EXPERIMENTAL RESEARCH ON VASCULAR PERMEABILITY CHANGES IN DIABETIC RETINOPATHY, USING FLUORESCEIN-LABELING TECHNIQUE

Key Words: experimental diabetes, alloxan, diabetic retinopathy, nephropathy, stem cell stimulation, methanandamide, FITC-BSA, extravasation of fluorescein, Langerhans' islets.

Summary

In our experimental research we induced diabetes by administering Alloxan.

We used two groups of animals (Wistar rats), one of them having an average body weight of 300 g (group A) and the other one of 425-475 g (group B).

The 125 mg/kg dose of Alloxan, administered intraperitonally, produced a higher mortality rate in group B, probably due to the overweight of the animals, a factor known to increase the vulnerability of insulin excreting pancreatic cells to exogenous toxic effects. This is why the Alloxan dose was reduced in case of group B, to 115 mg/kg.

During the experiments the blood glucose level and body weight of animals was measured at different intervals.

In order to determine vascular permeability, we applied the fluorescein-labeling technique, administering bovine serum albumin labeled with this substance just before the exsanguination of the animals. Fluorescence was measured in the blood plasma of the sacrificed animals and in the microscopical sections of the retina and kidney. The obtained data were statistically compared and correlated.

These determinations were done in the case of untreated and treated diabetic animals as well as nondiabetic ones.

In group A the induced diabetes was treated with the stem cell stimulator Olimpiq® StemXCell SL. The diabetic animals of group B were treated with the endocannabinoid derivative Methanandamide.

The animals of both groups were divided in three subgroups: a nondiabetic control group, an untreated diabetic group and a treated diabetic group.

In the group of animals treated with stem cell stimulator we observed the following:

Body weight loss in both diabetic groups with no statistically important difference, and body weight gain in the nondiabetic group.

Decreased blood glucose level in the treated diabetic group as well as in the untreated one with no statistically important difference, and minimal fluctuations in the nondiabetic group.

Significantly decreased vascular permeability in the treated diabetic group in comparison with the untreated one, both in retinal and renal tissues, with a positive and statistically significant correlation. The difference between the control group and the untreated diabetic group was significant, and the difference between the control group and the treated diabetic group was insignificant.

In the group of animals treated with Methanandamide we observed the following:

Body weight loss in the untreated diabetic group and body weight gain in the treated diabetic group, with statistically important difference. This body weight gain can be the consequence of increased appetite which is a characteristic effect of cannabinoids.

A tendency towards decreased and stable blood glucose levels in the treated diabetic group after the moment of starting treatment in comparison with the untreated diabetic group. Differences have been statistically significant between the three groups.

A statistically significant increase of vascular permeability in the diabetic groups in comparison with the nondiabetic one, the difference between the treated diabetic groups and the untreated one being significant from a statistical point of view. The correlation between values of the retinal and renal tissue was positive and statistically significant.

Based on these two studies, we succeeded to demonstrate for the first time that modifications of vascular permeability were much more important in the case of animals treated on the long run with stem cell stimulating substances, in comparison with the ones treated with Methanandamide. The mechanism appears to be the regeneration of the structures of the vascular wall, especially of the endothelium, which is the blood–retinal barrier.

We also demonstrated for the first time the positive and statistically significant correlation between retinal and renal fluorescence. Modifications in the fluorescence

of different tissues have been parallel, which demonstrates the precision of the experimental method used.

We proved the usefulness of the method for quantifying vascular permeability modifications caused by the influence of different substances, as well as for quantifying the extravasation of fluorescein–labeled albumin within the vascular bed. The fluorescence of different tissues can be compared with the fluorescence of plasma, and the data presented constitute a novelty from this point of view.

Our results highlight the importance and usefulness of the methods based on the analysis of fluorescence intensity at the level of the eye fundus, used in the ophthalmic practice to diagnose diseases with a modification of vascular permeability of the choroid and retina, diabetic retinopathy and macular degeneration, respectively, etc.

The stem cell stimulator complex Olimpiq® StemXCell SL has proven to be a useful choice of treatment for complications of diabetes associated with the extravasation of different blood components from the capillaries into the adjacent tissues, such as retinopathy or nephropathy.

As far as the reduction of blood glucose level with Methanandamide is concerned, we recommend this substance to be used in combination with an FAAH inhibitor (an enzyme which breaks down cannabinoids) for the treatment of early stages of diabetic retinopathy.

In the case of the animals which were given Alloxan, fewer Langerhans' islets were visible and they presented cellular necrobiosis, fibrosis and hyalinization, whereas in the case of the group which was not given Alloxan, the islets were intact.

We have noticed a certain degree of endocrine cell regeneration at the edge of the Langerhans' islets, which can explain the decreasing tendency of blood glucose level of the experimental animals.

10.09.2012

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