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Novel role of curcumin combined with bone marrow transplantation in reversing experimental diabetes: Effects on pancreatic islet regeneration, oxidative stress, and inflammatory cytokines

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ABSTRACT

Therapeutic utility of bone marrow transplantation in diabetes is an attractive approach. However, the oxidative stress generated by hyperglycemia may hinder β-cell regeneration. The present study was undertaken to investigate the therapeutic potential of curcumin, a dietary spice with antioxidant activity, bone marrow transplantation, and their combined effects in the reversal of experimental diabetes. Diabetes was induced in mice by multiple low doses of streptozotocin. After the onset of diabetes, mice were treated with curcumin (10 mM; 100 μl/mouse, i.p., for 28 days) or received a single bone marrow transplantation (10^6 unfractionated bone morrow cells), or both. Parameters of diabetes, integrity of pancreatic islets, pancreatic oxidative stress markers, and serum pro-inflammatory cytokines, were evaluated. Treatment with either curcumin or bone marrow transplantation significantly reversed streptozotocin-induced hyperglycemia/glucose intolerance, hypoinsulinemia, and damage of pancreatic islets. Interestingly, combination of curcumin and bone marrow transplantation elicited the most profound alleviation of such streptozotocin-evoked anomalies; including islet regeneration/insulin secretion. On the other hand, curcumin, either alone or combined with bone marrow transplantation, blunted the pancreatic lipid-peroxidation, up-regulated activities of the antioxidant enzymes, and suppressed serum levels of TNF-α and IL-1β. Curcumin and single bone marrow transplantation proved their therapeutic potential in reversing diabetes when used in combination. Curcumin, via its antioxidant and anti-inflammatory effects, evidently enhanced the ability of bone marrow transplantation to regenerate functional pancreatic islets. Hence, the use of natural antioxidants combined with other therapeutic regimens to induce pancreatic regeneration is a promising strategy in the management of diabetes.

1. Introduction

Diabetes mellitus is a serious chronic metabolic disease that predisposes to ill health and multiple-organ dysfunction (Kim et al., 2006). A reduction of β-cell mass in the pancreas is a pathophysiological hallmark in the development of both type 1- and type 2-diabetes (Rosenberg, 1995). Therefore, regeneration of pancreatic islets is certainly a worthwhile therapeutic goal that would substantially ameliorate diabetes and lessen its complications (Risbud and Bhonde, 2002).

Recent animal and clinical studies have suggested that tissue regeneration can occur upon the introduction of bone marrow derived stem cells that have multiple effects (Colman, 2004). It was hypothesized that such pleuripotent stem cells may be able to grow as adult pancreatic islet cells (Lechner and Habener, 2003); however, its value in diabetes is controversial. Early studies by Slack (1995) indicated the failure of islet regeneration following treatment with streptozotocin or alloxan and suggested that these drugs target, in addition to the differentiated β-cells, the potential stem or transit cells. Contrary to this report, more recent studies have shown that streptozotocin does not destroy the intra-islet stem cell reserve in experimentally-diabetic animals (Banerjee and Bhonde, 2003). In support, Ianus et al. (2003) demonstrated that bone marrow cells can differentiate into functionally competent pancreatic β-cells; thereby rationalizing for the cell-based approach in treatment of diabetes.

Streptozotocin, a monofunctional nitrosourea derivative, is one of the most commonly used agents to induce diabetes in experimental animals (Szkudelski, 2001). Several lines of evidence suggest that the diabetogenic capacity of streptozotocin relies on its ability to damage β-cells and induce oxidative stress, along with up-

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regulation of iNOS and nuclear factor kappa B (NF-κB) (Ohkuwa et al., 1995; Stosić-Grujicić et al., 2001).

Natural products have been proven effective against many diseases; like cancer, arthritis, Alzheimer disease, and diabetes (Kanitkar et al., 2008; El-Azab et al., 2009; El-Mowafy et al., 2010; Moon et al., 2010; Zhang et al., 2010). Curcumin, the naturally occurring yellow pigment isolated from the rhizomes of the plant *Curcuma longa*, has been shown to possess antioxidant, anti-tumor, and anti-inflammatory properties (Fujisawa et al., 2004; El-Azab et al., 2009). At the cellular and molecular levels, such effects for curcumin are mediated by free-radical scavenging, up-regulation of defense proteins; such as heme oxygenase-1 and reduced glutathione, and suppression of pro-inflammatory/pro-apoptotic cytokines/transcription factors; like TNF-α and NF-κB (Motterlini et al., 2000; Weber et al., 2006). The potential of curcumin as a hypoglycemic agent has been studied in animals (Hussain, 2002; Pari and Murugan, 2005); yet with conflicting results. Although many aspects of curcumin cytoprotection had been revealed, there has been a gap of knowledge regarding its therapeutic potential in reversing the effects of experimental streptozotocin-induced diabetes.

Currently, in multiple low doses of streptozotocin-induced diabetes, we probed the individual and combined effects of curcumin and bone marrow transplantation on key elements of glycemic control, pancreatic islet integrity and function, pancreatic oxidative stress level, and levels of serum pro-inflammatory cytokines (TNF-α and IL-1β). This would ultimately improve our knowledge as to whether abrogation of oxidative stress and cytokine-mediated inflammation would boost the transformation of bone marrow cells into functional, insulin secreting pancreatic β-cells.

2. Materials and Methods

2.1. Chemicals and Reagents

Streptozotocin was obtained from MP Biomedicals, LLC. (France). *Curcumin* was purchased from Bio Basic Inc. (Canada). Commercially available kits (Bio-Diagnostic, Egypt) were used for determining the enzymes superoxide-dismutase, catalase, glutathione peroxidase, and the lipid-peroxide marker malondialdehyde. Serum insulin levels were determined using *Ultra Sensitive Mouse Insulin Enzyme Linked Immunosorbent Assay (ELISA) Kit* (Crystal Chem Inc., USA). *Mouse tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β) Immunoassay kits [Quantikine, ELISA]* were purchased from R&D systems (Minneapolis, USA). All other chemicals were obtained from Sigma (St. Louis, MO, USA).

2.2. Animals

All animal procedures and the experimental protocols were carried out in accordance with the *Guide for the Care and Use of Laboratory Animals*. Swiss albino mice weighing 20 to 25 g were obtained from the Egyptian Organization for Biological Products and Vaccines (Vacsera, Egypt) and housed under controlled temperature (25 ± 1 °C) on a 12 h light/dark cycle. Food and water were allowed *ad libitum* during the study period.

2.3. Induction of Diabetes

The animals were acclimatized for one week before initiation of the experiment. After overnight fasting, diabetes was induced by multiple intraperitoneal injections of freshly prepared streptozotocin (40 mg/kg of bodyweight) dissolved in 0.1 M chilled citrate buffer (pH 4.5) for 5 consecutive days. The control group received the vehicle alone. The animals were allowed to drink 5% glucose solution overnight to prevent initial streptozotocin-induced hypoglycemic mortality. Forty eight hours after last streptozotocin dose, fasting blood glucose levels were monitored and animals with blood glucose levels >200 mg/dl were considered diabetic and assigned for different treatment regimens.

2.4. Bone Marrow Cell Isolation and Transplantation

Bone marrow from normal mice was flushed from the medullary cavities of femurs using a 25 gauge needle and suspended in phosphate-buffered saline. Cells after isolation were checked for viability by Trypan blue dye exclusion method and counted using hemocytometer. Recipient diabetic mice (non-irradiated) were transplanted intravenously through tail vein approximately 10^6 unfractionated bone marrow cells, as a single injection (Banerjee et al., 2005).

2.5. Pharmacological Treatment

Forty diabetic mice were randomly assigned to 4 groups, 10 mice each, and were treated as follows. The first group received vehicle injection and served as diabetic control. A second group received intravenous, single bone marrow transplantation. A third group was treated with curcumin (10 mM; 100 μl/mouse, i.p.) for 28 days. It was freshly dissolved in DMSO to a final concentration of 1 M, and then further diluted in phosphate-buffered saline (Gururaj et al., 2002; El-Azab et al., 2011). A fourth group received a combination of single bone marrow transplantation and curcumin. Additional eight normal mice received vehicle injection and served as normal control. All treatments started two days after the last dose of streptozotocin, i.e. after the onset of diabetes. At the end of experiment, seven surviving mice from each group were randomly selected for further analysis.

2.6. Intraperitoneal Glucose Tolerance Test

After fasting overnight, all groups of mice were injected with 2 g/kg body weight of glucose intraperitoneally. Glucose disposal was analyzed by measuring random blood glucose of mice at different time points 0, 30, 60, and 90 min. Intraperitoneal glucose tolerance test was performed the day before scarifying.

2.7. Blood Glucose Estimation

At the end of experiment; i.e. 30 days after last dose of streptozotocin, blood samples were obtained by tail prick and glucose was measured by Accu-check go blood glucose meter (Roche Diagnostic, Germany) in all groups.

2.8. Collection of Serum and Tissue Samples

Blood samples were collected through the orbital sinus, under light ether anesthesia, centrifuged at 1000 × *g* for 15 min, serum samples were separated and stored at −20 °C for the determination of insulin, TNF-α, and IL-1β levels. After cervical dislocation, pancreata were excised and divided into 2 portions. One portion was rinsed in cold saline, plotted dry, weighted, and frozen immediately at −80 °C for homogenization. The other portion was fixed in 10% neutral buffered formalin for histological examination.

2.9. Insulin Levels

Serum insulin levels were determined using ELISA following the protocol given by the manufacturer (Crystal Chem Inc.). In brief, to a 96-well microplate pre-coated with polyclonal antibody specific for mouse insulin, 95 μl sample diluent and 5 μl samples were added. Recombinant mouse insulin was used to set up the standard curve. After incubation for 2 h at 4 °C, the wells were washed and polyclonal anti-mouse insulin antibodies conjugated to horseradish peroxidase

were added. Incubation was continued for 30 min at room temperature, plates were washed; substrate solution was added to each well and incubated for 40 min. The enzyme reaction yielded a blue product that turned yellow when the stop solution was added. The O.D at 450 nm (correction wavelength set at 630 nm) was measured using microplate reader (Metertech, M960). Intra- and inter-assay precision CVs are ≤10.0%.

2.10. Antioxidant Enzyme Activities in Pancreas

The frozen individual tissue samples were homogenized in 50 mM phosphate buffer (10% W/V), pH 7.4, using Glas-Col tissue homogenizing system (Cole-Parmer, Vernon Hills, USA). Supernatants obtained upon centrifugation at 8000×*g* at 4 °C were used for measuring the activity of the antioxidant enzymes superoxide-dismutase, catalase, and glutathione peroxidase using standard spectrophotometric assays. Briefly, superoxide-dismutase activity in the tissue homogenate was determined by generating superoxide radicals by the photochemical reduction of phenazine methosulphate, which reduces nitroblue tetrazolium into a blue-colored compound, formazone. Superoxide-dismutase quenches free oxygen radicals and inhibits reduction of nitroblue tetrazolium, which was measured at 560 nm (Nishikimi et al., 1972). Catalase assay was carried out by checking the rate of hydrogen peroxide degradation at 510 nm in the presence of the homogenate (Aebi, 1984). Glutathione peroxidase catalyzes the reduction of hydrogen peroxide with reduced glutathione as hydrogen donor. The reaction was monitored spectrophotometrically at 340 nm (Lawrence and Burk, 1976).

2.11. Lipid Peroxidation Level in Pancreas

Lipid peroxidation is a good way of evaluating oxidative stress-induced damage to tissues. Hence levels of malondialdehyde as thiobarbituric acid-reactive substances were measured in tissue homogenate by the method of Ohkawa et al. (1979). Briefly, thiobarbituric acid reacts with malondialdehyde in acidic medium at temperature of 95 °C for 30 min to form colored substances. The resultant pink color representative of thiobarbituric acid-reactive substances was measured at 534 nm.

2.12. Serum Cytokine Levels

Levels of the pro-inflammatory mediators, TNF-α and IL-1β, in serum were determined using ELISA following the manufacturer's protocol (R&D systems). In brief, to a 96-well microplate pre-coated with polyclonal antibody specific for mouse TNF-α or IL-1β, 50 µl assay diluent and 50 µl samples were added. Recombinant mouse TNF-α or IL-1β was used to set up the corresponding standard curve, respectively. After incubation for 2 h at room temperature, the wells were washed and corresponding polyclonal antibodies conjugated to horseradish peroxidase were added. Incubation was continued for 2 h, plates were washed; substrate solution was added to each well and incubated for 30 min. The enzyme reaction yielded a blue product that turned yellow when the stop solution was added. The O.D at 450 nm (correction wavelength set at 570 nm) was measured using microplate reader (Metertech, M960). Intra- and inter-assay precision CVs for TNF-α were 4.3–9% and 6.1–9.2%, respectively. For IL-1β, intra- and inter-assay precision CVs were 1.5–4.4% and 2.8–6.1%, respectively.

2.13. Histological and Morphometric Analysis

Pancreata were excised, and then fixed in 10% neutral buffered formalin. Tissues were then processed for paraffin embedding, subsequent serial sectioning, and stained with hematoxylin/eosin (H&E) to allow the assessment of pancreatic islet morphology in different treatment groups. Islets were observed from every tenth serial section, morphometric measurements were performed using "Laica Qwin 500" image analyzer computer system (Cambridge, England).

2.14. Statistical Analysis

All data were expressed as mean ± S.E.M. Distribution of the data was verified to be normal using Tests of Normality (SPSS package). Statistical significance was tested by one way analysis of variance (ANOVA) followed by Bonferroni *post hoc* analysis.

3. Results

3.1. Body Weight

Initial and final body weights are represented in Fig. 1. Comparing animals' body weights at the end of the study with the corresponding starting weights showed that individual treatment with curcumin or bone marrow transplantation had no marked effect on body weight. Curcumin administration or single bone marrow transplantation only slightly ($P>0.05$) reversed the weight loss observed in streptozotocin group. Conversely, however; animals co-treated with curcumin and bone marrow transplantation showed significantly increased final body weight ($P\leq0.05$), as compared to their initial value.

3.2. Intraperitoneal Glucose Tolerance Test

The intraperitoneal glucose tolerance test of diabetic mice treated with both curcumin and bone marrow transplantation showed comparable values to that of the normal animals, whereas all other diabetic groups exhibited an abnormal pattern, consonant with persistent hyperglycemia (Fig. 2). Thus, a rise in blood glucose levels 30 min after glucose administration was observed in all groups. However, further glucose clearance was observed only in normal and curcumin/bone marrow transplantation-treated mice, while the untreated mice exhibited delayed glucose clearance, along with consistent hyperglycemia. Individual treatment with either curcumin or bone marrow transplantation significantly enhanced glucose clearance as compared with untreated diabetic group; however, they failed to normalize it.

3.3. Blood Glucose Level

Fig. 3 shows the initial and final fasting blood glucose levels in untreated and treated diabetic mice. The group treated with only multiple low doses of streptozotocin exhibited sustained hyperglycemia at the end of the study period. Although single treatment with curcumin or MBT showed a significant ($P\leq0.001$) reduction in blood

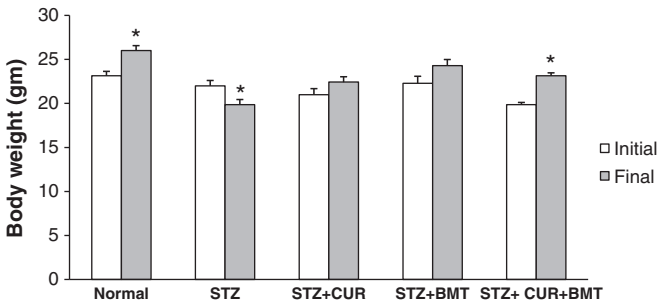

Fig. 1. Differential and combined effects of curcumin (CUR, 10 mM; 100 µl/mouse, i.p.) and single bone marrow transplantation (BMT, 10^6 un-fractionated cells) on initial and final body weights in multiple low doses of streptozotocin (STZ)-induced diabetic mice. Values are expressed as mean ± S.E.M. Data were analyzed using student *t*-test. *$P\leq0.05$ with respect to corresponding initial body weight. Each group consisted of 7 mice.

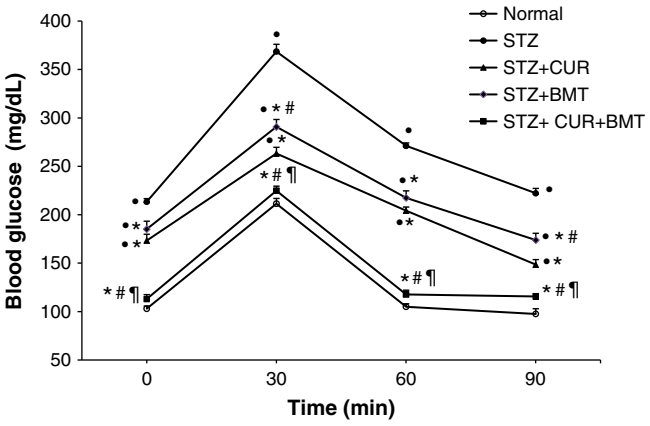

Fig. 2. Differential and combined effects of curcumin (CUR, 10 mM; 100 µl/mouse, i.p.) and single bone marrow transplantation (BMT, 10^6 un-fractionated cells) on glucose tolerance test in multiple low doses of streptozotocin (STZ)-induced diabetic mice. All groups of mice were fasted overnight then injected with 2 g/kg body weight of glucose intraperitoneally. Glucose disposal was analyzed by measuring random blood glucose of mice at different time points 0, 30, 60, and 90 min. Values are expressed as mean ± S.E.M. Data were analyzed and compared at each single time point using ANOVA followed by Bonferroni *post hoc* test. $^{\bullet}P \leq 0.05$ with respect to normal, $^{*}P \leq 0.05$ with respect to STZ, $^{\#}P \leq 0.05$ with respect to STZ + CUR, $^{\P}P \leq 0.05$ with respect to STZ + BMT at corresponding time point. Each group consisted of 7 mice.

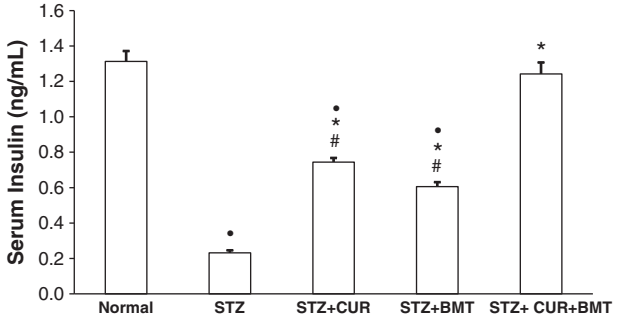

Fig. 4. Differential and combined effects of curcumin (CUR, 10 mM; 100 µl/mouse, i.p.) and single bone marrow transplantation (BMT, 10^6 un-fractionated cells, i.v.) on serum insulin levels in multiple low doses of streptozotocin (STZ)-induced diabetic mice. Serum insulin levels were measured using ELISA (Crystal Chem Inc.). Values are expressed as mean ± S.E.M. All data were analyzed using ANOVA followed by Bonferroni *post hoc* test. $^{\bullet}P \leq 0.001$ with respect to normal, $^{*}P \leq 0.001$ with respect to STZ, $^{\#}P \leq 0.001$ with respect to STZ + CUR + BMT. Each group consisted of 7 mice.

glucose levels as compared to untreated diabetic group, they failed to return blood glucose to normal levels. The combined treatment with curcumin and bone marrow transplantation significantly ($P \leq 0.001$) reduced blood glucose levels to approach normal values at the end of the study.

3.4. Serum Insulin Level

To gain insights into the insulin secretory capacity of pancreatic islets and its relationship with blood glucose/glucose tolerance, we monitored serum insulin levels before and after single and combined treatments with curcumin and bone marrow transplantation (Fig. 4). Exposure to multiple low doses of streptozotocin significantly ($P \leq 0.001$) reduced the serum insulin concentrations; consonant with destruction of pancreatic β-cells. Individual treatment of diabetic mice with curcumin or bone marrow transplantation significantly enhanced insulin secretion ($P \leq 0.001$), yet still to a lower extent than the normal levels ($P \leq 0.001$), implying partial recovery of the β-cell secretory capacity. Intriguingly, however; combined treatment with both curcumin and bone marrow transplantation substantially elevated serum insulin concentrations to near normal levels. This indicates that the combination regimen used (curcumin with bone marrow transplantation) strikingly promoted β-cell regeneration to restore normal insulin production.

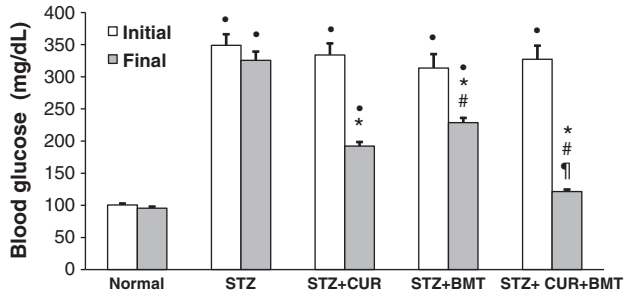

Fig. 3. Differential and combined effects of curcumin (CUR, 10 mM; 100 µl/mouse, i.p.) and single bone marrow transplantation (BMT, 10^6 un-fractionated cells, i.v.) on initial and final blood glucose levels in multiple low doses of streptozotocin (STZ)-induced diabetic mice. Values are expressed as mean ± S.E.M. All data were analyzed using ANOVA followed by Bonferroni *post hoc* test. $^{\bullet}P \leq 0.001$ with respect to corresponding normal, $^{*}P \leq 0.001$ with respect to corresponding STZ, $^{\#}P \leq 0.001$ with respect to corresponding STZ + CUR, $^{\P}P \leq 0.001$ with respect to corresponding STZ + BMT. Each group consisted of 7 mice.

3.5. Pancreatic Lipid Peroxidation and Antioxidant Enzymes

To study the differential and combined effects of curcumin and bone marrow transplantation on oxidative stress induced by multiple low doses of streptozotocin, the concentration of malondialdehyde and activities of antioxidant enzymes (superoxide-dismutase, catalase, and glutathione peroxidase) in pancreas were measured. A significant ($P \leq 0.001$) rise in pancreatic malondialdehyde levels was detected in untreated diabetic group as well as in diabetic group that had received only bone marrow transplantation, indicating increased lipid peroxidation that also remained unresponsive to bone marrow transplantation. The administration of curcumin for 28 days, either alone or in combination with bone marrow transplantation, blunted the mounting malondialdehyde levels in the pancreas ($P \leq 0.001$) (Fig. 5). On the other hand, antioxidant enzyme activities were found to be significantly decreased in the pancreas of untreated diabetic mice ($P \leq 0.05$), indicating breakdown of antioxidative defense (Table 1). Bone marrow transplantation managed to significantly enhance superoxide-dismutase and catalase activities, as compared to

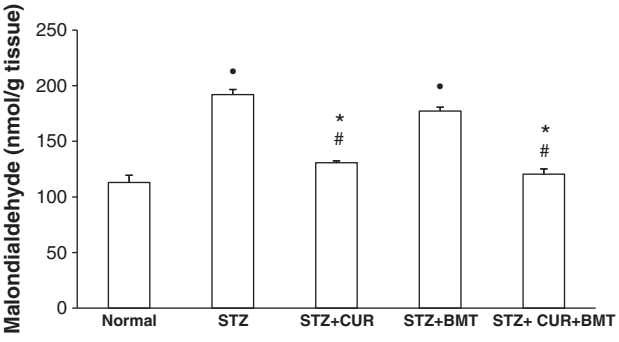

Fig. 5. Differential and combined effects of curcumin (CUR, 10 mM; 100 µl/mouse, i.p.) and single bone marrow transplantation (BMT, 10^6 un-fractionated cells, i.v.) on lipid peroxidation in multiple low doses of streptozotocin (STZ)-induced diabetic mice. Tissue levels of malondialdehyde as thiobarbituric acid-reactive substances were measured spectrophotometrically at 534 nm. Values are expressed as mean ± S.E.M. All data were analyzed using ANOVA followed by Bonferroni *post hoc* test. $^{\bullet}P \leq 0.001$ with respect to normal, $^{*}P \leq 0.001$ with respect to STZ, $^{\#}P \leq 0.001$ with respect to STZ + BMT. Each group consisted of 7 mice.

the streptozotocin group ($P \leq 0.05$), yet to a level that remained lower than those of normal mice ($P \leq 0.01$), implying partial up-regulation of these enzymes. Bone marrow transplantation didn't change the activity of glutathione peroxidase which was found lower than normal value. On the other hand, treatment of diabetic mice with curcumin, either alone or in combination with bone marrow transplantation, normalized the activities of superoxide-dismutase, catalase and glutathione peroxidase, indicating the crucial role of curcumin in restoration of the pancreatic antioxidative machinery.

3.6. Serum Cytokine Levels

Inflammation and necrosis of the pancreas are indicators of early stages of diabetogenesis (Steer et al., 2006) and hence we have evaluated serum levels of the pro-inflammatory mediators, TNF-α and IL-1β. Mice treated with multiple low doses of streptozotocin showed increased serum levels of TNF-α and IL-1β, by 10.2- and 9-fold; respectively, compared to the normal group (Fig. 6). Diabetic mice that had received only bone marrow transplantation showed no significant difference from the cytokine levels of the diabetic animals, implying the persistence of inflammatory response to streptozotocin. By contrast, the treatment of diabetic mice with curcumin, either alone or in combination with bone marrow transplantation, significantly lowered serum levels of TNF-α and IL-1β ($P \leq 0.001$ vs. streptozotocin and/or bone marrow transplantation diabetic groups) down to near normal group levels.

3.7. Histological Evaluation of Pancreas

Histological examination of the H&E-stained paraffin sections of mouse pancreas subjected to multiple low doses of streptozotocin showed severe reduction in the mass of the islets of Langerhans in addition to necrosis and inflammation. Bone marrow transplantation into recipient streptozotocin-diabetic mice significantly, yet incompletely, induced pancreatic regeneration ($P \leq 0.05$). Pancreatic regeneration after bone marrow transplantation was evidenced by the appearance of small islets (Fig. 7); many of which existed near the duct, consonant with new islet formation. Similar observation was detected in the mice treated with curcumin/bone marrow transplantation, however, with higher number of small islets and the lack of necrosis and inflammation.

3.8. Image Analysis

Image analysis of normal and newly generated islets in different treatment groups has been performed on H&E-stained paraffin sections of pancreas. Islet mean area and mean diameter of normal group were found to be $6305.45 \pm 226.54\ \mu m^2$ and $87.58 \pm 5.30\ \mu m$, respectively (Fig. 7). Mice treated with multiple low doses of streptozotocin showed a significant ($P \leq 0.001$) drastic reduction in both area and diameter to become $331.74 \pm 46.23\ \mu m^2$ and $20.92 \pm 2.18\ \mu m$, respectively. The treatment with curcumin or bone marrow transplantation resulted in a significant ($P \leq 0.05$) increase in islets mean area as well as diameter. The combination therapy of curcumin and bone marrow transplantation resulted in further significant ($P \leq 0.001$) increments in islet mean area and mean diameter to reach $5235.41 \pm 165.72\ \mu m^2$ and $78.89 \pm 2.91\ \mu m$, respectively (Fig. 7).

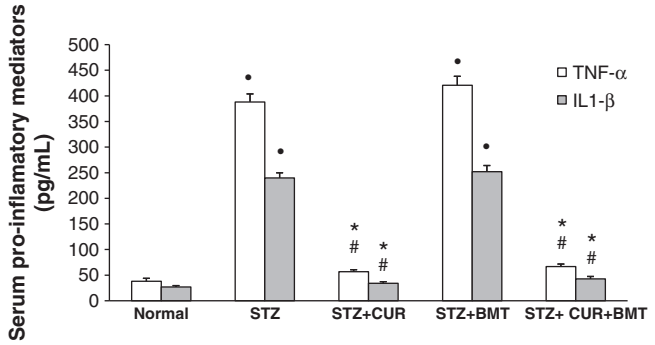

Fig. 6. Differential and combined effects of curcumin (CUR, 10 mM; 100 μl/mouse, i.p.) and single bone marrow transplantation (BMT, 10^6 un-fractionated cells, i.v.) on pro-inflammatory mediators in multiple low doses of streptozotocin (STZ)-induced diabetic mice. Serum levels of TNF-α and IL-1β were measured using ELISA (R&D). Values are expressed as mean ± S.E.M. All data were analyzed using ANOVA followed by Bonferroni *post hoc* test. •$P \leq 0.001$ with respect to corresponding normal, *$P \leq 0.001$ with respect to corresponding STZ, #$P \leq 0.001$ with respect to corresponding STZ + BMT. Each group consisted of 7 mice.

Table 1
Differential and combined effects of curcumin (CUR) and single bone marrow transplantation (BMT) on pancreatic activity of antioxidant enzymes in multiple low doses of streptozotocin (STZ)-induced diabetic mice.

	Superoxide dismutase	Catalase	Glutathione peroxidase
Normal	37.79 ± 2.10	23.78 ± 1.06	3.26 ± 0.11
STZ	24.28 ± 0.78[a]	13.27 ± 0.73[a]	1.85 ± 0.10[a]
STZ + CUR	36.51 ± 1.13[b, c]	25.02 ± 0.94[b, c]	3.34 ± 0.15[b, c]
STZ + BMT	30.54 ± 0.70[a, b]	19.38 ± 0.63[a, b]	2.13 ± 0.08[a]
STZ + CUR + BMT	34.13 ± 1.35[b, c]	24.35 ± 0.70[b, c]	2.93 ± 0.09[b, c]

Activities of antioxidant enzymes in pancreatic tissues were evaluated spectrophotometrically and expressed as U/g tissue. Values are mean ± S.E.M. All data were analyzed using ANOVA followed by Bonferroni *post hoc* test. a: Significantly different from corresponding normal, b: significantly different from corresponding STZ, c: significantly different from corresponding STZ + BMT. $P \leq 0.05$ was selected as the level of significance. Each group consisted of 7 mice.

4. Discussion

Diabetes mellitus, the most common endocrine disease, is a group of disorders of varying etiology and pathogenesis. Effective management of diabetes is a pivotal global need that is yet to be established. Modern drugs, including insulin and other hypoglycemic agents, control the blood glucose level only when they are regularly administered, but these treatments are tedious and tend to pose several clinical challenges (Jin et al., 2008). However, medicinal herbs may offer a similar degree of efficacy without so many troublesome side effects.

Curcumin has been known as an antioxidant, anti-inflammatory, anti-proliferative and cytoprotective agent (Phan et al., 2001; Grandjean-Laquerriere et al., 2002; Fujisawa et al., 2004; Kanitkar et al., 2008). Likewise, its efficacy as an antidiabetic agent has been widely targeted (Babu and Srinivasan, 1995; Srinivasan et al., 2003). Albeit, all such studies merely sought the potential of curcumin in preventing diabetes or its complications; rather than an actual "remedy" after diabetes had already occurred.

The pancreas has been reported as the organ with the lowest levels of antioxidant enzymes; consequently, pancreatic β-cells are exceptionally vulnerable to detrimental actions of oxidative stress (Tiedge et al., 1997). The cytotoxic, diabetogenic action of streptozotocin is mediated mostly by reactive oxygen species (Lei et al., 2005). It has been also reported that destruction of β-cells by streptozotocin remains such incomplete that the resulting mice are hyperglycemic but do not die (Colman, 2004). This view goes well with the currently observed minimal insulin secretion in untreated diabetic mice. Alternatively, after streptozotocin treatment; endogenous β-cell regeneration and/or transdifferentiation of liver cells into pancreatic ones have been postulated (Fernandes et al., 1997; Kim et al., 2007). Interestingly, the latter routes for insulin production can be promoted by antioxidants (Alvarez et al., 2004). These envisions prompted us to further evaluate the palliative role of curcumin, when used alone and

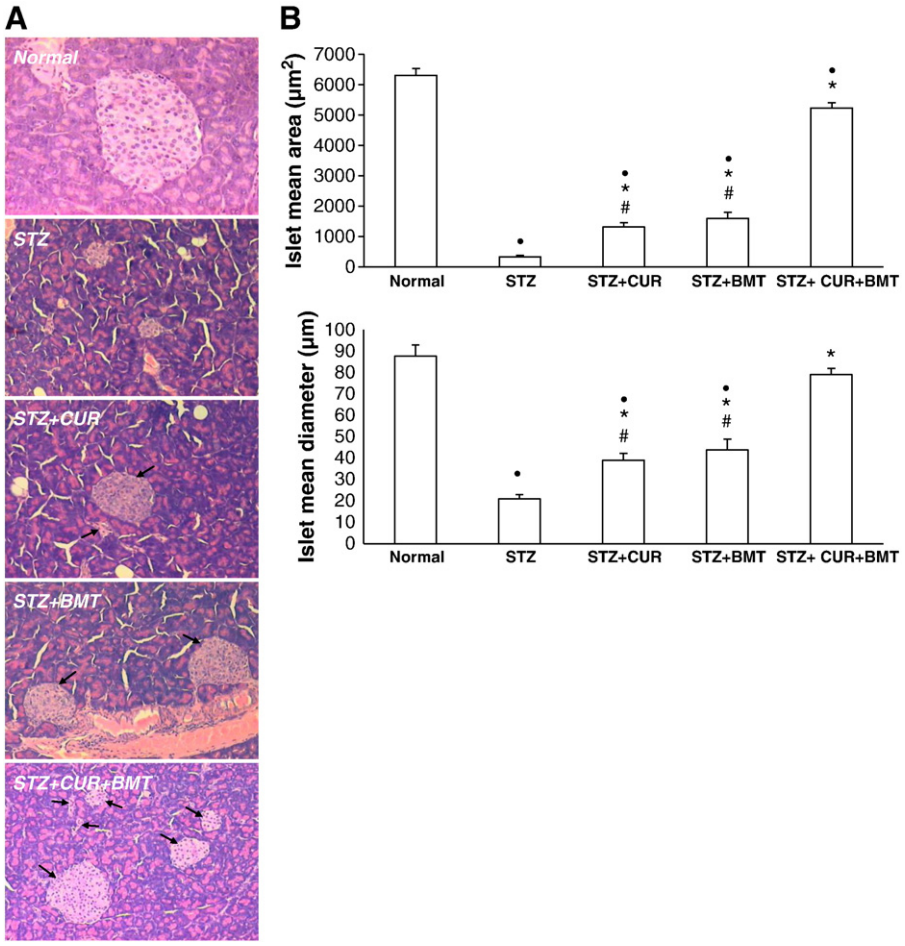

Fig. 7. Differential and combined effects of curcumin (CUR, 10 mM; 100 μl/mouse, i.p.) and single bone marrow transplantation (BMT, 10^6 un-fractionated cells, i.v.) on histopathological and morphometric analysis of pancreatic sections in multiple low doses of streptozotocin (STZ)-induced diabetic mice. Photomicrographs are representative to H&E stained pancreatic sections in different groups at ×200 magnification (A). Morphometric measurements were performed using "Laica Qwin 500" image analyzer to determine islet mean area (upper panel) and islet mean diameter (lower panel) in different groups (B). Values are expressed as mean ± S.E.M. All data were analyzed using ANOVA followed by Bonferroni *post hoc* test. $^{\bullet}P \leq 0.001$ with respect to normal, $^{*}P \leq 0.05$ with respect to STZ, $^{\#}P \leq 0.001$ with respect to STZ + CUR + BMT. Arrows indicate newly generated islets. Each group consisted of 7 mice.

in combination with a single bone marrow transplantation, against diabetes induced by multiple low doses of streptozotocin.

Our results demonstrate that single injection of bone marrow, although managed to reduce blood glucose level, remains insufficient to restore normoglycemia. This agrees with the findings of Banerjee et al. (2005) that only multiple injections (at least three) of bone marrow could achieve normoglycemia over a long period of time. Similarly, in the current study, curcumin treatment, though did not normalize hyperglycemia, it reduced it to a better extent than the single bone marrow transplantation regimen. Unlike their individual responses, the combined regimen of curcumin with single bone marrow transplantation virtually normalized blood glucose concentrations. Underpinnings of such synergistic response appear to involve enhanced β-cell regeneration (islet neogenesis) and insulin secretion; as clearly revealed by current histological and hormonal results.

In reversing the sequelae of diabetes, the molecular triggers whereby curcumin confers synergy with bone marrow transplantation appear to be multi-factorial. First, curcumin abates oxidative stress due to reactive oxygen species and lipid-peroxidation by reducing hyperglycemia, and enhancing endogenous antioxidant machinery (glutathione peroxidase, catalase, superoxide-dismutase, and reduced glutathione, and GSH). This certainly protects residual pancreatic islets and newly bone marrow transplantation-generated ones against cellular damage evoked by reactive oxygen species or advanced-glycation end products (Saxena et al., 1993; Donnini et al., 1996; Feillet-Coudray et al., 1999; Venkateswaran and Pari, 2002; Ugochukwu et al., 2004). We have clearly revealed that pancreatic oxidative stress was most sensitive to co-treatment with curcumin/bone marrow transplantation in diabetic mice. Second, curcumin obliterates inflammation and immune response; as evident by its ability to suppress production of the cytokines, tumor necrosis factor (TNF)-α and interleukin (IL)-1β. This certainly creates a favorable systemic and pancreatic environment to foster bone marrow transplantation and islet neogenesis.

It is worthwhile evaluating the utility of this combined curcumin/bone marrow transplantation regimen in the management of insulin-dependent (type-1) diabetes. This is an autoimmune disease that is characterized by selective destruction of insulin-producing β-cells found in the pancreatic islets of Langerhans (Bach, 1994). The development of diabetes is initiated with insulitis, in which leukocytes migrate to and invade the islets. This is followed by an overt, insulin deficient diabetes phase that is distinguished by destruction of the majority of β-cells (Mathis et al., 2001). Interestingly, adult mice given multiple low doses of streptozotocin develop insulin-dependent diabetes that is quite similar to the autoimmune forms of type-1 diabetes (Rees and Alcolado, 2005).

Cytokines produced by inflammatory cells are believed to contribute to the dysfunction of β-cell during the development of

autoimmune-driven diabetes (Rabinovitch and Suarez-Pinzon, 1998). Thus, IL-1 inhibits insulin secretion and blunts islet cell viability (Mandrup-Poulsen et al., 1985). Further; alone or jointly with interferon-γ and TNF, IL-1 can induce the β-cell nitric oxide synthase and hence nitric oxide production, along with other reactive oxygen species; to disrupt glucose utilization by β-cells. Therefore, the existence in type-1 diabetes of such surge of inflammatory and immune-stimulatory players pose an ample threat that would hamper the recruitment of newly introduced BM-cells into pancreatic islets (Grandjean-Laquerriere et al., 2002; Gautam et al., 2007; Sharma et al., 2007; Kanitkar et al., 2008; Shirley et al., 2008; Weisberg et al., 2008). Accordingly, combining curcumin; as an established anti-inflammatory and immune modulatory drug; with bone marrow transplantation would likely boost and preserve the process of islet regeneration; which was evidently proven true in this study.

On the other hand, caveats that can stand as a potential barrier against the clinical use of curcumin in type-1 diabetes can relate mostly to its limited bioavailability. However, phase I clinical trials have shown that curcumin is safe even at high doses (12 g/day) in humans. Furthermore, myriad of approaches; such as liposomal preparations, use of adjuvants, new congeners, or nanoparticles have been fruitfully undertaken to overcome limited solubility and bioavailability of curcumin (Anand et al., 2007). Accomplishing this in the near future may well bring this agent to the forefront of natural drugs in the treatment of diabetes.

5. Conclusions

Current data show for the first time that either bone marrow transplantation or curcumin can significantly ameliorate experimental diabetes induced by multiple low doses of streptozotocin, and restore the power of pancreatic antioxidant machinery. Curcumin also reduced elevated levels of both lipid peroxidation and serum pro-inflammatory cytokines. A strikingly novel finding of this study is that co-administration of curcumin with single bone marrow transplantation elicited virtually complete reversal of islet destruction and its consequences; thus indicating the superiority of combined treatment to individual regimens. Further, this observed synergy in the effects of curcumin and bone marrow transplantation opens new, unconventional therapeutic avenues in the management of diabetes.

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References

Aebi, H., 1984. Catalase in vitro. Meth. Enzymol. 105, 121–126.
Alvarez, J.F., Barbera, A., Nadal, B., Barcelo-Batllori, S., Piquer, S., Claret, M., Guinovart, J.J., Guinovart, N., Gomis, R., 2004. Stable and functional regeneration of pancreatic beta-cell population in nSTZ-rats treated with tungstate. Diabetologia 47, 470–477.
Anand, P., Kunnumakkara, A.B., Newman, R.A., Aggarwal, B.B., 2007. Bioavailability of curcumin: problems and promises. Mol. Pharmacol. 4, 807–818.
Babu, P.S., Srinivasan, K., 1995. Influence of dietary curcumin and cholesterol on the progression of experimentally induced diabetes in albino rat. Mol. Cell. Biochem. 152, 13–21.
Bach, J.F., 1994. Insulin-dependent diabetes mellitus as an autoimmune disease. Endocr. Rev. 15, 516–542.
Banerjee, M., Bhonde, R.R., 2003. Islet generation from intra islet precursor cells of diabetic pancreas: in vitro studies depicting in vivo differentiation. JOP 4, 137–145.
Banerjee, M., Kumar, A., Bhonde, R., 2005. Reversal of experimental diabetes by multiple bone marrow transplantation. Biochem. Biophys. Res. Commun. 328, 318–325.
Colman, A., 2004. Making new beta cells from stem cells. Semin. Cell Dev. Biol. 15, 337–345.
Donnini, D., Zambito, A.M., Perella, G., 1996. Glucose may induce cell death through a free radical-mediated mechanism. Biochem. Biophys. Res. Commun. 219, 412–417.
El-Azab, M.F., Hishe, H.Z., Moustafa, Y.M., El-Awady, E.E., 2009. Effect of resveratrol and curcumin on tumor volume and survival in Ehrlich ascites carcinoma-bearing mice. Bull. Fac. Pharm. Cairo Univ. 47, 207–223.
El-Azab, M., Hishe, H., Moustafa, Y., El-Awady, E., 2011. Anti-angiogenic effect of resveratrol or curcumin in Ehrlich ascites carcinoma-bearing mice. Eur. J. Pharmacol. 652, 4–17.
El-Mowafy, A.M., El-Mesery, M.E., Salem, H.A., Al-Gayyar, M.M., Darweish, M.M., 2010. Prominent chemopreventive and chemoenhancing effects for resveratrol: unraveling molecular targets and the role of CRP. Chemotherapy 56, 60–65.
Feillet-Coudray, C., Rock, E., Coudray, C., Grzelkowska, K., Azais-Braesco, V., Dardevet, D., Mazur, A., 1999. Lipid peroxidation and antioxidant status in experimental diabetes. Clin. Chim. Acta 284, 31–43.
Fernandes, A., King, L.C., Guz, Y., Stein, R., Wright, C.V., Teitelman, G., 1997. Differentiation of new insulin-producing cells is induced by injury in adult pancreatic islets. Endocrinology 138, 1750–1762.
Fujisawa, S., Atsumi, T., Ishihara, M., Kadoma, Y., 2004. Cytotoxicity, reactive oxygen species — generating activity and radical scavenging activity of curcumin and other related compounds. Anticancer Res. 24, 563–570.
Gautam, S.C., Gao, X., Dulchavsky, S., 2007. Immunomodulation by curcumin. Adv. Exp. Med. Biol. 595, 321–341.
Grandjean-Laquerriere, A., Gangloff, S.C., Le Naour, R., Trentesaux, C., Hornebeck, W., Guenounou, M., 2002. Relative contribution of NFkB and AP-1 in the modulation by curcumin and pyrrolidine dithiocarbamate of the UVB-induced cytokine expression by kertinocytes. Cytokine 18, 168–177.
Gururaj, A., Belakavadi, M., Venkatesh, D., Marmé, D., Salimath, B., 2002. Molecular mechanisms of anti-angiogenic effect of curcumin. Biochem. Biophys. Res. Commun. 297, 934–942.
Hussain, H.E., 2002. Hypoglycemic, hypolipidemic and antioxidant properties of combination of curcumin from *Curcuma longa*, Linn. and partially purified product from *Abroma augusta*, Linn. in streptozotocin induced diabetes. Ind. J. Clin. Biochem. 17, 33–43.
Ianus, A., Holz, G.G., Theise, N.D., Hussain, M.A., 2003. In vivo derivation of glucose-competent pancreatic endocrine cells from bone marrow without evidence of cell fusion. J. Clin. Invest. 111, 843–850.
Jin, L., Xue, H.Y., Jin, L.J., Li, S.Y., Xu, Y.P., 2008. Antioxidant and pancreas-protective effect of aucubin on rats with streptozotocin-induced diabetes. Eur. J. Pharmacol. 582, 162–167.
Kanitkar, M., Gokhale, K., Galande, S., Bhonde, R.R., 2008. Novel role of curcumin in the prevention of cytokine-induced islet death in vitro and diabetogenesis in vivo. Br. J. Pharmacol. 155, 702–713.
Kim, S.H., Hyun, S.H., Choung, S.Y., 2006. Anti-diabetic effect of cinnamon extract on blood glucose in db/db mice. J. Ethnopharmacol. 104, 119–123.
Kim, S., Shin, J.S., Kim, H.J., Fisher, R.C., Lee, M.J., Kim, C.W., 2007. Streptozotocin-induced diabetes can be reversed by hepatic oval cell activation through hepatic transdifferentiation and pancreatic islet regeneration. Lab. Invest. 87, 702–712.
Lawrence, R.A., Burk, R.F., 1976. Glutathione peroxidase activity in selenium deficient rat liver. Biochem. Biophys. Res. Commun. 71, 952–958.
Lechner, A., Habener, J.F., 2003. Stem/progenitor cells derived from adult tissues: potential for the treatment of diabetes mellitus. Am. J. Physiol. Endocrinol. Metab. 284, 259–266.
Lei, Y.C., Hwang, J.S., Chan, C.C., Lee, C.T., Cheng, T.J., 2005. Enhanced oxidative stress and endothelial dysfunction in streptozotocin-diabetic rats exposed to fine particles. Environ. Res. 99, 335–343.
Mandrup-Poulsen, T., Bendtzen, K., Nielsen, J.H., Bendixen, G., Nerup, J., 1985. Cytokines cause functional and structural damage to isolated islets of Langerhans. Allergy 40, 424–429.
Mathis, D., Vence, L., Benoist, C., 2001. Beta-cell death during progression to diabetes. Nature 414, 792–798.
Moon, D.O., Kim, M.O., Choi, Y.H., Park, Y.M., Kim, G.Y., 2010. Curcumin attenuates inflammatory response in IL-1beta-induced human synovial fibroblasts and collagen-induced arthritis in mouse model. Int. Immunopharmacol. 10, 605–610.
Motterlini, R., Foresti, R., Bassi, R., Green, C.J., 2000. Curcumin, an antioxidant and anti-inflammatory agent, induces heme oxygenase-1 and protects endothelial cells against oxidative stress. Free Radic. Biol. Med. 28, 1303–1312.
Nishikimi, M., Appaji, N., Yagi, K., 1972. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem. Biophys. Res. Commun. 46, 849–854.
Ohkawa, H., Ohishi, N., Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95, 351–358.
Ohkuwa, T., Sato, Y., Naoi, M., 1995. Hydroxyl radical formation in diabetic rat induced by streptozotocin. Life Sci. 56, 1789–1798.
Pari, L., Murugan, P., 2005. Effect of thetrahydrocurcumin on blood glucose, plasma insulin and hepatic key enzymes in streptozotocin induced diabetic rats. Basic Clin. Physiol. Pharmacol. 16, 257–274.
Phan, T.T., See, P., Lee, S.T., Chan, S.Y., 2001. Protective effects of curcumin against oxidative damage on skin cells in vitro: its implications for wound healing. J. Trauma 51, 927–931.
Rabinovitch, A., Suarez-Pinzon, W.L., 1998. Cytokines and their roles in pancreatic islet beta-cell destruction and insulin-dependent diabetes mellitus. Biochem. Pharmacol. 55, 1139–1149.
Rees, D.A., Alcolado, J.C., 2005. Animal models of diabetes mellitus. Diabet. Med. 22, 359–370.

Risbud, M.V., Bhonde, R.R., 2002. Models of pancreatic regeneration in diabetes. Diab. Res. Clin. Pract. 58, 155–165.
Rosenberg, L., 1995. In vivo cell transformation: neogenesis of beta cells from pancreatic ductal cells. Cell Transplant. 4, 371–383.
Saxena, A.K., Srivastava, P., Kale, R.K., Baquer, N.Z., 1993. Impaired antioxidant status in diabetic rat liver. Effect of vanadate. Biochem. Pharmacol. 45, 539–542.
Sharma, S., Chopra, K., Kulkarni, S.K., Agrewala, J.N., 2007. Resveratrol and curcumin suppress immune response through CD28/CTLA-4 and CD80 co-stimulatory pathway. Clin. Exp. Immunol. 147, 155–163.
Shirley, S.A., Montpetit, A.J., Lockey, R.F., Mohapatra, S.S., 2008. Curcumin prevents human dendritic cell response to immune stimulants. Biochem. Biophys. Res. Commun. 374, 431–436.
Slack, J.M.W., 1995. Developmental biology of the pancreas. Development 121, 1569–1580.
Srinivasan, A., Menon, V.P., Periaswamy, V., Rajasekaran, K.N., 2003. Protection of pancreatic beta cell by the potential anti-oxidant bis-o-hydroxycinnmoyl methane, analogue of natural curcuminoid in experimental diabetes. J. Pharm. Sci. 6, 327–333.
Steer, S.A., Scarim, A.L., Chambers, K.T., Corbett, J.A., 2006. Interleukin-1 stimulates beta-cell necrosis and release of the immunological adjuvant HMGB1. PLoS Med. 3, 253–265.
Stosić-Grujicić, S., Maksimović, D., Badovinac, V., Samardzić, T., Trajković, V., Lukić, M., Mostarica Stojković, M., 2001. Antidiabetogenic effect of pentoxifylline is associated with systemic and target tissue modulation of cytokines and nitric oxide production. J. Autoimmun. 16, 47–58.
Szkudelski, T., 2001. The mechanism of alloxan and streptozotocin action in B cells of rat pancreas. Physiol. Res. 50, 536–546.
Tiedge, M., Lortz, S., Drinkgern, J., Lenzen, S., 1997. Relation between antioxidant enzyme gene expression and antioxidative defense status of insulin-producing cells. Diabetes 46, 1733–1742.
Ugochukwu, N.H., Bagayoko, N.D., Antwi, M.E., 2004. The effects of dietary caloric restriction on antioxidant status and lipid peroxidation in mild and severe streptozotocin-induced diabetic rats. Clin. Chim. Acta 348, 121–129.
Venkateswaran, S., Pari, L., 2002. Antioxidant effect of *Phaseolus vulgaris* in streptozotocin-induced diabetic rats. Asia Pac. J. Clin. Nutr. 11, 206–209.
Weber, W.M., Hunsaker, L.A., Roybal, C.N., Borovnikova-Marjon, E.V., Abcouwer, S.F., Royer, R.E., Deck, L.M., Vander Jagt, D.L., 2006. Activation of NF kappa B is inhibited by curcumin and related enones. Bioorg. Med. Chem. 14, 2450–2461.
Weisberg, S.P., Leibel, R., Tortoriello, D.V., 2008. Dietary curcumin significantly improves obesity-associated inflammation and diabetes in mouse models of diabesity. Endocrinology 149, 3549–3558.
Zhang, C., Browne, A., Child, D., Tanzi, R.E., 2010. Curcumin decreases amyloid-beta peptide levels by attenuating the maturation of amyloid-beta precursor protein. J. Biol. Chem. 285, 28472–28480.