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EFFECT OF PANCREATIC CELLS CULTURE TRANSPLANTATION IN EXPERIMENTAL DIABETES MELLITUS

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Abstract:

Aim. To study possibility of insular and contrainsular hormones in alloxan diabetes after pancreatic cells culture transplantation.

Alloxan allows to model diabetes mellitus in rats with similar pathophysiological shifts as in type 1 diabetes mellitus in humans. After transplantation of pancreas cell culture insulin, C-peptide, thyroxine, and triiodothyronine level increases, corticosterone concentration is decreased that is associated with the complete normalization of hormones; this regularity is stable till the end of the experiment. Stabilization of hormones normalizes carbohydrate metabolism in rats.

Materials and methods. 80 rats with alloxan diabetes were shared into two equal groups: I – control – non-cured animals, II – experimental – after pancreatic cells culture transplantation. Males and females were even on both groups, animals' age made 12-14 months, body mass – 244.23±20.31 g. Diabetes mellitus was modeled by subcutaneous introducing Alloxan solution dosed 200 mg/kg. We investigated the main parameters of carbohydrate metabolism (insulin, corticosterone, C-peptide, thyroxin, and triiodothyronine) by radioimmunological method with standard reactives set.

Results. In the group after transplantation of pancreatic cellular culture on the 1st day we noticed evident increase of insulin level up to 12.7±0.69 mkIU/l, i.e. 6,6 times more in comparison with database and 3,6 times more above the norm (3,53±0,21 mkIU/l). By the 7th day insulin concentration is decreased down to 3.69±0.07 mkIU/l ($p<0,001$). By the 14th day insulin level makes 3.45±0.08 mkIU/l that is less of previous index ($p<0,05$) and does not differ from the norm ($p>0.05$), and remains within these limits till the end of the 1st month of post-operation period. C-peptide level as well as insulin on the 1st day after transplantation evidently increases 9,8 times comparing with database that is twice more above the norm (0.73±0.02 ng/ml). By the 7th day it unevidently elevates up to 1.59±0.08 ng/ml, and by the 14th day falls for 1,9 times ($p<0.001$). By the end of the 1st month it remains stable and makes 0.85±0.09 ng/ml that is 5,6 times more above database ($p<0.001$).

Conclusions. Thus, our experiments demonstrated using Alloxane tetrahydrate to model experimental diabetes mellitus and studying different pathological processes in rats. On proved the role of contrainsular hormones misbalance in advanced diabetes mellitus course. Transplantation of pancreatic β-cells normalize hormonal content and glucose level in rats.

***Key words**: alloxan diabetes, cell transplantation, contrainsular hormones.*

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INTRODUCTION:

Despite the great number of studies dealing with pathophysiological shifts in diabetes mellitus (DM) several problems remain opened and need further study as in experiment as in clinical conditions. The great importance belongs to experimental diabetology [1, 2, 4]. Alloxan diabetes is a universal model of type 1 DM and enables to study totality of β-cells destruction and misbalance of insular and contrainsular hormones [1, 3-5].

Aim is to study possibility of insular and contrainsular hormones in alloxan diabetes (AD) after pancreatic cells culture transplantation.

MATERIAL AND METHODS:

80 rats with AD were shared into two equal groups: I – control – non-cured animals, II – experimental – after pancreatic cells culture transplantation. Males and females were even on both groups, animals' age made 12-14 months, body mass – 244.23±20.31 g. DM was modeled by subcutaneous introducing Alloxan solution dosed 200 mg/kg.

Cellular culture was obtained from newborn rabbits as follows: preliminarily trypsinized pancreas is mechanically divided into fragments of 1 mm^3 and underwent heat trypsinization in 37°C within 5 min in 0.25% trypsin solution. Trypsin activity is arrested with adding 5% cattle serum.

We investigated the main parameters of carbohydrate metabolism (insulin, corticosterone, C-peptide, thyroxin, and triiodothyronine) by radioimmunological method with standard reactives set.

RESULT AND DISCUSSION:

In AD group in 3 months we found stable glucose level (12.1±0.49 mmol/l) that doubtfully increased by the 6th month to 13,21±1,83 mmol/l, by the 9th month – 19.08±2,37 mmol/l. Comparatively with the primary level (1 month of AD) the glucose level was increased 1,4 times and differed evidently (t=2.21; p<0,05). This process was accompanied with stable high level of glycosylated hemoglobin that within 6 months was made 5,59±0,41%, by the 9th month doubtfully (t=2,21; p<0,05) increased up to 6,21±0,54% that is 1,12 times more of the primary level. Animals from the control group did not survive above 9 months and died due to DM complications.

1-6 months after transplantation we see evident stable level of glycemia within 6 months (4.44±0.07 mmol/l) and by the 12th month glucose concentration evidently increased up to 5.64±0.09 mmol/l (p<0.001) that is 1,27 more comparatively 6-month-index. It's 2.45 times less of database and 1.3 times more of the standard (4,31±0,13 mmol/l, p<0.001).

In AD group initially in normal indexes of insulin and C-peptide the corticosterone level is on the normal level but if insulin and C-peptide are below standard for a long time corticosterone concentration progressively decreases. Thyroxine and triiodothyronine level is decreased just after decrease of insulin and C-peptide level fall. Thus, low concentration if insular hormones are associated with pathologic increase of corticosterone and drop of thyroxine and triiodothyronine concentration.

In the group after transplantation of pancreatic cellular culture on the 1st day we noticed evident increase of insulin level up to 12.7±0.69 mkIU/l, i.e. 6,6 times more in comparison with database and 3,6 times more above the norm (3,53±0,21 mkIU/l) (table 1). By the 7th day insulin concentration is decreased down to 3.69±0.07 mkIU/l (p<0,001). By the 14th day insulin level makes 3.45±0.08 mkIU/l that is less of previous index (p<0,05) and does not differ from the norm (p>0.05), and remains within these limits till the end of the 1st month of post-operation period. C-peptide level as well as insulin on the 1st day after transplantation evidently increases 9,8 times comparing with database that is twice more above the norm (0.73±0.02 ng/ml). By the 7th day it unevidently elevates up to 1.59±0.08 ng/ml, and by the 14th day falls for 1,9 times (p<0.001). By the end of the 1st month it remains stable and makes 0.85±0.09 ng/ml that is 5,6 times more above database (p<0.001).

Cortirosterone level drops within 24 hrs down to 152.3±2.39 nmol/l, i.e. 1,3 times more above the norm. By the 7th day it insignificantly falls (p<0.05) and makes 138.32±4.69 nmol/l, but by the 14th day it decreases 1,3 times (p<0.001) down to 107.5±3.2 nmol/l that evidently differs from the norm (p>0.05). By the end of the 1st month corticosterone level unevidently increases up to 339.0±8.4 nmol/l that is 1,8 times less from preoperative database (p<0,001) and does not evidently differ from the norm.

Thyroxine within 24 hrs evidently increases 1,8 times and makes 36.36±0.57 nmol/l and statistically does not differ from the standard (39.54±1.93 nmol/l). By the 7th day thyroxine level insignificantly decreased down to 34.2±1.5 nmol/l (p>0,05) and remained within this diapason during a month. By the end of the 1st month it was 37.14±0.74 nmol/l that is 1,8 times more of initial concentration (p<0,001) and statistically does not differ from the norm.

Triiodothyronine level within 24 hrs after transplantation was 2.15±0.06 nmol/l that is twice more of database (p<0,001). Further it remained stable and by the end of the 1st month was 2.78±0.07 nmol/l that 2,6 times more above the database and 1,2 time more of the norm (p<0,05).

Table 1. Levels of proinsular and contrainsular hormones in rats of group III within a month (M±m)

Parameter	database	24 hours	7 days	14 days	1 month
Insulin, mkIU/l	1,94±0,12	12,71±0,69 ****	3,69±0,07 ****	3,45±0,08 *	3,51±0,07 **, #
C-peptide, ng/ml	0,15±0,01	1,48±0,03 ****	1,59±0,08 **	0,83±0,12 ****	0,85±0,09 **, #
Corticosterone, nmol/l	355,2±21,2	456,9±7,2 ****	414,96±14,07 *	322,41±9,6 ****	337,02±8,4 **, #
Thyroxine, nmol/l	20,12±0,82	36,36±0,57 ****	34,2±1,5 **	36,12±0,69 **	37,14±0,74 **, #
Triiodothyronine, nmol/l	1,08±0,09	2,15±0,06 ****	2,44±0,14 **	2,64±0,06 **	2,78±0,07 **, #

*- evident difference between previous and compared parameter (p<0,05); **- unevident difference between previous and compared parameter (p>0,05); ***- unevident difference between initial and final results (p<0,05); **** - evident difference between previous and compared parameter(p<0,001); # - evident difference between initial and final results (p<0,05)

Remote shifts of hormones after transplantation of pancreatic cellular culture (table 2) demonstrated stability of insulin concentration within 6 months (3.44±0.05 mkIU/l) and by the end of the 12th month decreased 1,1 time comparatively with the 6th month (p<0,001) and 1,7 times more above database (p<0,001), statistically does not differ from the norm.

Table 2. Proinsular and contrainsular hormones level in rats of III group within 12 months (M±m)

Parameter	database	1 month	3 months	6 months	12 months
Insulin, mkIU/l	1,94±0,12	3,51±0,07	3,47±0,08 **	3,44±0,05 **	3,21±0,02 ****, #
C-peptide, ng/ml	0,152±0,01	0,85±0,09	0,86±0,07 **	0,859±0,08 **	0,847±0,07 **, #
Corticosterone, nmol/l	355,2±25,2	337,02±8,4	340,26±14,8 **	330,72±17,4 **	251,72±20,7 **, #
Thyroxine, nmol/l	20,12±0,82	37,14±0,74	41,52±2,5 **	41,23±1,8 **	40,27±1,98 **, #
Triiodothyronine, nmol/l	1,08±0,09	2,78±0,07	2,54±0,06 *	2,49±0,02 **	2,46±0,14 **, #

*- evident difference between previous and compared parameter (p<0,05); **- unevident difference between previous and compared parameter (p>0,05); ***- unevident difference between initial and final results (p<0,05); **** - evident difference between previous and compared parameter(p<0,001); # - evident difference between initial and final results (p<0,05)

C-peptide level within 12 months evidently remained stable 0.847±0.07 ng/ml that is 5,6 times more above pre-operation level and evidently above the norm ($p<0,05$). Corticosterone concentration also was evidently stable till the end of the 12th month (251.72±20.7 nmol/l) and 1,7 time below preoperative database, and evidently did not differ from the norm ($p>0.05$). Thyroxine level by the 3rd month unevidently increased up to 41.52±2.1 nmol/l and statistically did not differ from the norm. Till the end of the 12th month it was stable (40.27±1.98 nmol/l) that was twice more above the norm ($p<0,001$) and did not differ from the norm ($p>0,05$).

Triiodothyronine concentration by the 3rd month insignificantly decreased down to 2.54±0.06 nmol/l and remained stable till the 12th month. By the end of the 12th month it made 2.46±0.14 nmol/l that was 2,3 times more above the preoperative index and did not differ from the norm ($p>0.05$).

Mathematical analysis cleared linear relationship between insulin and C-peptide ($\chi=0,61$) but very weak, this dependence can't be described with equation of linear regression because $RI<0,8$. Relationship between insulin, corticosterone, thyroxine, and triiodothyronine is absent because $\chi<0,4$. Relationship between C-peptide and thyroxine, and triiodothyronine is weak linear ($\chi=0,47$ and 0.51 respectively), but between C-peptide and corticosterone absent ($\chi=0,31$). But between parameters of corticosterone and thyroxine there is a linear link of high degree ($\chi=0,91$). Correlation may be described with the equation of liner regression: $K=(286,8-4,32\cdot T_4)\cdot 3$ if $RI=0,83$, $F=28,31$, $p<0,0018$, $SEOE=14,9$. Co-relation between corticosterone and triiodothyronine is subordinated to the linear link equation ($\chi=0,96$): $K=(268,9-59\cdot T_3)\cdot 3$ if $RI=0,92$, $F=70,8$ при $p<0,00015$, $EOE=9,9$. Between thyroxine and triiodothyronine there is also the high degree linear link ($\chi=0,89$) with the following equation of linear regression: $T_4=11,39\cdot T_3-9,4$ if $RI=0,76$, $F=20,8$, $p<0,00385$, $SEOE=9,5$. Relations between other hormones aren't subordinated to linear/non-linear regression ($RI<0,8$). But the analysis of co-relations within 1-12 months cleared relations subordination to the equation of non-liner regression with high degree of process description and minimal standard deviation of estimation. So, co-relation between insulin and corticosterone may be described as \=0,3·SQR(K/3), if RI=0,99, F=2253,7, p<0,00001, SEOE=0,16; between insulin and C-peptide - $I=47,79\cdot logC$ if $RI=0,99$, $F=520,6$, $p<0,00002$, $SEOE=0,3$; between insulin (I) and thyroxine - $I=0,54\cdot SQRT_4$, if $RI=0,99$, $F=1751,4$, $p<0,000001$, $SEOE=0,18$; between insulin and triiodothyronine – $I=8,29\cdot logT_3$, if $RI=0,99$, $F=9360,5$, $p<0,000001$, $SEOE=0,13$; between C-peptide (C) and triiodothyronine – $C=2,1\cdot logT_3$, if $RI=0,99$, $F=1450,1$, $p<0,000001$, $SEOE=0,05$; between C-peptide and thyroxine – $C=0,14\cdot SQRT_4$, if $RI=0,99$, $F=1168$, $p<0,000001$, $SEOE=0,02$; between C-peptide and corticosterone – $C=0,08\cdot SQRK$, if $RI=0,99$, $F=18473$, $p<0,000001$, $SEOE=0,0154$; between corticosterone (K) and thyroxine – $K=(3,7\ SQRT_4)\cdot 3$, if $RI=0,99$, $F=1584$, $p<0,000001$, $SEOE=2,21$; between corticosterone and triiodothyronine – $K=(0,24\cdot SQRT_3)\cdot 3$, if $RI=0,99$, $F=1456,9$, $p<0,000001$, $SEOE=0,15$; between thyroxine and triiodothyronine - $T3=0,4\cdot SQRT_4$, if $RI=0,99$, $F=828,56$, $p<0,0001$, $SEOE=0,2$.

CONCLUSION:

Thus, our experiments demonstrated using Alloxane tetrahydrate to model experimental DM and studying different pathological processes in rats. On proved the role of contrainsular hormones misbalance in advanced DM course. Transplantation of pancreatic β-cells normalize hormonal content and glucose level in rats.

List of symbols and abbreviations:

AD - alloxan diabetes
DM - diabetes mellitus

REFERENCES:

1. Mozheyko LA. Experimental models for studying diabetes mellitus part 1. alloxan diabetes. Journal of Grodno State Medical University, 2013;3(43):26-29.
2. Shevtsova LM, Bykova OS, Fedorova NP, Grigor'eva MV, Maksimiuk NN. Myocardium morphologic reorganization in experimental diabetes. Bulletin of Novgorod State University. Yaroslav the Wise, 2015;2(85):146-150.
3. Vyatkin VA, Butolin EG, Ivanov VG. Effect of chondroitin sulfate on the type I collagen metabolism in the compact bone in alloxan-induced rats. Kazan medical journal, 2015;96(5):802-806.
4. Mykhaylichenko VYu., Stolyarov SS. The effect of pancreatic cell culture transplantation in alloxan diabetes mellitus in rats in the experiment. International Journal of Applied and Basic Research, 2015;9(4):670-672.
5. Lesova ZT, Abdreshov SN, Mamataeva AT, Rebezov MB. Using of soya milk and a-tocopherol to reduce the alloxan diabetes rate in rats. AIC of Russia, 2016;75(1):30-34.