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Full Length Research Paper

Gentamicin and erythromycin modify post prandial glucose excursion in New Zealand rabbits.

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The influence of gentamicin and erythromycin on glucose excursion after oral glucose loads was evaluated in twelve New Zealand rabbits, weighting between 1278 and 1861 g. The rabbits were randomized into three equal groups and were given either 5 mg/kg of Gentamicin i.m., 50 mg/kg of Erythromycin per oral or 0.9% Saline. One hour later, all the rabbits were given 2.5 g/kg of glucose per oral in 5 mls of 0.9% saline. Blood glucose levels were determined just before oral glucose loads, then every 1 h for 4 h, using commercial glucometer. Gentamicin pre-treatment significantly increased the peak glucose level (1 h) and significantly reduced the half-life of the rise in glucose level compared to controls. On the other hand, erythromycin pre-treatment caused significant increase in the glucose levels at 3 and 4 h while the peak glucose level (1 h) was not significantly different. The perturbation of glycemic response to glucose load revealed by both drugs may be clinically important in persons undergoing screening for glycemia and patients under care.

Key words: Gentamicin, erythromycin, glucose, insulin, rabbits, kinetics, prandial.

INTRODUCTION

Gentamicin is a bactericidal, broad spectrum aminoglycoside that is widely used in bacterial infections, especially sepsis by gram negative bacteria (Edson and Terrel, 1987). Erythromycin is a bacteriostatic macrolide used widely in clinical environment, especially as perioperative medications (Narchi, 1993). Both antibiotics have non antibiotic effects including neuromuscular bloc-kage (Gentamicin) (Brownsberger and Morrelli, 1988), gastrointestinal prokinetic activity (Erythromycin) (Zatman et al., 2001) as well as modulation of ion channels. Some calcium and potassium channel modulators have been shown to modify glucose kinetic (Cansu and Korkmaz, 2008; Doyle and Egan, 2003). Gentamicin has been shown to block calcium and potassium sensitive channels in guinea pig with inhibitory concentration fifty (IC_{50} of 3.2 µM (a clinically achievable level) (Christope et al., 2001). Erythromycin has been shown to block potassium channels of wide diversity from the inward rectifier channels in the heart (Jeyaraj et al., 2008) to the Human ether- a-go-go related gene (HERG) channel in some cancer cells (Chen ,2006) at low therapeutic multiples(13.9-21.0 Um). Macrolides have also been shown to inhibit L-type calcium channel (Gluais et al., 2003) and calcium activated potassium channels of rabbit's colon (Lu et al., 1999). Therefore, it appears plausible that Gentamicin or Erythromycin may modify glucose excursion in response to glycemic loads. To the best of our knowledge, no study has been conducted to evaluate this *in vivo*. It would be clinically important to define the extent and rate of such effects because in many clinical situations, perturbation of blood glucose status may contribute either to the ongoing illness or affects the rate and extent of recovery (Juneja, 2008; Krinsley, 2008; Wiener et al., 2008). This study was conducted to evaluate the changes in blood glucose induced by Gentamicin or Erythromycin (*in vivo*), define its pattern and derive a hypothesis for further exploration.

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Abbreviations: A1/2; Absorption half-life, **D1/2**; Distribution half-life, **E1/2**; Elimination half-life, **Pt**: Time to peak glucose level and **AUC0-4**: Area under the circumference at 0 to 4 h after glucose load.

MATERIALS AND METHODS

Animals and drugs

Twelve adult male New Zealand rabbits weighting 1278-1861 g pur-

chased from a commercial breeder were used for the study. The rabbits were de-wormed and assessed by a veterinary physician to be healthy before use. They were then acclimatized for 45days during which they were housed at temperature of 27-32^{o}C under 12 h light/darkness cycle and fed *ad-libitum* with water and antibiotic-free standard rabbit chow. The animals were randomized into 3 equal groups (Control, Gentamicin and Erythromycin treated groups) using computer generated random numbers. All animals were fasted, except ad libitum water, for 16 hours before dosing. The control group was given 1 mls of 0.9% saline orally and had sham injection. The Gentamicin group was given 1 of 0.9 mls saline per oral and also had 5 mg/kg i.m. of Gentamicin sulphate (Gentalek) (Lek d.d., Pharmaceutical and Chemical Company, Ljubljana, Slovenia), while the Erythromycin group was given 50 mg/kg of Erythromycin stearate (Erotab) (Hovid BHD, Perak, Malaysia) dissolved in 1 ml of 0.9% saline per oral and also had sham injecttion. One hour later, fasting blood sugar was estimated in all groups. Animals were then given 2.5 g/kg glucose in 5 mls of 0.9 % saline orally. Blood glucose was then determined every one hour, for four hours after oral glucose by bleeding the marginal ear vein directly into the test strip of a commercial glucose meter based on the glucose oxidase method(One Touch BasicR . Lifescan Inc, California, USA).

Statistics

Data were analyzed using GraphPad Prism version 5.00 for Windows (GraphPad Software, California, USA). Point estimates of the blood sugar was summarized as mean ±standard deviation rather than standard error of the mean (SEM) because we considered that variations in measurement are more likely to be biological rather than measurement precision. The kinetics of glucose in the presence of a given drug may be more predictive of glucose levels after repeated glucose loads (e.g. regular feeding) than point estimates at a given time. We, therefore, evaluated the excursion of blood sugar over time using the pharmacokinetic software, PK Solutions 2.0 (Summit Research Services, Montrose, USA). Glucose excursions in 0-4hours were the kinetic of interest because we considered data extrapolated beyond that attributable to the glucose load (that is the time taken for blood glucose to baseline in the control) to be spurious. We assumed a two compartment model whereby blood was taken as compartment 1 and all sites as com--partment 2. We calculated and compared the rate of rise in blood glucose; taken as the absorption half life (A1/2), time to peak blood glucose (Pt); taken as the time to maximum glucose concentration, the rate of fall of blood glucose after the peak; taken as the distribution half life (D1/2) and the observed terminal rate of fall; taken as the terminal elimination half life (E1/2). We also calculated the total glucose load handled by the body by integrating the area under the curve from time 0 to 4 h (AUC0-4). Using hierarchal approach, the glucose excursion-time data were determined for each animal separately and then presented as mean ±standard deviation.

We used parametric statistics to compare point estimates of blood glucose because this is normally distributed in the general population, but we used non parametric statistics to compare all secondary data because the distribution of these in the general population is uncertain. As regards the point estimates of blood glucose, the comparison of interest was pair-wise and was between control and Gentamicin treated rabbits and between control and Erythromycin treated rabbits. We, therefore, used unpaired t-test with Welch's post test for this comparison. On the other hand, the comparison of interest in the derived blood glucose excursion-time data included comparison between Gentamicin and Erythromycin treated rabbits. This was done because differences in the curves were not expected to be dependent on the absolute value of blood glucose at any point in time but was dependent on the relationship between blood glucose over time (that is the equations of the curves). Such difference may suggest a difference in mechanism. We, therefore, used Kruskal-Wallis test with Dunn's post test for this comparison. All comparisons were performed at a level of significance of 0.05. At this pre-specified level of significance, a group size of 4 has 50% power to detect 0.49 changes (Harvey, 1995). Four animals per group were considered adequate (and conserves animal) in this exploratory, hypotheses-generating study.

RESULTS

Considering the point estimates of blood glucose, there was no significant difference in the fasting blood glucose levels of the control, gentamicin and erythromycin treated groups (5.76±0.78, 5.13±0.73 and 5.71±0.56 mmol/l respectively, P>0.050, all by pair wise comparisons). The blood glucose levels at one hour after oral glucose were significantly higher in the Gentamicin treated group in comparison to control (9.67±0.33 versus 8.11±0.12, P = 0.001). This was reversed in the second hour when the blood glucose levels became significantly lower in the gentamicin treated group in comparison to the control (6.53±0.08 versus 7.47±0.04, P=0.001). There was no significant difference (P>0.05) in all other blood glucose levels between the gentamicin treated group and control. On the other hand, there was no significant difference between the blood glucose levels of the Erythromycin treated group and control at 1 h (8.00±1.61 versus 8.11 ± 1.76, P= 0.93) and 2 h (7.83 ± 3.27 versus 7.47 ± 1.50, P= 0.85) after oral glucose load but the blood glucose levels in the Erythromycin treated group were significantly higher at 3 h (7.32 ± 0.17 versus 5.74 ± 0.79, P= 0.008) and 4 h (6.40 ± 0.27 versus 5.69 ± 0.23, P= 0.00) after oral glucose load.

Analysis of the kinetics of glucose excursion (Figures 1 and 2) revealed a significant difference in the half–life (A1/2) of increase in blood glucose between the Gentamicin treated group and control (0.45 ± 0.10 versus 0.59 ± 0.04 respectively, P=0.040) and between Genta-micin and Erythromycin (0.45±0.10 versus 0.69± 0.11 respectively, P=0.022) but not between Erythromycin treated group and control (0.69± 0.11 versus 0.59±0.04 respectively, P=0.142). The half-life of fall of the blood glucose level after the peak (D1/2) was significantly different between all groups. It was significantly lower with the Gentamicin treated rabbits than controls and much lower in comparison to Erythromycin treated group (0.44±0.16 versus 0.81±0.18; p=0.021, and versus 9.44±2.16; P=0.0002, respectively). The terminal half life of blood glucose (E1/2) of control and Gentamicin treated rabbits was not significantly different from each other (3.17±1.64 versus 4.42±2.36, p=0.422) but both were significantly lower than that of the Erythromycin treated groups (P<0.0001). In all groups, the time to peak concentration (Pt) were not significantly different from each other (P>0.051).There was no significant difference in AUC0-4 between control and Gentamicin treated group (P=0.453) but AUC0-4 of the Erythromycin treated group

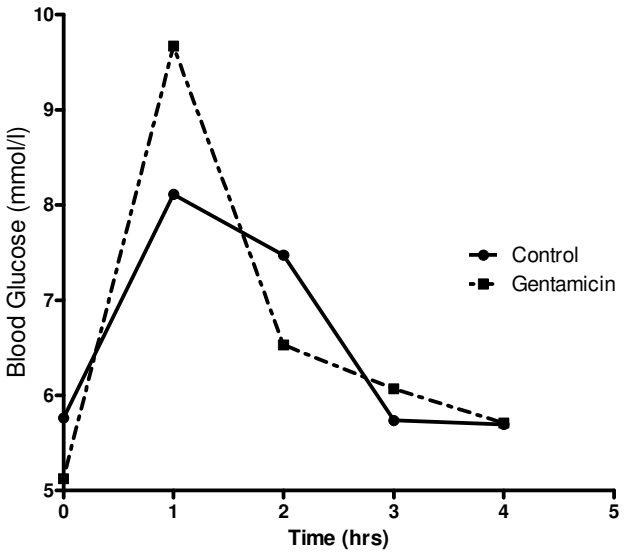

Figure 1. Blood glucose excursion over time after oral glucose loads in Gentamicin treated versus control rabbits.

was significantly higher than control (31.82±0.12 versus 29.95±0.79; p=0.003) and significantly higher than Gentamicin treated group (31.82 versus 30.28±0.18; p=0.0001).

DISCUSSION

This study revealed striking perturbations of blood glucose excursions after oral glucose loads in rabbits pretreated with either gentamicin or oral erythromycin.

In the Gentamicin treated group, the rapid rise and fall in blood sugar in the first two hours after the oral glucose load may be clinically important in patients undergoing screening for diabetes and in the intensive care unit, where frequent or continuous blood glucose monitoring is routine. The awareness of the possible effect of Gentamicin on glucose loads may lead to fewer misinterpretations and better coordinated and appropriate intervenetions. In the Erythromycin group, blood glucose kinetic is perturbed after the peak glucose level, resulting in retardation in the rate of blood glucose decline in comparison to controls. This may have similar clinical implication to Gentamicin but in addition, would suggest that Erythromycin may adversely influence glycemic control.

Both Gentamicin and Erythromycin also appear to perturb point estimates of post-prandial blood glucose levels. Such perturbations are clinically important because the risk to health from glycemia has been demonstrated to have no threshold and that across the range of even normal glycemia, every (small) change in blood glucose leads to an increase in health hazard (Chien et al.,2008)

Although, as previously stated, the hypothesis that guided the choice of gentamicin-erythromycin antibiotics and the design of this study was based on ion channels modulations , we did not set out and did not perform experiments to confirm if and what ion channels (or pathways) are involved in any observed perturbation of blood glucose. However, reasonable explanations may be deducted from the extent and pattern of perturbation considering this study and previous studies. Such explanations may at least guide development of a hypothesis for further exploration. In this regard, the crescendo–decreescendo pattern in the Gentamicin treated group could be explained by an initial inhibition of insulin release by Gentamicin until the inhibition is suddenly overcome, followed by rapid insulin release. This scenario appears plausible because at least one previous ex vivo study has shown that Gentamicin inhibits glucose induced release of insulin by isolated pancreatic islets cells and that this inhibition may be rapidly overcome by high glucose and potassium levels (Boschero and Delattre, 1985). Though our study is *in vivo,* inhibition of insulin release similar to the *in vitro* pattern is expected to lead to a continuing and rapid increase in blood sugar until a threshold sugar level that can overcome the inhibition is reached. This is expected to then lead to a rapid fall in blood glucose followed by slower oscillations. Our findings, as depicted in Figure 1 would appear to fit such a scenario.

The suggestion of the data from the Erythromycin treated rabbits appears to contradict reports from previous studies which suggested that Erythromycin improved glycemic control in type 2 diabetic patients (Ueno et al., 2000), decreased fasting hyperglycemia in the same type of subjects (Ueno et al., 2001) and decreased circulating growth hormone levels in non diabetic subjects(Ueno et al., 2006). It may be worthy of note that our study subjects are non diabetic and that the focus of the study was (precisely) on response to a glucose load. Also, the hypothesis of previous studies was based on, and the results explained to be due to, motilin receptor dependent modulation of gastric emptying or insulin -like growth factor 1(Ueno et al., 2000, 2001 , 2006), which may not be operative in fasted rabbits that have been given oral glucose in saline. Our findings (Figure 2) may be expected if Erythromycin induces a late compromise of the rate or quantity of insulin release by pancreatic islets. Such a compromise would be expected to occur early if due to ion channel perturbation. Late compromise may be expected from disturbance of the synchronization of insu-lin secretion within and between pancreatic islets (Hellman et al., 2006; Meda, 2003; Grapengiesser at al., 2004). Such synchronization has been recognized to be mediated via diffusion of ATP (Hellman et al., 2006) through gap junctions (between B-cells within islets) (Meda, 2003) and via purinergic neural activity between different islets (Grapengiesser at al., 2004). Erythromycin is known to act as leaky perforin on cell membranes (Barker, 2005). Such perforins may modulate synchro-nization between beta-cells, and has also been shown to reduce the re-

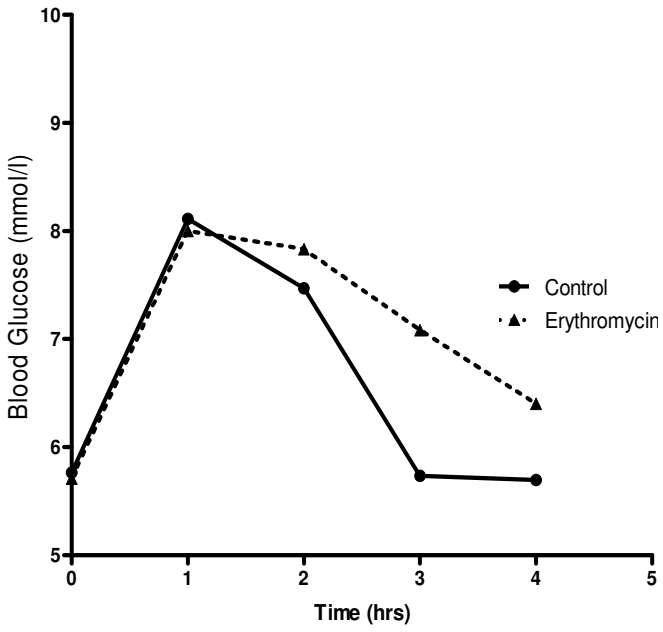

Figure 2. Blood glucose excursion over time after oral glucose loads in Erythromycin treated versus control rabbits.

lease of pancreatic polypeptides via puri-nergic neural pathways (Witteman, 1994). Furthermore, cholinergic recaptors on B-cells are known to enhance glucose-induced insulin release (Gautam, 2007). It is interesting that Erythromycin has been demonstrated to act as partial agonist on cholinergic receptors (Irokawa et al., 1999). Our findings may be new, but may be inter-preted, in retrospect, by effects of Erythromycin on cell membranes and receptors that have been described in previous studies.

This study revealed clinically important modulations of blood sugar excursion by Gentamicin and Erythromycin after glucose loads. The clinical importance is probably wide and because of these perturbations may need further exploration, especially considering the hypotheses it has suggested.

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