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Promising anti-diabetic effect of dextran sulfate sodium: Is it its clinical come back?



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ABSTRACT

Clinical studies showed that dextran sulfate sodium (DSS) alleviates stroke, diabetic retinopathy and hypercholesterolemia, yet its mechanism of action was unrevealed. This study show that DSS reduces hyperglycemia, plasma insulin and enhances glucose utilization by attenuating ROS production, suggesting a novel therapeutic use of DSS in diabetes and its complication.

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1. Introduction

Type 2 diabetes mellitus (T2DM) presents a huge global burden both in developed and developing countries [1]. Nowadays, there is no therapeutic intervention that claims a continuous glucose-lowering effect or can be a potential cure for diabetic complications [2].

Dextran sulfate sodium (DSS) has been used as an anticoagulant [3], hypocholesterolemic [3], antiviral agent [4,5] and showed persistent effect on acute cerebral infarction [6] in clinical settings. However, there is a scarcity in data on the effect of DSS on blood glucose or glucose metabolism. Schicho et al. and Dong et al. are the only two groups to report a link between DSS and blood glucose [7,8]. In a non-diabetic setting, both studies reported a marked reduction of glucose

levels and attenuation of the Krebs cycle intermediates of DSS-treated mice when compared to their controls [7,8].

Interestingly, our current study is the first to show that DSS lowers blood glucose levels through reduction of reactive oxygen species (ROS) production in the pancreas. Our findings are of great interest in the discovery of an anti-diabetic drug with a completely unique and novel mechanism of action.

2. Materials and methods

2.1. Animal studies

MKR male mice that spontaneously develop non-obese T2DM were used (The Jackson Laboratory, USA) together with their control FVB-NJ. All of the animal studies were approved by the Animal Care and Use Committee of the American Univer-

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sity of Beirut, Lebanon. Blood glucose levels were assessed daily using the standard method [9] (glucometer, Accu-Chek, USA).

2.2. Oral starch tolerance test

After 8 h of fasting, mice were challenged orally with starch (Sigma, USA) at a dose of 3 g/kg body weight which was administered via oral gavage. Blood samples were taken from the tail tip at baseline (before treatment), 30, 60, 90, 120, 150 and 180 min after the starch challenge for blood glucose determination [10].

2.3. Sacrifice

At the end of the experiment, mice were anaesthetized and sacrificed. Blood and organs were immediately collected and stored at -80°C for further analysis. Plasma insulin was determined using the Cayman chemical kits (USA) and following the manufacturer's instructions.

2.4. Detection of intracellular superoxide in pancreatic tissue

We used pancreatic tissue for evaluation of superoxide production by HPLC according to standard protocol [11]. Results are expressed as the amount of EOH produced (nmol) normalized for DHE consumed.

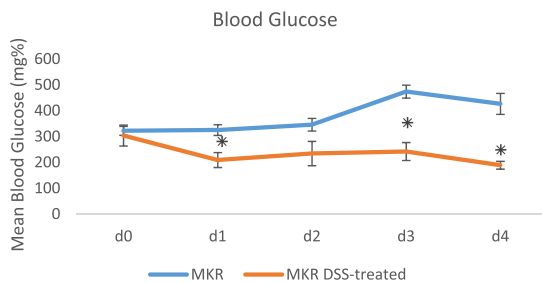
2.5. Statistical analysis

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) (v24.0) [12]. All of the results are expressed as mean \pm SEM (standard error of mean). We used a two-tailed student's *t* test to determine significance with $p \leq 0.05$ as statistically significant.

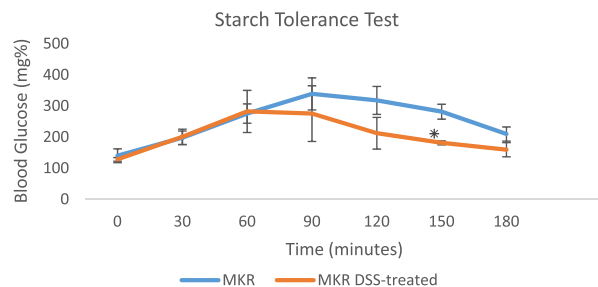
3. Results

Four groups of 4 mice each were used and divided as follow: 2 FVB-NJ groups and 2 MKR groups. The first group of FVB-NJ and MKR served as control while the other groups were administered 2.5% of DSS (Mwt = 35–50 kDa) (MPBiomedicals, USA) solution in autoclaved water for four days. This dose was chosen to mimic the clinical dose used to study other indications for DSS [13]. At baseline, MKR mice showed higher glucose levels when compared to their control littermates (234.75 ± 26.36 vs 176 ± 5.45 mg/dL). Treatment with DSS did not alter glucose levels within FVB-NJ groups (147.75 ± 8.98 vs 141.75 ± 9.99 mg/dL) yet it shows a significant decrease in the MKR DSS-treated mice when compared to the MKR vehicle-treated mice starting day 1 and sustained for the whole time of the study (Fig. 1A and Table 1A). Noteworthy, food was weighed daily to monitor its consumption and it was shown that it was the same across different groups and it did not change after DSS administration.

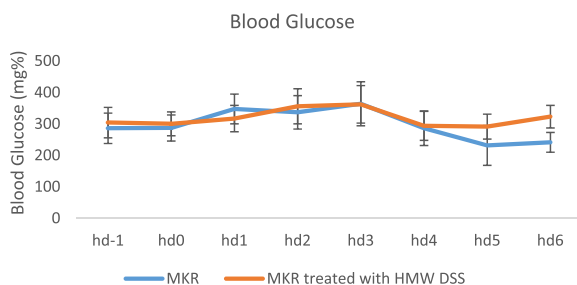
A Difference in mean blood glucose in DSS-treated MKR mice (35–50 kDa) (n=4) compared to non-treated mice (n=4).



B Oral starch tolerance test performed on MKR (n=4) and MKR DSS-treated groups after DSS (35–50 kDa) administration (n=3).



C Difference in mean blood glucose in MKR HMW DSS group (200 kDa) (n=5) compared to non-treated group (n=4).



D Difference in mean blood glucose in MKR DSS-treated group (n=3) compared to non-treated group (n=4) after administration of HMW DSS (200 kDa).

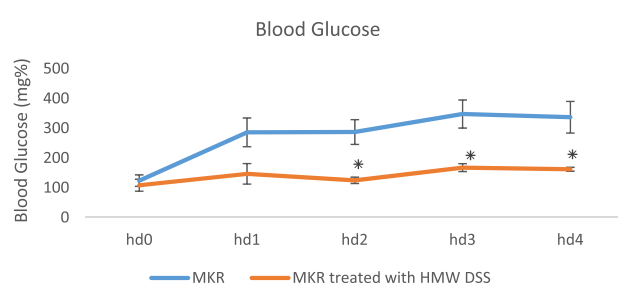


Fig. 1 – Dextran sulfate sodium reduces blood glucose in MKR mice. DSS: Dextran sulfate sodium; d: day. HMW DSS: high molecular weight dextran sulfate sodium; hd: day after administration of HMW DSS. * significant at $p < 0.05$ compared to MKR group. Values are represented as mean \pm standard error.

Table 1 – Blood glucose levels of MKR mice throughout the study. DSS: Dextran sulfate sodium, HMW DSS: High molecular weight dextran sulfate sodium. *significant at $p < 0.05$ compared to MKR group. Values are expressed as mean \pm standard error.

(A) Blood glucose before and after administration of dextran sulfate sodium (35–50 kDa)							
Variable	MKR group (n = 4)		MKR DSS-treated group (n = 4)			p-value	
Blood Glucose (mg%)							
Baseline	322.00 \pm 17.25		303.75 \pm 40.54			0.693	
Day 1	325.00 \pm 20.80		209.00 \pm 28.91			0.017*	
Day 2	345.75 \pm 24.38		234.25 \pm 46.98			0.080	
Day 3	473.75 \pm 25.10		242.00 \pm 34.71			0.002*	
Day 4	426.25 \pm 40.69		189.00 \pm 15.13			0.002*	
(B) Starch tolerance test							
Time (minutes)	0	30	60	90	120	150	180
Blood glucose (mg%) for MKR group (n = 4)	140.00 \pm 22.14	197.75 \pm 21.71	275.5 \pm 30.97	338.75 \pm 51.44	317.75 \pm 44.91	281.5 \pm 24.01	209.75 \pm 22.74
Blood glucose (mg%) for MKR DSS-treated group (n = 3)	128.33 \pm 5.81	200.67 \pm 24.70	282.33 \pm 67.63	275.33 \pm 89.50	212.33 \pm 50.90	181.33 \pm 6.44	159.33 \pm 22.88
(C) Blood glucose before and after administration of high molecular weight dextran sulfate sodium (200 kDa)							
Blood Glucose (mg%)	MKR group (n = 4)		MKR treated with HMW DSS group (n = 5)			p-value	
Baseline	287.00 \pm 41.54		300.20 \pm 37.9			0.808	
Day 1	347.50 \pm 47.27		317.00 \pm 41.95			0.632	
Day 2	336.75 \pm 53.18		356.00 \pm 55.83			0.809	
Day 3	364.00 \pm 69.95		362.20 \pm 59.63			0.984	
Day 4	286.25 \pm 54.96		293.80 \pm 46.10			0.915	
(D) Blood glucose before and after administration of high molecular weight dextran sulfate sodium (200 kDa) to MKR mice previously treated with DSS (35–50 kDa)							
Blood Glucose (mg%)	MKR group (n = 4)		MKR treated with HMW DSS group (n = 3)			p-value	
Baseline	123.75 \pm 19.25		108.25 \pm 20.13			0.598	
Day 1	286.00 \pm 48.31		146.50 \pm 34.51			0.057	
Day 2	287.00 \pm 41.54		124.75 \pm 10.64			0.026*	
Day 3	347.50 \pm 47.27		167.50 \pm 13.19			0.027*	
Day 4	336.75 \pm 53.18		162.0 \pm 6.56			0.040*	

Table 2 – Gross characterization of MKR and dextran sulfate sodium-treated MKR mice. DSS: Dextran Sulfate Sodium. * significant at $p < 0.05$ compared to MKR group. Values are expressed as mean \pm standard error.

Variable	Control group (n = 4)	DSS group (n = 3)	p-value
Body Weight (gm)	24.10 \pm 0.895	21.60 \pm 0.984	0.115
Blood Glucose (mg%)	241.50 \pm 31.436	142.00 \pm 3.0551	0.044*
Plasma Insulin (μ U/mL)	40.0 \pm 1.730	33.57 \pm 0.900	0.032*
Colon Length (cm)	6.30 \pm 0.78	4.30 \pm 0.44	0.104
Heart Weight (gm)	0.1363 \pm 0.0112	0.1047 \pm 0.0227	0.232
Heart Weight to Body Weight Ratio	0.0056 \pm 0.0004	0.0048 \pm 0.0009	0.395
Kidney Weight (gm)	0.1823 \pm 0.0081	0.1733 \pm 0.0067	0.457
	0.1870 \pm 0.0162	0.1600 \pm 0.0058	0.229

In order to elucidate the mechanism of action of DSS, oral starch tolerance test was performed on the MKR groups of mice. Our results show no significant difference in the blood glucose levels between the MKR and the MKR DSS-treated mice in the ascending portion of the tolerance curve suggesting that DSS did not affect the digestion nor the absorption

process (Table 1B and Fig. 1B). To further confirm our finding, MKR mice were treated with 2.5% HMW-DSS solution (Mwt = 200 kDa) (Sigma, USA), a non-absorbed form of DSS. Interestingly, throughout the whole treatment length, HMW DSS failed to achieve any changes in blood glucose levels as it is described with the absorbed DSS form (Table 1C and

Fig. 1C). Strikingly, when mice previously treated with DSS (35–50 kDa) were administered another 2.5% HMW-DSS solution, the combination succeeded to induce a significant more prominent reduction of hyperglycemia (Table 1D and Fig. 1D).

Moreover, our results show no glucose release in the urine of DSS-treated mice after the treatment, while DSS treatment succeeded in reducing plasma insulin significantly (Table 2).

ROS production in pancreatic tissues was also measured. Our results show a significant decrease in ROS production in the presence of DSS ($0.000569 \pm 2.2E-05$ vs $0.00119 \pm 2.7E-05$ pmol/umol) suggesting that the DSS effect is potentiated by reducing the deleterious effect of ROS [11] thus enhancing the function of the pancreatic cells.

4. Discussion

To our knowledge, this is the first study to report an anti-diabetic effect for DSS, and its potential mechanisms of action.

Our results show that DSS exerted a biologically reliable anti-diabetic effect in MKR mice starting from day 1 and remained for the whole study period. The effect of DSS on blood glucose was reported only twice in literature [7,8] but in non-diabetic setting and the mechanism of blood glucose reduction was not revealed. Concomitant with these findings, our data show the same hyperglycemic-reduction effect which is explained by ROS production in the pancreas. ROS has been described to be injurious for β -cells integrity and was shown to reduce pancreatic function [14]. Our group and others have previously shown that this ROS production is through NADPH oxidases (NOXs) [11,15–19] which suggest that DSS inhibits ROS production generated by NOXs.

Also, DSS has been used previously for clinical indications as an infusion or as tablets [5,6,20]. Our results could revive this compound again as an oral hypoglycemic for patients with DM.

5. Statement of interests

Authors declare no conflict of interests and received no funding.

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