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The activity of lysosomal enzymes of rabbit's pancreas during treated experimental diabetes

According to the literature, the activity of pancreatic lysosomal enzymes changes during acute pancreatitis, pancreatic neoplasm and induced experimental diabetes (1–5). For that reason, we decided to follow it during treated experimental diabetes mellitus. Hydrogen chloride of pioglitazon and repaglinid were administered to treat. We would like to thank Takeda Chemicals Industries from Japan for those medicines.

MATERIAL AND METHODS

The investigations were carried on 60 New Zealand rabbits, male adults. The material was divided into the following groups: Group 1 – 10 rabbits which received i.v. one dose of physiological salt solution and amylaceous gruel during 8 weeks; Group 2 – 10 rabbits which received i.v. one dose of physiological salt solution and pioglitazon orally every day; Group 3 – 10 rabbits which received i.v. one dose of physiological salt solution and repaglinid orally every day; Group 4 – 10 rabbits with 8-week diabetes receiving amylaceous gruel every day; Group 5 – 10 rabbits with 8-week diabetes receiving pioglitazon every day; Group 6 – 10 rabbits with 8-week diabetes receiving repaglinid every day.

Diabetes was provoked like in the previous study (3). Administration of pioglitazon was started on the 13th day after giving alloxan. It was administered every day at the same time, before feeding, as the 1 ml pioglitazon suspension in 5% amylaceous gruel prepared *ex tempore*. The medicine was administered with a syringe without a needle directly into oral cavity of rabbit. The animals from the control group received 1 ml pure amylaceous gruel. Pioglitazon and repaglinid were administered in the dose 1 mg/1kg body mass which corresponds to $^1/_4$ daily human dose. The rabbits were killed by i.v. injection of sodium penthobarbitalum in the dose 60 mg/kg body mass and then bled. The segments of pancreas were taken for the investigation and this material was rinsed with physiological salt solution, desiccated and frozen at –80 degrees Celsius. The activity of lysosomal enzymes was marked like in the previous study (3). T-test was used to evaluate statistical differences between groups.

RESULTS

Table 1 presents the activity of free pancreatic lysosomal enzymes of rabbit during treated experimental diabetes and Table 2 presents connecting ones. According to Table 1, the activity of free acid phosphatase in rabbits treated with pioglitazon was higher by 70.8% and in rabbits treated with

repaglinid was higher by 53.5% than in the control group, the difference being statistically significant. In the group of rabbits with untreated diabetes the activity of this enzyme was higher by 43.3% than in the control group. In the group with diabetes treated with repaglinid it was higher by 52.9% than in the group with untreated diabetes. The activity of free acid phosphatase in rabbits with diabetes receiving repaglinid was lower by 12.15% in comparison with the group with untreated diabetes. According to Table 2, the activity of connecting acid phosphatase in the rabbits receiving pioglitazon and repaglinid was only slightly higher than in the control group. It was higher by 58.1% in the group of rabbits with diabetes than in the control group. The activity of connecting acid phosphatase only slightly decreased in the group receiving pioglitazon in comparison with the group of rabbits with untreated diabetes but in rabbits treated with repaglinid this activity was lower by 35.5%.

Table 1. The activity of free fractions of lysosomal enzymes of rabbit's pancreas during treated experimental diabetes estimated in μg/mg protein/1hour of incubation

Group	1	2	3	4	5	6
Acid phosphatase	0.0529 ±0.0242	0.0904 ±0.0077	0.0812 ±0.0343	0.0758 ±0.0343	0.1159 ±0.0363	0.0666 ±0.0149
β-galactosidase	0.0348 ±0.0077	0.0411 ±0.0035	0.0354 ±0.0044	0.0265 ±0.0044	0.0351 ±0.0048	0.0281 ±0.0032
NAGL	0.0540 ±0.0275	0.1556 ±0.0132	0.1072 ±0.0173	0.0726 ±0.0173	0.0987 ±0.0431	0.0516 ±0.0090
Cathepsin D	74.1198 ±53.8669	55.8585 ±4.7253	54.4478 ±5.8699	41.7331 ±5.8699	60.4948 ±4.1305	79.9348 ±16.1307
Cathepsin L	61.8320 ±49.6755	115.8476 ±9.800	166.3575 ±93.2250	195.2566 ±93.2250	174.3404 ±117.4315	68.2054 ±21.383
Lipase	0.1122 ±0.0727	0.1834 ±0.0155	0.1389 ±0.0442	0.1593 ±0.0442	0.1946 ±0.0403	0.1444 ±0.0019
Sulphatase	0.0003 ±0.0002	0.0005 ±0.0001	0.0004 ±0.0001	0.0004 ±0.0001	0.0006 ±0.0002	0.0066 ±0.0001μμ

The activity of free β-galactosidase in the group with pioglitazon administration was higher by 18.1% than in the control group, but at rabbits with repaglinid administration, it was higher only slightly. In the group of rabbits with diabetes there was recorded decrease of the activity of this enzyme by 23.9%.Comparing rabbits treated with pioglitazon and rabbits with untreated diabetes, there was observed the increase of activity of free β-galactosidase by 32.4% in the group receiving pioglitazon, observed also to a small degree in the group treated with repaglinid. The activity of connecting β-galactosidase was higher by 21.6% in the group of rabbits receiving pioglitazon than in the control group. It was also slightly higher in the group treated with repaglinid. In the group of rabbits with diabetes the activity of these enzymes was only slightly lower than in the control group. In the group of rabbits treated with pioglitazon it was lower by 29.3% than in the group with untreated diabetes but in the group treated with repaglinid it was eqal.

Regarding the activity of free N-acetyl-β-D-glucosaminidase (NAGL), it was higher by 188.1% in the group receiving pioglitazon and by 98.5% in the group receiving repaglinid than in the control group, which is statistically significant. In the group with untreated diabetes it was higher by 34.4% than in the control group. The activity of NAGL was higher by 39.5% in the group treated with pioglitazon than in the group with untreated diabetes but in the group treated with repaglinid, it was lower by 28.9%. The activity of connecting NAGL in rabbits receiving pioglitazon was higher by

45.7% than in the control group. Also in rabbits receiving repaglinid it was higher by 28.5%.The activity of this enzyme in the group with untreated diabetes was slightly lower than in the control group. Comparing rabbits with treated and untreated diabetes, a slight decrease of activity of connecting NAGL was noticeable in the group treated with pioglitazon and an increase by 65.9% in the group treated with repaglinid, which is statistically significant.

Table 2. The activity of connecting fractions of lysosomal enzymes of rabbit's pancreas during treated experimental diabetes estimated in μg/mg protein/1 hour of incubation

Group	1	2	3	4	5	6
Acid phosphatase	0.0310 ±0.0242	0.0354 ±0.0030	0.0348 ±0.0165	0.0490 ±0.0185	0.0474 ±0.0144	0.0316 ±0.0050
β-galactosidase	0.0088 ±0.0087	0.0107 ±0.0009	0.0099 ±0.0046	0.0082 ±0.0046	0.0058 ±0.0018	0.0092 ±0.0002
NAGL	0.0221 ±0.0087	0.0322 ±0.027	0.0284 ±0.0065	0.0217 ±0.0065	0.0209 ±0.0090	0.0360 ±0.0104
Cathepsin D	71.7869 ±54.8206	151.4951 ±12.8156	113.1735 ±86.4003	111.6908 ±86.4003	47.8162 ±22.5191	214.5721 ±86.6729
Cathepsin L	71.1777 ±8.2531	54.3441 ±4.5972	126.9918 ±14.0009	301.7239 ±142.0009	441.8038 ±218.5600	181.5698 ±67.3126
Lipase	0.0696 ±0.0296	0.0867 ±0.0073	0.0781 ±0.0205	0.0844 ±0.0205	0.0862 ±0.0193	0.0840 ±0.0134
Sulphatase	0.0003 ±0.0001	0.0004 ±0.0001	0.0004 ±0.0002	0.0003 ±0.0002	0.0003 ±0.0001	0.0004 ±0.0001

Regarding the activity of free cathepsin D in both groups with pioglitazon and repaglinid it was lower by 25% in comparison with the control group. The activity of this enzyme was also lower by 38.3 % in the group with untreated diabetes than in the control group. In the group receiving pioglitazon it was higher by 44.9% and in the group receiving repaglinid it was higher by 91.5% in comparison with the group with untreated diabetes, which is statistically significant. The activity of connecting cathepsin D increased by 111% in the group treated with pioglitazon and by 57.7% in the group treated with repaglinid in comparison with the control group, which is statistically significant. The activity of this enzyme in rabbits with diabetes was higher by 55.6% than in the control group, which is statistically significant. The activity of connecting cathepsin D decreased by 57.2% in comparison with rabbits with untreated diabetes but in the group treated with repaglinid it was higher twice, which is statistically significant.

The activity of free cathepsin L was higher by 87.4% in the group receiving pioglitazon and by 169% in the group treated with repaglinid than in the control group. Also in the group with untreated diabetes it was higher by 215.8% in comparison with the control group, which is statistically significant. In the group treated with pioglitazon a slight decrease of activity of this enzyme was noticeable and in the group treated with repaglinid this decrease was significant – by 65.1%.The activity of connecting cathepsin L decreased in the rabbits receiving pioglitazon by 23.7% and in the group treated with repaglinid it increased by 78.4% in comparison with the control group, which is statistically significant. In the group with untreated diabetes it increased over four times in comparison to the control group, which is statistically significant. The increase of activity of connecting cathepsin L by 46.4% was

noticeable in the group receiving pioglitazon and its decrease by 39.9% was noticeable in the group receiving repaglinid.

Regarding the activity of free lipase, it was higher by 63.4% in rabbits treated with pioglitazon and by 23.8% in rabbits treated with repaglinid than in the control group. In the group with untreated diabetes, it was higher by 41.9% in comparison with the control group. Comparing the animals with diabetes, the activity of this enzyme increased by 22.2% in rabbits treated with pioglitazon and slightly decreased in the group receiving repaglinid beside rabbits with untreated diabetes. The activity of connecting lipase was higher by 24.6% in the group receiving pioglitazon and by 12.25 in the group receiving repaglinid than in the control group. Also in the group with diabetes it was higher by 21.3% than in the control group. Comparing the animals with treated and untreated diabetes, the values of activity of connecting lipase was almost equal.

The activity of free and connecting sulphatase was very low and only slight differences were noticeable between particular experimental groups.

From our experiment we can conclude that there was increased the activity of four free enzymes: acid phosphatase, β-galactosidase, NAGL and cathepsin D in rabbits treated with repaglinid in comparison with untreated rabbits. Among connecting enzymes, the higher activity of cathepsin L and lipase was observed in this group. Regarding rabbits treated with repaglinid, there was the higher activity of free β-galactosidase and cathepsin D and connecting NAGL, cathepsin D. The activity of β-galactosidase was equal in this group.

REFERENCES

1. Duffy M. J.: The role of proteolytic enzymes in cancer invasion and metastasis. Clin. Exp. Med., 10, 145, 1992.
2. Gorelick F., Malovick L.: Lysosomal enzymes and pancreatitis. Gastroenterol., 109, 620, 1995.
3. Wójtowicz Z. et al.: The activity of lysosomal enzymes of rabbit's gingival mucosa during induced experimental diabetes. Annales UMCS, Sectio D, 59, 2004.
4. Wójtowicz Z. et al.: The activity of lysosomal enzymes of rabbit's pancreas during experimental diabetes. Annales UMCS, Sectio D, 60, 2005.
5. Yamaguchi N. et al.: Characterisation of cathepsin L-like enzyme secred from the human pancreatic cancer cell line HPC-YP. Cancer Res., 50, 658, 1990.

SUMMARY

The investigations were carried on 60 New Zealand rabbits. Diabetes was provoked by means of an i.v. injection of alloxan. Pioglitazon and repaglinid were administered to treat induced diabetes. They were administered every day, orally as 1 ml suspension in 5% amylaceous gruel. The rabbits were killed and bled. The segments of pancreas were taken for the investigation and the material was frozen at –80 degrees Celsius and then it was homogenized in the solution of saccharose. The activity of lysosomal enzymes was marked with the proper substrates. In the group of rabbits treated with pioglitazon activity of free acid phosphatase, β-galactosidase, NAGL and cathepsin D was higher than in the group with untreated diabetes. Regarding connecting enzymes in this group, the activity of connecting cathepsin L and lipase was higher. In the group of rabbits treated with repaglinid, higher activity of free β-galactosidase and cathepsin D and connecting NAGL and cathepsin D was noticeable. The activity of connecting β-galactosidase was like in the compared group.

Aktywność enzymów lizosomalnych trzustki u królika w przebiegu leczonej cukrzycy doświadczalnej

Badania przeprowadzono na 60 królikach rasy nowozelandzkiej białej. Cukrzycę u królików wywoływano przez dożylne podanie alloksanu. W leczeniu cukrzycy zastosowano pioglitazon i repaglimid, które podawano codziennie doustnie w postaci 1 ml zawiesiny w 5 % kleiku skrobiowym. Króliki usypiano i skrwawiano. Do badań pobierano trzustkę, którą zamrażano do –80°C, a następnie homogenizowano w roztworze sacharozy. Aktywność enzymów lizosomalnych oznaczano przy użyciu specyficznych substratów. U królików leczonych pioglitazonem stwierdzono większą aktywność wolnych enzymów lizosomalnych: fosfatazy kwaśnej, β-galaktozydazy, NAGL i katepsyny D w porównaniu ze zwierzętami nieleczonymi. W przypadku enzymów związanych większą aktywność miała katepsyna L i lipaza. U królików leczonych repaglimidem większą aktywność miały β-galaktozydaza i katepsyna D. Co do enzymów związanych większą aktywność miały NAGL i katepsyna D oraz jednakową β-galaktozydaza.