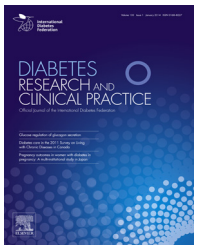
Contents available at ScienceDirect

Diabetes Research and Clinical Practice

journal homepage: www.elsevier.com/locate/diabres

International Diabetes Federation



Invited review

Cereulide food toxin, beta cell function and diabetes: Facts and hypotheses

Roman Vangoitsenhoven [a], *Michael Maris* [a], *Lut Overbergh* [a], *Joris Van Loco* [b], *Chantal Mathieu* [a], *Bart Van der Schueren* [a,*]

[a] *Clinical and Experimental Endocrinology, KU Leuven, Leuven, Belgium*
[b] *Scientific Institute of Public Health, Brussels, Belgium*

ARTICLE INFO

Article history:
Received 23 April 2015
Accepted 28 April 2015
Available online 6 May 2015

Keywords:
Bacillus cereus
Cereulide
Food toxin
Environmental pollutant
Mitochondria
Diabetes mellitus

ABSTRACT

The incidence of both type 1 and type 2 diabetes is increasing and although environmental pollutants are believed to be potential culprits, the extent to which they can be held responsible remains uncertain. Some bacterial strains of the *Bacillus cereus* produce a toxin, cereulide, which is frequently found in starchy meals and which is difficult to eradicate from the food chain as it is highly resistant to heat, acidity and proteolysis. While cereulide is well known to cause acute emetic toxicity when ingested at high doses, several in vitro studies have shown that also extremely low doses of cereulide can be toxic, with beta cells being particularly sensitive. Mechanistically, such low doses impair the mitochondrial activity of the beta cells thereby leading to hampered insulin secretion and cell death, both key traits in the pathophysiology of diabetes. In vivo studies of chronic or repeated low dose exposure to cereulide are currently lacking, but should be performed to further clarify the true relevance of cereulide as a potential environmental contributor to the ongoing diabetes epidemic.

Contents

* *Corresponding author.*
E-mail address: Bart.vanderschueren@uzleuven.be (B. Van der Schueren).
http://dx.doi.org/10.1016/j.diabres.2015.04.029

1. Introduction

The incidence of type 1 and type 2 diabetes continues to rise, resulting in the ongoing worldwide diabetes epidemic. Both forms of diabetes are characterized by hyperglycemia, mainly due to a deficient insulin secretion. In type 1 diabetes the beta cells are destructed in an auto-immune reaction, while in type 2 diabetes a relative insulin deficiency develops as a result of insulin resistance and stressed beta cells. However, beta cell failure and beta cell death are central processes in the course of type 1 and type 2 diabetes.

The pathophysiology of the failing beta cell has been studied extensively and is thought to be a consequence of both genetic predisposition and environmental factors [1]. Although sedentary lifestyle and Western diet are the main drivers of the rising prevalence of type 2 diabetes, a role for several environmental pollutants has been demonstrated over the years as well. Persistent organic pollutants (POPs) including polychlorinated biphenyl (PCB) and organochlorine pesticides (OCPs), dioxins, phthalates, xenostrogens or cadmium have all been linked with a higher prevalence of type 2 diabetes [2].

As the insulin producing beta cell has been shown to be very susceptible to cereulide's toxicity, we will review here the evidence for a potential role for this common food toxin in the onset of diabetes.

1.1. *Cereulide toxin and its prevalence*

Bacillus cereus (*B. cereus*), an endospore-forming gram positive bacterium, has been known to cause 2 different forms of food poisoning, either a diarrheal-type syndrome or an emetic-type syndrome. The strains that cause the emetic-type food poisoning produce cereulide, a cyclic dodecadepsipeptide that acts as a potassium ionophore on the inner mitochondrial membrane. As the maintenance of the mitochondrial membrane potential is compromised, the driving force for oxidative phosphorylation in the electron chain is lost, and thereby the option of aerobic energy production, causing a huge stress on cells with high metabolic demands, such as the beta cell [3]. Structurally, cereulide closely resembles valinomycin, another potassium ionophore, which is used frequently as a mitochondrial uncoupling agent in basic research experiments. Cereulide is heat stable and resistant to extreme alkaline and acid environments, making it difficult to eradicate from the food chain and digestive tract. Moreover, it is highly lipophilic (log Kow 5.96), causing it to stick to various surfaces, and possibly allowing accumulation in human tissues [4].

Although *B. cereus* is ubiquitous in the environment, the emetic cereulide producing strains are usually rare (1–2% of all *B. cereus*). On the other hand, the incidence of *B. cereus* toxin-related food poisoning is increasing in Europe [5] and a recent study in Germany showed that the prevalence of emetic strains of *B. cereus* is underestimated [6]. Cereulide has been implicated in food intoxications worldwide and it has been found in several dishes, including rice, pasta and potatoes but also in vegetables and infant meals [7–10]. In general, starchy nutrients, especially if stored improperly are the highest risk for cereulide contamination. A study performed in Belgian Chinese-style restaurants showed that up to 56% of the served fried rice dishes contained *B. cereus*, and that cereulide toxin could be found in 7.4% of such meals, albeit in low concentrations (approximately 4 ng/g food) [11,12].

At high doses, cereulide is well known to cause acute mostly self-limiting food poisoning, with nausea and emesis occurring a few hours after the meal [13]. In rare cases, the food intoxication can be fatal, with an estimated lethal dose of 8 μg/kg body weight [10]. Cases of acute toxicity typically report hepatotoxicity, ranging from hepatitis to hepatic failure, but cereulide has also been detected in the spleen and plasma of intoxicated patients, suggesting that cereulide enters both portal and systemic circulation.

Taken together, these characteristics make chronic or repeated low grade exposure likely.

1.2. *Effects of cereulide in beta cell models*

Different studies have evaluated the effect of cereulide on the function of beta cells, making use of in vitro beta cell models. Virtanen et al. demonstrated in 2008 that fetal porcine islets of Langerhans exposed to 1 ng/ml of cereulide for 2 days have reduced insulin content and increased cell death [14]. Hoornstra and colleagues found that 8–24 h exposure to 10 ng/ml cereulide caused disruption in the islet-like growth pattern in murine insulinoma cells (MIN6), followed by a decrease in cell density and an appearance of necrotic and pyknotic cells [15]. Moreover, MIN6 cells stained with JC1-dye showed a green fluorescence after exposure to cereulide, indicating loss of mitochondrial membrane potential, and thus of energized mitochondria. Interestingly, cell death in the MIN6 cells occurred at 100 times lower concentration as compared to human keratinocytes (HaCaT), porcine spermatozoa or murine fibroblasts (L929) [15].

Studies from our group confirmed the increased sensitivity to cereulide of different beta cell models when compared to other mammalian cell lines [16]. As such, rat and mouse beta cell lines (INS-1E and MIN6) and freshly isolated murine pancreatic islets showed very high rates of apoptosis after exposure to 5 ng/ml cereulide for 24 h, whereas human hepatocellular carcinoma (HepG2) and renal fibroblast cells (COS-1) remained viable when exposed to the same concentration. Hoechst/propidium Iodide staining and electron microscopy revealed that the observed cell death was mainly due to apoptosis, with only a minor proportion of cells dying by necrosis. This was further supported by increased caspase 3/7 activation, elevated cytochrome C release into the cytoplasm and upregulation of pro-apoptotic mRNA markers, such as CCAAT/-enhancer-binding protein homologous protein (CHOP), in MIN6 cells [16].

Transmission electron microscopy showed that mitochondria of the MIN6 cells were swollen and disintegrated after exposure to 0.5 ng/ml cereulide. At this dose, reactive oxygen species culminated more than twofold, and basal respiration rate was reduced to half, as compared to unexposed MIN6 cells.

Presumably, such high mitochondrial toxicity underlies the impaired insulin secretion, since normal insulin secretion is initiated by aerobic glycolysis in the mitochondria to generate