Diabetic Complications Consortium

Application Title: Adipose-derived MSCs Treatment of Diabetes and Diabetic Bladder Dysfunction

Principal Investigator: Guiming Liu, M.D., Ph.D.

1. Project Accomplishments:

In the past year, we have performed 4 experiments examining effects of rat adipose-derived mesenchymal stem cells (aMSCs) on hyperglycemia and bladder dysfunction in streptozotocin (STZ)-induced diabetic rats. In the first experiment, we induced diabetes mellitus (DM) in Sprague Dawley rats by i.p. injection of STZ, which selectively targets and is toxic to pancreatic beta cells. Three days later, we injected single passage rat aMSCs (1.5 million cells) though the tail vein. Since our main interest was to determine if aMSCs can reverse diabetic bladder dysfunction (DBD) in the face of unresolved, prolonged hyperglycemia, we aimed to destroy enough beta cells with STZ to prevent recovery of glucose control after injection of aMSCs. However, at 6 weeks after aMSC injection, we found that both hyperglycemia and DBD were reversed in the rats that received aMSCs. We believe that the reversal of hyperglycemia after STZ was due to repair and/or proliferation of surviving beta cells in response to the aMSCs, and that the reversal of DBD was due to the restoration of glycemic control. In the subsequent experiments, we varied the time of injection of aMSCs from 0 days to 4 weeks after STZ injection, to establish a time interval when aMSCs could no longer reverse hyperglycemia. However, aMSC treatment of STZ-diabetic rats failed to either reverse the hyperglycemia or improve the DBD in any of these rats, even when the aMSCs were injected 3 days or immediately after STZ, suggesting that the aMSCs in these experiments lacked their normal restorative properties.

We consulted with Dr. Arnold I. Caplan, Professor of Biology and Director of the Skeletal Research Center at Case Western Reserve University, who is a pioneer in MSC biology and therapeutic applications, regarding our inconsistent results, but have not yet identified the cause(s). Preparation of aMSCs for therapeutic applications is complex, requiring optimization of cell extraction procedures, culture medium, and serum, as well as characterization of a MSCs from different rats before injection. Second, the injection route is important. aMSCs injected into the tail vein may remain lodged in the tail and not reach the general circulation. Injection of aMSCs into the bladder or heart (the latter is a difficult technique) may be a better choice. Third, since the mechanisms of aMSCs therapeutic effects are not clear, standardization of the protocol is not straightforward, and the causes of the problems encountered are difficult to identify. We believe aMSC treatment will be an effective method. However, a better understanding of the mechanisms of aMSC effects and the factors that affect the therapeutic capacities of aMSCs will likely be required before aMSCs can be a consistently efficacious treatment modality. For our study, more experiments are needed to screen and optimize the aMSCs, medium, and injection methods, which cannot be performed through this one-year pilot award.

As an alternative approach, we determined if DM-induced alterations in the bladder can be reversed by insulin replacement treatment initiated 3 weeks after injection of STZ in male Sprague-Dawley rats. We found that insulin treatment for 8 weeks reversed both hyperglycemia and polyuria in diabetic animals, which was demonstrated by normalization of blood glucose, HbA1c, and 24-hour urinary habits. In addition, body weight, bladder weight, and percentage change of bladder components (smooth muscle, collagen, urothelium) in total bladder cross-sectional area were reversed to almost normal levels, and the DBD as measured by cystometry was mostly reversed by 8 wks of insulin treatment. Similar alterations of bladder components and function were seen in diuretic rats, and were also reversed by removal of 5% sucrose for 8 weeks. In summary, short term (3-week induction) diabetic and polyuria-induced functional alterations of the bladder can be mostly reversed in rats.

2. Specific Aims:

Aim 1: To examine the effects of aMSCs on hyperglycemia reversal and DBD at different DM stages in STZ-induced diabetic rats.

Results: We have performed 4 experiments examining effects of aMSCs on hyperglycemia and bladder dysfunction in STZ-diabetic male Sprague Dawley rats, by injecting aMSCs immediately (0 days), 3 days, 1 week, 2 weeks, and 4 weeks after STZ injection. In the first experiment, first passage or sixth passage aMSCs (1.5 million cells) were injected into the tail vein 3 days after i.p. injection of STZ, and 6 weeks later we found that both hyperglycemia and DBD (Table 1) were reversed in rats that received first passage (Figure 1), but not sixth passage (Figure 2,) aMSCs. In the subsequent experiments, although we used only first passage aMSCs, we failed to see reversal of STZ-induced hyperglycemia or improvement of DBD by aMSC treatment at any time point, even when the aMSCs were injected 3 days or immediately after STZ injection (data not shown).

Figure 1. Change of blood glucose in DM rats that received first passage aMSCs

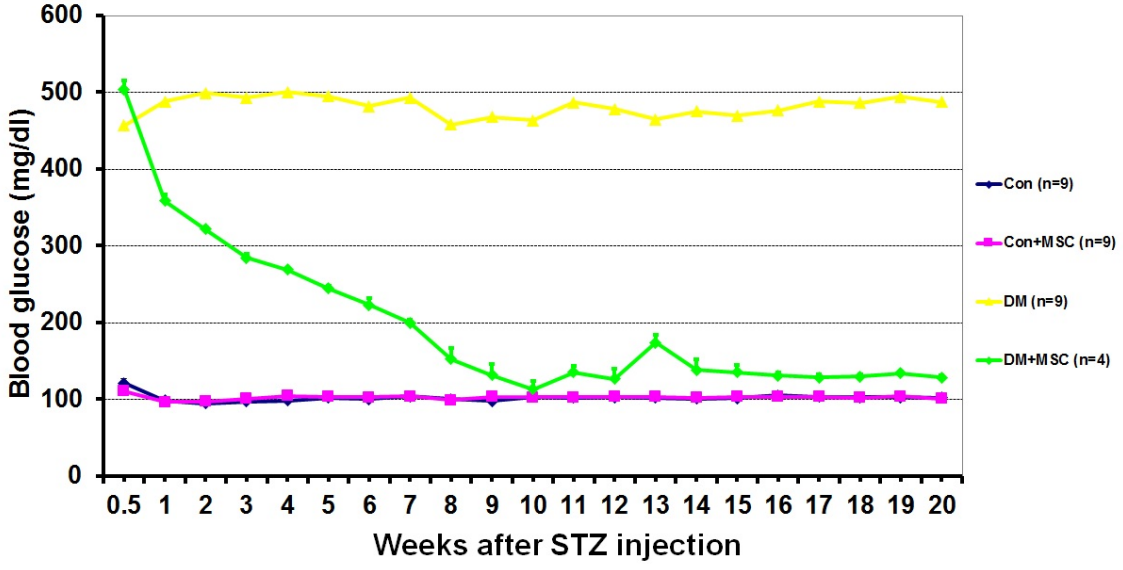

Figure 2. Change of blood glucose in DM rats that received sixth passage aMSCs

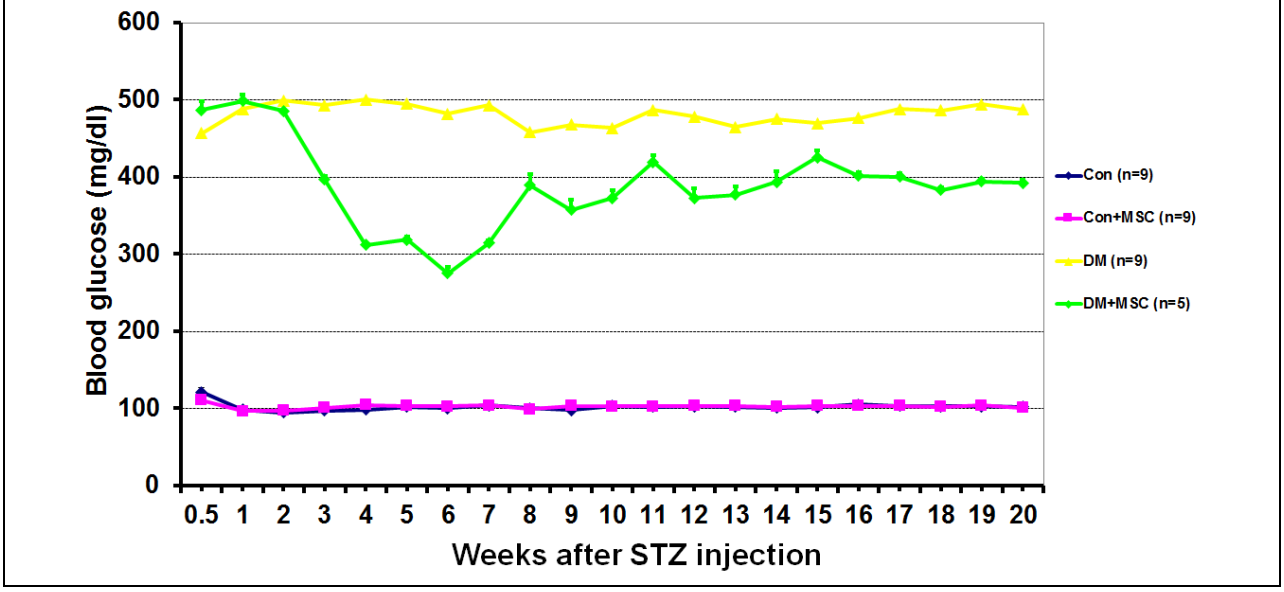

Table 1: 24-hour urinary habits of rats that received first passage aMSCs

	3 weeks after aMSCs treatment			6 weeks after aMSCs treatment		
	# Urinary Events	Void volume	Total Voided	# Urinary Events	Void volume	Total Voided
Control	14.3 ± 0.9	0.61 ± 0.13	8.4 ± 1.5	15.3 ± 3.0	0.63 ± 0.08	10.1 ± 3.0
Control + aMSCs	15.5 ± 2.2	0.5 ± 0.06	7.6 ± 0.7	20.5 ± 3.3	0.45 ± 0.03	9.5 ± 2.0
DM	52.7 ± 6.0*	1.9 ± 0.04*	98.2 ± 9.2*	50.5 ± 10.7*	2.1 ± 0.12*	107.2 ± 25.8*
DM + aMSCs	32 ± 17.7#	0.85 ± 0.34#	44.5 ± 37.4#	22.8 ± 6.0	0.76 ± 0.11	19.3 ± 7.8

*significantly different from corresponding values in control, control+aMSCs, and DM+aMSCs groups (P <0.01).
#significantly different from corresponding values in control, control+aMSCs, and DM groups (P <0.01).

Aim 2: To compare the effects of administration of aMSCs vs. conditioned medium (CM) from aMSCs cultured with standard fetal bovine serum, normal rat serum, or diabetic rat serum, on hyperglycemia and DBD in STZ-induced diabetic rats.

Results: Since we did not see consistent treatment effects of aMSCs, we did not perform the experiments proposed in this Aim. Instead, we determined if DM-induced alterations in the bladder can be reversed by insulin replacement initiated 3 weeks after induction of DM with STZ. Male Sprague-Dawley rats were randomly distributed into 8 groups (n=16): 3-wk and 11-wk age-matched controls, 3-wk and 11-wk STZ-induced DM, 3-wk DM followed by treatment with insulin for 8 wk, 3-wk and 11-wk 5% sucrose-induced diuresis, and 3-wk 5% sucrose-induced diuresis followed by removal of 5% sucrose for 8 wk. We found that insulin treatment reversed hyperglycemia and polyuria in diabetic animals successfully, which was demonstrated by normalization of blood glucose, HbA1c (Table 2) and 24-hour urinary habits (Table 3). In addition, body weight, bladder weight, and percentage change of bladder components (smooth muscle, collagen, urothelium) in total bladder cross-sectional area were reversed to almost normal levels (Table 4), and the DBD as measured by cystometry was mostly reversed by 8 wks of insulin treatment

(Table 5). Similar alterations of bladder components and function were seen in diuretic rats, and were also reversed by removal of 5% sucrose for 8 weeks. In summary, short term (3-week induction) diabetes- and polyuria-induced functional alterations of the bladder can be mostly reversed in rats.

Table 2: Body weight, blood glucose, HbA1C, and bladder weight in different groups (n=16).

	Body weight (gm)		Blood glucose (mg/dl)		HbA1C (%)	Bladder weight (mg)
	Initial	**Final**	**Initial**	**Final**	**Final**	**Final**
Con 3w	309.9±6.1	367.3±4.6	88.8±4.13	120.0±3.1	5.4±0.2	93.8±2.7
Con 11w	302.8±2.3	436.5±6.9[a]	90.7±5.6	119.2±6.7	5.4±0.1	95.3±4.3
DM 3w	301.1±4.9	311.9±7.5	96.0±11.3	459.0±14.3[b]	11.0±0.3[b]	177.5±6.3[c]
DM 11w	306.6±5.0	323.2±7.3	99.1±6.0	439.2±17.9[b]	11.1±0.3[b]	171.9±5.7[c]
DM 3+8w	305.6±6.7	422.8±9.1[a]	96.4±6.0	124.6±17.4	6.2±0.3	120.5±3.2[d]
DIU 3w	315.9±5.7	367.6±5.4	111.0±11.2	124.0±7.9	5.5±0.1	174.0±7.7[c]
DIU 11w	317.8±2.3	451.1±7.6[a]	88.2±4.7	117.9±9.6	5.6±0.1	171.2±5.8[c]
DIU 3+8w	315.4±3.8	445.3±7.9[a]	115.3±5.7	110.8±6.5	5.2±0.1	120.0±4.7[d]

Values are expressed as means plus or minus standard error of the mean.
[a]significantly different from the corresponding value in the Con 3w, DM 3w, DM 11w, and DIU 3w groups (P<0.05).
[b]significantly different from the corresponding values in the Con 3w, Con 11w, DM 3+8w, DIU 3w, DIU 11w and DIU 3+8w groups (P<0.001).
[c]significantly different from the corresponding values in the Con 3w, Con 11w, DM 3+8w, and DIU 3+8w groups (P<0.05).
[d]significantly different from the corresponding values in the Con 3w, Con 11w, DM 3w, DM 11w, DIU 3w and DIU 11w groups (P<0.05).

Table 3: 24-hour urinary habits in different groups (n=8)

	24 hr Urinary Events (#)	Void Volume/Event (ml/event)	24 hr Total Void Volume (ml)
Con 3w	16.0±1.52	0.6±0.08	9.40±1.28
Con 11w	16.7±1.43	0.7±0.06	10.9±0.76
DM 3w	63.5±3.89[a]	2.0±0.18[b]	125.8±9.10[e]
DM 11w	42.3±5.86[a]	2.2±0.33 [b]	86.7±10.71[f]
DM 3+8w	21.7±4.59	0.9±0.12	20.8±5.42
DIU 3w	56.1±5.97[a]	1.3±0.08[c]	75.0±9.22[f]
DIU 11w	42.3±5.90[a]	1.8±0.20[d]	76.6±13.89[f]
DIU 3+8w	22.0±1.53	0.7±0.08	16.0±1.86

Values are expressed as means plus or minus standard error of the mean.
[a]significantly different from the corresponding value in the Con 3w, Con 11w, DM 3+8w and DIU 3+8w groups (P<0.01).
[b]significantly different from the corresponding values in the Con 3w, Con 11w, DM 3+8w, DIU 3w, and DIU 3+8w groups (P<0.001).
[c]significantly different from the corresponding values in the Con 3w, Con 11w, DM 3w. DM 11w, DIU 11w, and DIU 3+8w groups (P<0.05).

[d]significantly different from the corresponding values in the Con 3w, Con 11w, DM 3+8w. DIU 3w, and DIU 3+8w groups (P<0.01).
[e]significantly different from the corresponding values in the Con 3w, Con 11w, DM 11w, DM 3+8w, DIU 3w, DIU 11w, and DIU 3+8w groups (P<0.001).
[f]significantly different from the corresponding values in the Con 3w, Con 11w, DM 3w, DM 3+8w, and DIU 3+8w groups (P<0.001).

Table 4: Areas of bladder wall components in different groups (n=8)

Group	Bladder cross-sectional area (mm^2)				Percentage of the total bladder tissue cross-sectional area (%)		
	total	muscle	collagen	urothelium	muscle	collagen	urothelium
Con 3w	10.7±.04	5.3±0.3	4.0±0.2	0.64±0.02	49.0±1.0	36.9±1.3	6.0±0.3
Con 11w	9.6±0.5	4.5±0.3	4.0±0.3	0.60±0.03	46.8±1.2	42.1±1.6	6.3±0.3
DM 3w	14.1±0.2[a]	8.1±0.2[a]	3.5±0.1	1.00±0.08[a]	57.4±1.3[a]	25.1±1.0[a]	7.1±0.6
DM 11w	13.3±0.5 [a]	7.7±0.3[a]	3.8±0.3	0.90±0.04[a]	57.7±1.0[a]	28.5±1.5[a]	6.8±0.4
DM 3+8w	11.3±0.6	5.8±0.3	4.1±0.3	0.71±0.03	51.3±1.2[b]	36.0±1.8	6.3±0.2
DIU 3w	14.5±0.4[a]	8.1±0.3[a]	4.0±0.2	0.87±0.06[a]	55.8±1.4[c]	27.2±0.6[a]	5.9±0.3
DIU 11w	13.8±0.5[a]	7.9±0.4[a]	4.5±0.3	0.88±0.05[a]	57.3±1.2[a]	32.3±1.7[d]	6.4±0.3
DIU 3+8w	10.8±0.3	5.5±0.2	4.0±0.3	0.66±0.05	51.2±1.1[b]	37.2±2.0	6.2±0.5

Values are expressed as means plus or minus standard error of the mean.
[a]significantly different from the corresponding value in the Con 3w, Con 11w, DM 3+8w and DIU 3+8w groups (P<0.05).
[b]significantly different from the corresponding value in the DM 3w, DM 11w, and DIU 11w groups (P<0.05).
[c]significantly different from the corresponding value in the Con 3w and Con 11w groups (P<0.05).
[d]significantly different from the corresponding value in the Con 11w, and DM 3w groups (P<0.05).

Table 5: Values of CMG parameters in different groups (n=8)

	Intecontraction Interval (sec)	Functional Capacity (ml)	Peak Pressure (cmH_2O)	Void Volume (ml)	Compliance (ml/cmH_2O)
Con 3w	855.3±72.8*	1.19±0.10	53.2±3.7	1.19±0.12	0.09±0.009
Con 11w	972.5±80.3*	1.35±0.11	54.8±3.3	1.28±0.10	0.11±0.007
DM 3w	798.0±122.5[#]	2.14±0.37[a]	83.8±12.8[b]	2.53±0.51[a]	0.18±0.024[a]
DM 11w	858.8±93.8[#]	2.39±0.26[a]	47.5±6.6	2.63±0.26[a]	0.19±0.025[a]
DM 3+8w	955.2±57.1*	1.33±0.08	53.7±2.9	1.28±0.09	0.10±0.008
DIU 3w	917.6±85.1[#]	2.55±0.24[a]	69.2±7.1	3.14±0.46[a]	0.18±0.018[a]
DIU 11w	1005.1±129.3[#]	2.79±0.36[a]	75.8±10.3	2.74±0.40[a]	0.21±0.034[a]
DIU 3+8w	930.8±152.7*	1.29±0.21	48.8±4.6	1.21±0.22	0.10±0.014

Values are expressed as means plus or minus standard error of the mean.
*infusion rate is 5 ml/hr; [#]infusion rate is 10 ml/hr.
[a]significantly different from the corresponding value in the Con 3w, Con 11w, DM 3+8w and DIU 3+8w groups (P<0.01).
[b]significantly different from the corresponding values in the Con 3w, Con 11w, DM 11w, DM 3+8w and DIU 3+8w groups (P<0.05).

3. Publications:

Nan Xiao, Yexiang Huang, Michael Kavran, Rania A. Elrashidy, Guiming Liu. The reversal of diabetes- and diuresis-induced alterations of bladder in rats. Submitted to the *Journal of Urology*, and DCC grant DK076169 was acknowledged.