipoprotein lipase activity, also the decreased serum HDL may be due to decrease synthesis of HDL-cholesterol from LDL-TG due to low lipoprotein lipase activity elevating fractional catabolic rate of apolipoprotein A-I (Laakso, 1993; Faure et al., 1997; Ding et al., 2005).

Also, in the present study high fructose feeding induced increase in free radical formation "as indicated by increase in TBARs levels". Fructose feeding can induce free radical formation by down regulation of hexose monophosphate (HMP) shunt enzymes that generate reduced environment in the form of NADPH and NADH (Nandhini et al., 2005). Furthermore, an increase in catabolism of fructose would result in energy depletion in cells, making them more susceptible to peroxidation (Oda et al., 1994). Enhanced lipid per-oxidation in fructose fed rats could be

associated with high circulating glucose, which enhances free radical production from glucose autoxidation and protein glycation.

Erythromycin treated rats showed significant decrease in blood glucose, serum insulin levels associated with improvement of insulin sensitivity. Erythromycin mimics the effects of gastrointestinal hormones motilin by binding to its receptor and acting as motilin agonist (Ueno et al., 2000). It was found that motilin stimulates insulin secretion and decreases blood glucose level at lower doses than that required to stimulate gastric contractile activity (Suzuki et al., 1998).

The improvement of insulin sensitivity by erythromycin may be attributed to its potential antioxidant effects (Miyachi et al., 1986; Labro et al., 1989) as evidenced by decrease in TBARs. In addition erythromycin decrease TNF- α (Iino et al., 1992; Mahgoub et al., 2005) which may explain the improvement of insulin sensitivity. The finding of Dan Dona et al. (1998) supports this result as they reported that a fall in TNF- α concentration may be attributed to the restoration of insulin sensitivity.

Erythromycin administration in rats produced significant decrease in TG, and LDL and increase in HDL associated with no changes in total cholesterol levels.

These result are in agreement with others (Ueno et al. 2000) and it could be explained on the basis that erythromycin improve insulin sensitivity.

On the light of this study it could be concluded that erythromycin treatment

has a beneficial effect in the reversal of insulin resistant and might prove useful in the treatment of non-hyperglycemic insulin resistance states such as obesity and impaired glucose tolerance as well as in the treatment of established non insulin dependant diabetes mellitus.

Table (1) : Diet composition of standard and high fructose diet(gm/100gm of dry weight)

	Standard diet	High fructose diet
Glucose	38	15.56
Fructose	-	33.64
Wheat starch	20	8.40
Casein	23	23
Cellulose	6	6
Corn oil	5	5
Salt mixture	7	7
Vitamins	1	1

Table (2) : Levels of serum glucose, insulin, insulin sensitivity index of control and experimental animals.

Parameters	CON	FRU	FRU + Erythromycin	CON + Erythromycin
Glucose (mg/dl)	81.22 ± 7.79	97.35 ± 2.37 ^{a*}	87.52 ± 6.22 ^{b*}	84.99 ± 6.62
Insulin (u U/ml)	54.21 ± 4.03	90.70 ± 4.00 ^{a*}	59.63 ± 5.49 ^{b*}	57.40 ± 8.04
Insulin resistance index (HOMA)	12.52 ± 1.24	21.59 ± 1.08 ^{a*}	13.56 ± 0.67 ^{b*}	12.7 ± 1.56

Values are means ± SE from 10 animals in each group. a- compared with CON.

b- compared with FRU. (P < 0.05).

$$\text{HOMA} = \frac{\text{Insulin (u U/L)} \times \text{glucose (m mol/L)}}{22.5}$$

Table (3): Levels of serum cholesterol, HDL, LDL and TG of control and experimental animals.

Parameters	CON	FRU	FRU + Erythromycin	CON + Erythromycin
1- Cholesterol (mg/dl)	80.5 ± 0.8	81.2 ± 0.9 ^{a NS}	79.9 ± 0.9 ^{b NS}	80.5 ± 0.7
2- HDL (mg/dl)	49.3 ± 0.4	25 ± 0.3 ^{a*}	46.9 ± 0.7 ^{b*}	47.9 ± 0.6
3- LDL (mg/dl)	37.8 ± 0.8	50.7 ± 0.9 ^{a*}	35.9 ± 0.7 ^{b*}	36.9 ± 0.5
4- TG (mg/dl)	94.6 ± 0.7	135.2 ± 1 ^{a*}	96.9 ± 0.6 ^{b*}	94.5 ± 0.8

Values are mean ± SE. a- compared with CON b- compared with FRU.

NS = Non – significant. (P < 0.05)

Table (4): Levels of serum TBARs and TNF-α in control and experimental animals.

Parameters	CON	FRU	FRU + Erythromycin	CON + Erythromycin
1- TBARs (n moles/ml)	1.64 ± 0.04	2.15 ± 0.10 ^{a*}	1.66 ± 0.09 ^{b*}	1.66 ± 0.04
2- TNF-α (pg/ml)	0.91 ± 0.1	3.84 ± 0.25 ^{a*}	0.98 ± 0.3 ^{b*}	0.95 ± 0.23

Values are mean ± SE. a- compared with control b- compared with FRU (P < 0.05)

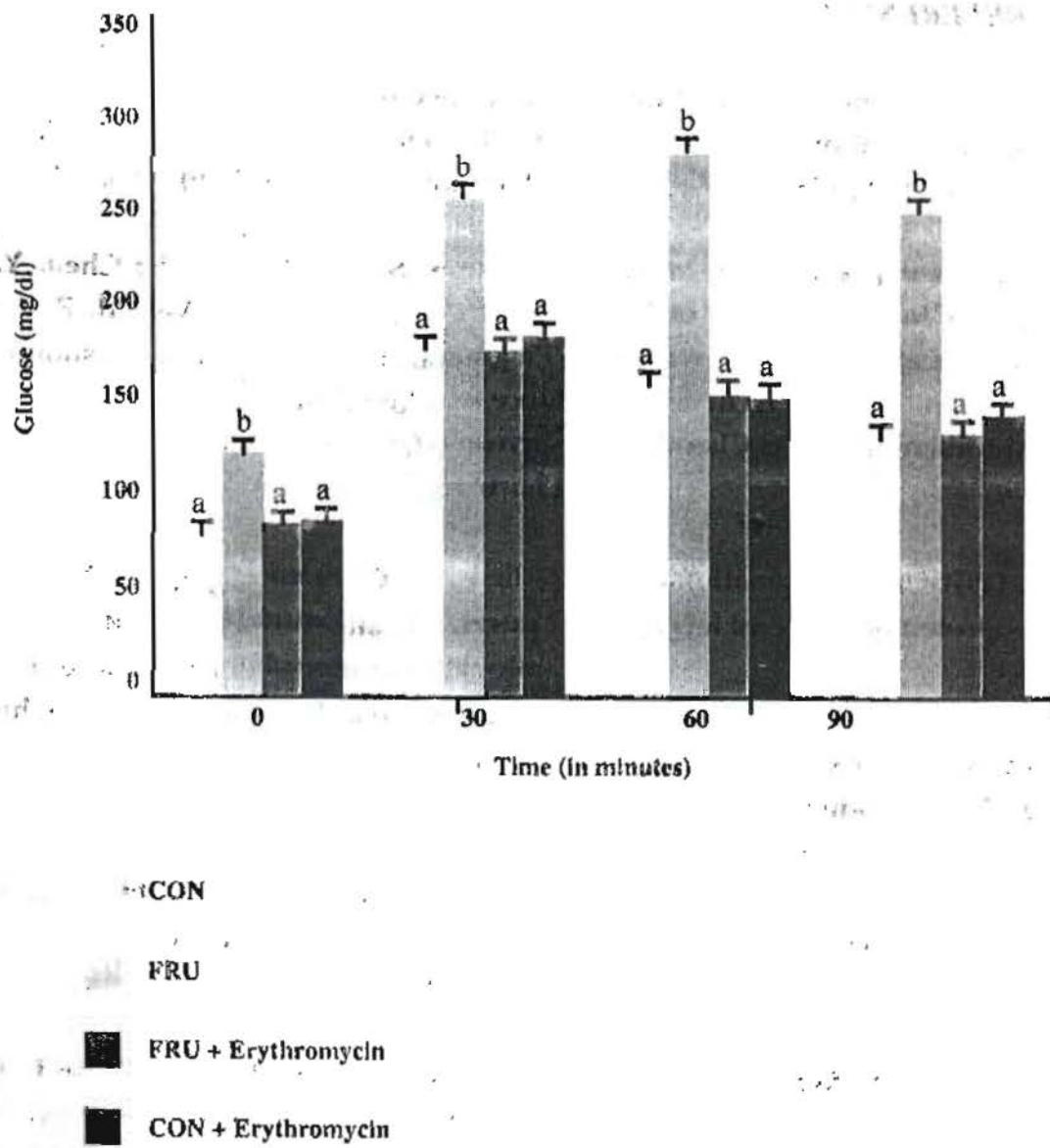


Fig. (1): Oral glucose tolerance test.

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تأثير الأريثرومايسين على مقاومة مفعول الأنسولين و معامل نخر الورم ألغا في الجرذان التي تغذت بسكر الفركتوز

د. أماني عبدالرحمن محمد شلبي

قسم الفارماكولوجي الإكلينيكي - طب المنصورة

أجرى هذا البحث لدراسة التأثير الإيجابي للأريثرومايسين على حساسية الأنسولين في الجرذان البيضاء والتي غذيت على كمية كبيرة من سكر الفركتوز وذلك لإحداث مقاومة لمفعول الأنسولين.

وقد استخدم لهذا البحث ٤٠ جرذاً أبيض قسمت إلى أربع مجموعات متساوية كالآتي:

المجموعة الأولى: مجموعة ضابطة غذيت بطعام قياسي.

المجموعة الثانية: مجموعة ضابطة غذيت بطعام يحتوي على نسبة عالية من سكر الفركتوز.

المجموعة الثالثة: تكونت من جرذان غذيت بطعام قياسي وأعطيت دواء الأريثرومايسين بجرعة ٤٠ مجم/كجم من وزن الجرذ.

المجموعة الرابعة: تكونت من جرذان غذيت بطعام يحتوي على نسبة عالية من الفركتوز وأعطيت الأريثرومايسين بنفس الجرعة السابقة.

ولقد تم إعطاء الدواء عن طريق الفم بواسطة أنبوبة مطاطية يوميا ولمدة ٣٠ يوماً.

وتم تقييم نتائج البحث بواسطة المعايير الآتية:

١- قياس نسبة السكر في الدم.

٢- قياس نسبة الأنسولين في الدم.

٣- قياس نسبة عامل نخر الورم - ألغا في الدم.

٤- قياس نسبة الدهون الثلاثية والكوليستيرول.

ويمكن تلخيص نتائج البحث كالآتي:

تغذية الجرذان بطعام يحتوي على كمية عالية من سكر الفركتوز أحدثت مقاومة لمفعول الأنسولين صاحبها زيادة في معامل نخر الورم ألغا ومستوى الشقوق الحرة ونسبة الدهون. كما أن إعطاء الأريثرومايسين أحدثت تأثير إيجابي على حساسية الأنسولين صاحبه نقص ذو دلالة إحصائية في مستوى معامل نخر الورم ألغا ومستوى الشقوق الحرة ونسبة الدهون.

وعلى ضوء هذه الدراسة يمكن استنتاج أن دواء الأريثرومايسين له تأثير إيجابي في معالجة مقاومة مفعول الأنسولين ومعالجة الخلل في نسبة الدهون المصاحبة لها وأن هذا التأثير يرجع إلى نقص التركيز في معامل نخر الورم ألغا ومستوى الشقوق الحرة. ولهذا يمكن أن يستخدم كعامل مساعد في علاج الحالات التي تعاني من مقاومة مفعول الأنسولين

1886

1887