The study of Dr. COA

Dr. COA improves Insulin Secretion and inhibits Pancreatic β cell death under Type I diabetic stress

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A. Summarizing the specific aims

Diabetes is one of the typical metabolic diseases in which blood glucose levels persist for a long time. This is a disease where insulin in the body is not produced properly or the cells in the body do not respond properly to insulin. It is a very frightening condition that leads to serious complications such as ketosis, hyperglycemic hypertrophy bicethonic coma, cardiovascular disease, stroke, chronic kidney failure, diabetic ulcer and blindness.
There are two main types of diabetes. First, type 1 diabetes is an autoimmune disorder caused by pancreatic β-cell loss that produces insulin. It is called insulin-dependent diabetes mellitus or juvenile diabetes mellitus because it is inherently insulin-deficient. However, the mechanisms of autoimmune reaction and cell death of pancreatic cells are still not clear. Type 2 diabetes is a disease caused by dysfunction of pancreas β-cells and insufficient insulin release in response to glucose in the surrounding tissues such as such as skeletal muscle (glucose disposal), liver (glucose production), and adipose tissue (lipolysis). This is called insulin resistance. The types of diabetes occur in older people and 90~95% of diabetes patients have type 2 diabetes. Therefore drug development and mechanisms are very actively studied.

As mentioned earlier, type 1 diabetes causes are not well known. It is deeply linked to the loss of function of pancreas β-cells that produce insulin. There are several factors, but the most common is the destruction of β-cells result from autoimmune diseases and apoptosis. Recently, several studies found that β-cells recruit Dendric cell (DC) cells by the secretion of exosome and then DC cell recruit the cytotoxic T cell, causing the β-cells death through autophagy. However, there are also many questions to solve mechanisms, and further, the study of countermeasures are still insufficient.

Here, we studied the effects of Dr.COA water on type 1 diabetes model by Streptozotocin (STZ) using INS-1 cells, which are the most representative pancreas β-cells.
Specific Aims:
Aim 1. Study the effect of Dr. COA water on Type 1 Diabetes cellular model
Aim 2. Study the effect of Dr. COA water on insulin secretion from pancreas β-cells

B. Background

Diabetes is a disease in which there is high blood glucose (blood sugar) levels. Glucose is the main energy source from foods and Insulin helps the glucose get into the cells by blood. Usually, the pancreas releases insulin to help the body store and use the sugar and fat from the food. Therefore, Diabetes occurs with abnormal pancreatic functions such as producing very little or no insulin.
With type 1 diabetes, the body does not make insulin because of the pancreas' failure to produce it.. With type 2 diabetes, the more common type, occurs with insulin resistance (Fig.1). Without enough insulin, the glucose stays in the blood. This is why high blood sugar levels are a common symptom of diabetes.

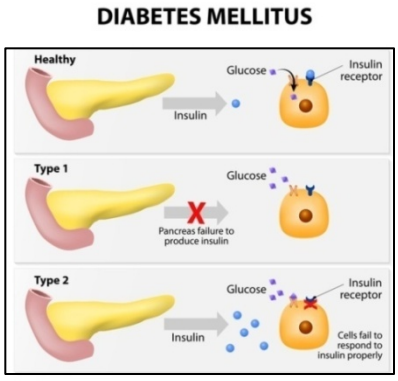

Fig. 1. Healthy pancreas and pancreas in type 1 and type 2 diabetes.
(NIH, Genetics Home Reference)

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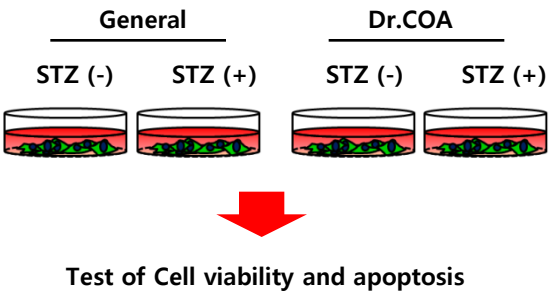
C. Methods

(1)



To study the effect of the Dr.COA water on Diabetes, we treated streptozotocin (STZ) to Rat insulin-secreting pancreatic β cell line INS-1 and then determined cell death. INS-1 cells were cultured in RPMI 1640 medium that was solved in general water or Dr. COA water during two days before STZ treatment. All results were performed in triplicate of at least three independent experiments. STZ-induced cell death was determined by cell viability and western blot (1).

Furthermore, insulin secretion assay was also determined to test the effect of Dr. COA on insulin secretion in INS-1 cells.(2)

(2)

D. Results

Figure 1.

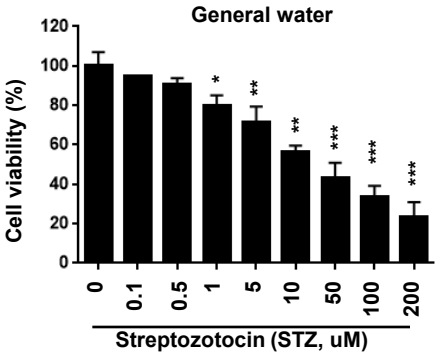
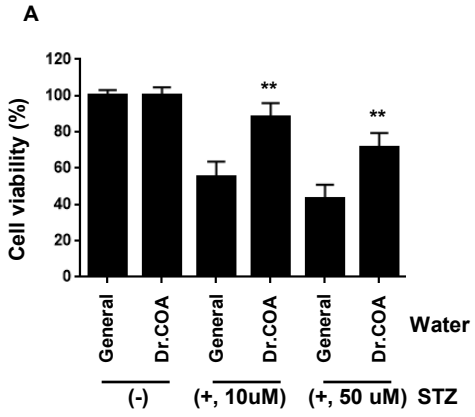
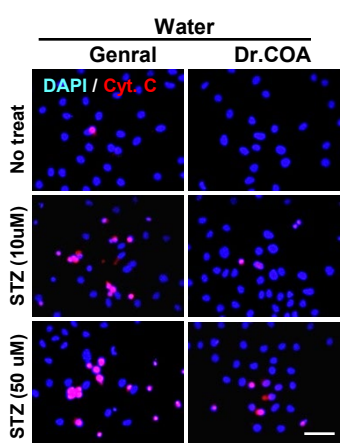


Figure 2.





To test cell viability, INS-1 cells were cultured in 96well plates and then exposed to STZ with a variety of concentrations for 24hr. We found that STZ-induced cell death in a dose-dependent manner. As previously demonstrated, the IC 50 of STZ was found to be 10~50uM (Figure 1). The IC 50 is the concentration of a drug or inhibitor that is required for a half inhibition in vitro.

To test the effect of Dr. COA on STZ-induced INS-1 cell death based on the IC 50, we cultured INS-1 with RPMI powder dissolved in general water or Dr. COA. After two days, cells were exposed to STZ with a IC 50 concentration. As shown in Figure 2A, INS-1 cell viability was markedly reduced by STZ with IC 50 concentrations while the cells with Dr.COA resulted in a significant improvement of cell viability. Similar results were obtained using Cytochrome C expression by immunofluorescence assay (IFA) (Figure 2B). Cytochrome C is a main marker of programmed cell death, especially Apoptosis. The expression of cytochrome C by STZ significantly decreased in cell cultured with Dr. COA.

These results suggest that Dr.COA might inhibit STZ-induced cell death on Pancreatic β-cell, INS-1.

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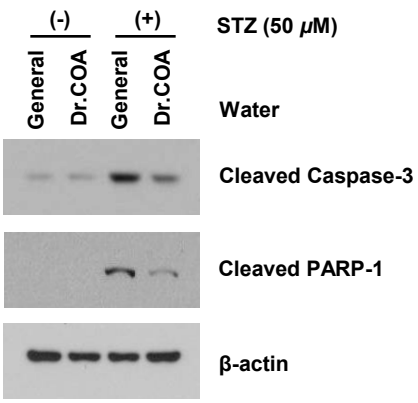
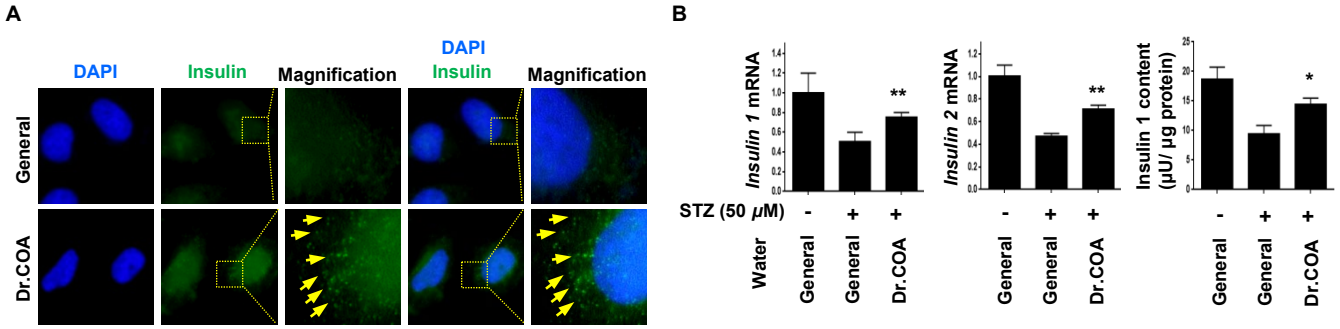
D. Results

Figure 3.



To test apoptosis, detection of cleavage Caspase-3 and Poly(ADP-ribose) polymerase (PARP1) and western blots were performed. During apoptosis, PARP1 is cleaved by activated caspase-3 (cleaved caspase-3). They are the final markers of apoptosis. We found cleaved caspase-3 and PARP1 levels increased in STZ-treated cells, but in the cell with Dr. COA, the expression of apoptosis markers significantly decreased (Figure 3). Together, we suggest the protective effect of Dr. COA on INS-1 cells might act as inhibitor of cell apoptosis. Especially, if the main reason of type 1 diabetes is the pancreas β cell death as several papers suggested, we could hypothesize that Dr. COA might help type 1 diabetes.

Figure 4.



Pancreatic β cell damage and insulin secretion dysfunction are main concerns on the diabetes study. Next, we tested effect of Dr. COA on insulin secretion in INS-1 cells. INS-1 cells were cultured in RPMI (dissolved in general water or Dr.COA) for two weeks and determined insulin secretion by IFA (3mM glucose, Figure 4A). Surprisingly, we found that insulin secretion improved significantly in the INS-1 cells with Dr. COA. In the cells with general water, we found slight insulin secretion, however, in INS-1 cells cultured in Dr. COA, it was clear and strong and about 80% of all β cells showed insulin secretion (data not shown). Furthermore, as shown in Figure 4B, decreased insulin secretion in damaged β cells by STZ was recovered in cells grown in Dr.COA.

These results suggest that Dr. COA might enhance help the β cell activity to increase a insulin secretion and protect against STZ-induced β cell damage and diabetes. However, more detailed studies are required through animal experiments and clinical trials, and the mechanisms are yet still not clear. Therefore, we need to study about it in detail as our further study.

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E. Conclusions

1. **Dr. COA protects INS-1 cells from STZ induced cellular damage.**
2. **Dr. COA is capable of protecting INS-1 cells through inhibition of β cell's apoptosis by damage.**
3. **Dr. COA has an effect of an improvement of insulin secretion in both basic and STZ-induced damage condition.**

F. Further Studies

1. **Dr. COA has the ability to help maintain homeostatic functions of β cells, especially the most fundamental problem of type 1 diabetes, and has the effect of excellently inhibiting cell death by STZ treatment.**
2. **Dr. COA plays a role in assisting insulin self-generation in pancreatic β cells. It is necessary to study the mechanism in future.**
3. **After establishing type 1 diabetes mice model, we need to study it more specifically.**
4. **Dr. COA has been proven to be effective for type 2 diabetes, and it is thought that more effective research results will be obtained when it is conducted in parallel with type 1 diabetes research..**