ER Stress Induces Beta-Cell Apoptosis

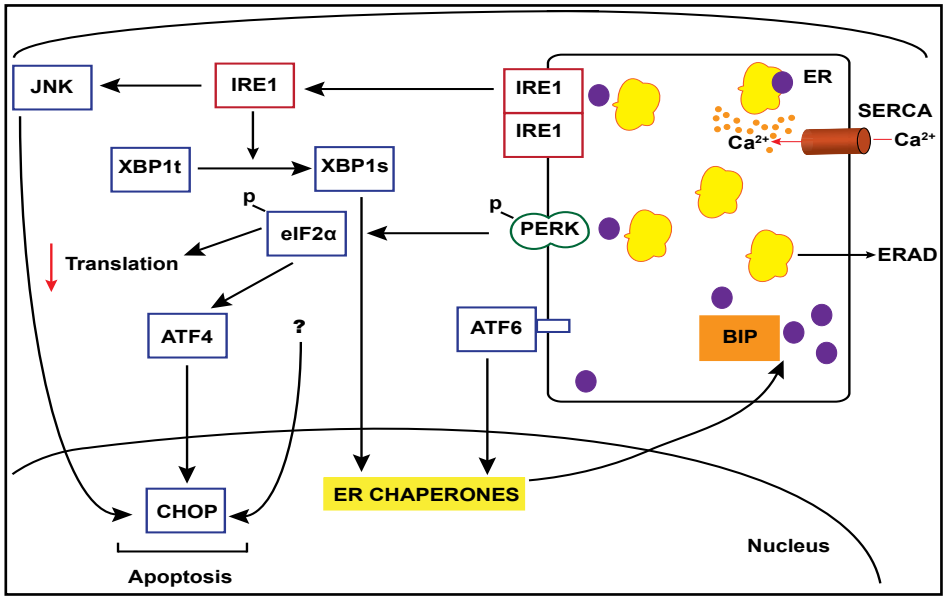
A growing consensus links the loss of beta-cell mass with the development of type 2 diabetes mellitus (T2DM). A lecture that was presented by D.L. Eizirik, MD, PhD, Laboratory of Experimental Medicine, Université Libre de Bruxelles, Brussels, Belgium, demonstrates that endoplasmic reticulum (ER) stress, induced by high levels of saturated free fatty acids (FFAs), triggers a response that leads to eventual beta-cell apoptosis. This observation suggests one or more new targets for the therapeutic maintenance of beta-cell populations.

Recent evidence suggests that the ER stress response plays a role in the pathogenesis of diabetes through the induction of beta-cell apoptotic pathways. This stress occurs in the presence of unfolded or misfolded proteins in the lumen of the ER and triggers the unfolded protein response (UPR); the end result is an upregulation of molecular chaperones to aid in protein folding or, failing that, in the face of chronic stress, the initiation of programmed cell death, or apoptosis (Eizirik et al. *Endocrine Reviews* 2008).

Saturated free fatty acids are candidate inducers of ER stress in T2DM. An investigation by Cunha et al. in Eizirik's laboratory (*J Cell Sci* 2008) exposed INS-1E (insulinoma) cells, FACS-purified rat primary beta-cells, and human islets to the unsaturated fat oleate and the saturated fat palmitate. Results showed a differential ER response based on fat saturation.

Palmitate induced the signaling of the pathways of 3 ER membrane-bound proteins, IRE1, and ATF6 – which probably are pro-cell survival in this case – but signals through the membrane-bound protein PERK as well, which can trigger apoptosis through the proapoptotic elF2alpha/ATF4/CHOP signaling cascade (Figure 1). This observation was verified through the use of the reagent salubrinal, a phosphatase inhibitor that is known to render the gatekeeper elF2alpha constitutively active. In the presence of oleate, salubrinal elicited the same UPR expression profile and cell death in INS-1E cells and FACS-purified rat primary beta-cells as those seen with exposure to the saturated fatty acid palmitate (Cnop et al. *Journal of Biological Chem* 2007). These results were replicated in human islet cell preparations (Ladriere, Eizirik, and Cnop. Unpublished data).

Figure 1. ER Stress Signaling Cascade.



Highlights from the
European Association for the Study of Diabetes 2008 Annual Meeting

Palmitate also induces IRE1 signaling, promoting cell survival through downstream chaperone activation or, alternately, tipping the scales toward apoptosis through activation of Jun kinase (JNK). This association was shown using JNK inhibitors. In vitro work also was able to verify the palmitate/UPR/CHOP association by knocking down palmitate-induced expression with the use of CHOP siRNA and thus partially protecting beta-cells against palmitate-induced apoptosis (Cunha et al. *J Cell Sci* 2008).

Conversely, IRE1 signaling that promotes cell survival through the production of chaperones (eg, BiP) was validated through the use of BiP overexpression to rescue an insulin-secreting cell line from lipid-induced apoptosis (Laybutt et al. *Diabetologia* 2007).

A final association is to be made between ER stress and the dysfunction and loss of beta-cells, as seen in diabetes. Studies have shown that in the islets of db/db (diabetic) mice, numerous ER stress factors were unregulated – this also is true of human islet preparations (Laybutt et al. *Diabetologia* 2007). More to the point, both the proapoptotic CHOP expression and the ER area were increased in islets from patients with T2DM (Huang et al. *Diabetes* 2007; Marchetti et al *Diabetologia* 2007). Interestingly, islets from obese patients also show some increase in cytosolic CHOP expression. This, and additional evidence, suggests that ER stress may be a common mediator of both beta-cell death and insulin resistance in T2DM (Eizirik et al. *Endocrine Reviews* 2008).

KIOM-79 Slows the Development of Diabetic Retinopathy in Animal Models

Treatment with KIOM-79, a novel mixture of 4 herbal extracts, appears to slow the development of retinopathy, according to a new animal study of diabetic eye disease. In addition to characterizing the treatment effects of KIOM-79, the study provides important insights into the pathophysiology of diabetic retinopathy.

Vascular Damage in Retinopathy

Researchers have previously observed the accumulation of advanced glycation end products (AGEs) in the neural retina and vascular cells of diabetic animals (Miura J et al. *J Diabetes Complications* 2003). In these animals, AGE accumulation appears to induce the programmed death of retinal pericytes and neuronal cells. Retinal pericyte apoptosis leads to a range of damaging events within the retina, including the development of microaneurysms, retinal hemorrhages, neovascularization, and permanent impairment of visual function.

KIOM-79 contains the extracts of 4 herbs: parched *Puerariae radix* and gingered *Magnoliae cortex, Glycyrrhizae radix*, and *Euphorbiae radix*. These agents inhibit AGE-induced apoptosis by preventing NFkB activation and lowering proapoptotic cytokine production. Jin Sook Kim, MD, Korea Institute of Oriental Medicine, Daejeon, Republic of Korea, described the effects of KIOM-79 on AGE-induced retinal damage.

Treatment Effects of KIOM-79

In the current study, Dr. Kim and colleagues treated 7-week-old male Zucker Diabetic Fatty (ZDF) rats with KIOM-79 (50 mg/kg) or placebo once daily for 13 weeks. Normal, untreated rats also were included as control animals. At the end of the treatment period, the retinas were harvested and examined for signs of vascular damage.

Compared with rats in the vehicle-treated group, KIOM-79-treated rats had significantly lower serum levels of AGEs ($p<0.05$) and lower numbers of AGE-positive retinal cells ($p<0.05$).

Retinas from the vehicle-treated group showed evidence of vascular damage when assessed by immunohistochemistry, including areas of retinal pericyte loss and the appearance of acellular capillaries. However, these vascular changes rarely were observed among retinas that were harvested from KIOM-79-treated rats (Figure 1, top panel).

Additional fluorescein staining allowed researchers to calculate retinal angiography lesion scores, which are an indication of the degree of retinopathy. When assessed by fluorescein staining, retinas from vehicle-treated ZDF rats showed severe signs of vascular damage, including vessel-narrowing, fluorescein leakage, and nonperfusion of fluorescein. By contrast, retinas from KIOM-79-treated diabetic rats showed significantly fewer changes in retinal angiography ($p<0.05$; Figure 1, middle panel).

These findings provide quantitative evidence to support the further study of KIOM-79, Dr. Kim said. "Treatment with KIOM-79 is useful in inhibiting the accumulation of AGEs in retinal tissue and has a preventive effect on the development of diabetic retinopathy," she concluded.