In vivo study of skin optical clearing in rats with alloxan diabetes of varying duration

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In the *in vivo* experimental and computational studies the effect of aqueous 70% glycerol solution on the skin of rats with model (alloxan) diabetes of different durations was studied, namely, the time dependences of the light attenuation coefficient in the skin of rats with model diabetes in vivo were obtained, the effectiveness and rate of optical clearing of the skin in vivo was assessed using OCT.

MATERIALS AND METHODS

Object of investigation

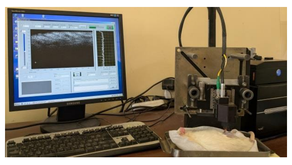
In vivo skin of white Wistar rats

Optical clearing agent (OCA) and RI (930 nm)

Aqueous 70% glycerol solution (1.4279)

Control group

Diabetic group



Protocol of *diabetes mellitus*

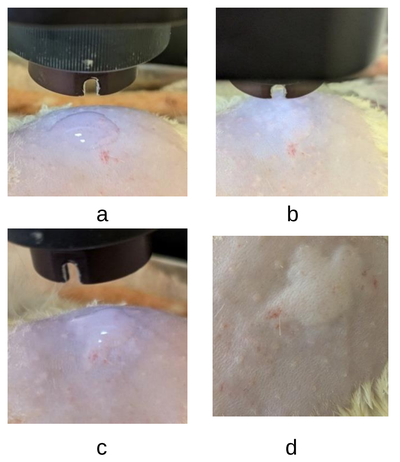
Diabetes mellitus was induced by injection of a single dose of alloxan (Acros Organic, Belgium) mixed with saline to experimental animals as 10 mg of alloxan per 100 g rat body mass.

Average level of glucose in blood

Day of measurement	Before injection	2 weeks after injection	1.5 months after injection	2 months after injection
Level of glucose in blood, mmol/l	5.3±0.7	35±5	25±6	25.4±3.1

OCT measurement

The Spectral Radar OCT System OCP930SR 022 (Thorlabs Inc., USA) with a wavelength of 930 nm was used to quantify the change in the optical properties of the skin. After hair removal from the mouse skin, a B-scan of the intact skin region with OCT was recorded. Then the solution was applied topically to the target skin area. Skin OCT scans were recorded every minute during the exposure of the skin area to the solution. The solution was removed each time before scanning and applied again after scanning.



The skin area under investigation with applied glycerol solution (a), during tomogram recording (b), after the solution application for few minutes (c), after measurements (d)

Data Processing

OCT scans were used to calculate the kinetics of light attenuation coefficient in the skin by approximating the dependence of the intensity of the reflected light $I(z,t)$ on the depth of the investigated region z of A-scan recorded at the time t by the equation:

$$I(z,t) = A_D \cdot \exp[-(\mu_t(t) \cdot z)] + y_0$$

The light penetration depth of the skin was determined from the coordinates, upon transition between which the signal intensity decreases by a factor of e (Δz = z1 - z2) before and optical clearing.

For estimation of characteristic time of skin optical clearing, the obtained time dependence of light attenuation coefficient in the skin was approximated by the equation:

$$\mu_{norm}(t) = \frac{\mu(t)}{\mu(t=0)} = A \cdot \exp\left(-\frac{t}{\tau}\right) + y_0 ,$$

where $\mu(t = 0)$ and $\mu(t)$ are the values of the light attenuation coefficient at the time $t = 0$ and t, respectively; A is the maximum degree of optical clearing of the sample, τ is the characteristic time of optical clearing of the sample, y_0 is the residual value of that can be achieved.

The efficiency of skin optical clearing was estimated from the kinetics of the light attenuation coefficient in skin as the ratio of the difference between the minimum and initial values of the light attenuation coefficient to its initial value:

$$OC_{eff} = \frac{\mu_{to} - \mu_{t\,min}}{\mu_{to}} \cdot 100\% .$$

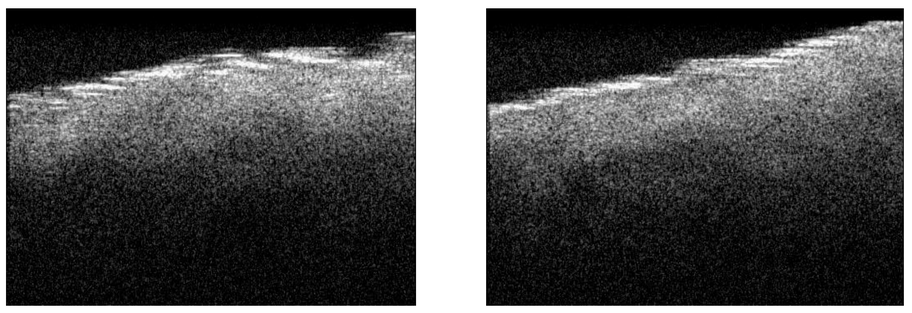
The glycerol diffusion coefficient (optical clearing rate) in the skin was restored from the kinetics of the of light attenuation coefficient in skin by minimizing the objective function:

$$f(D) = \sum_{i=1}^{N_t} \left(\mu_t^{teor}(D,t_i) - \mu_t^{exp}(t_i)\right)^2 ,$$

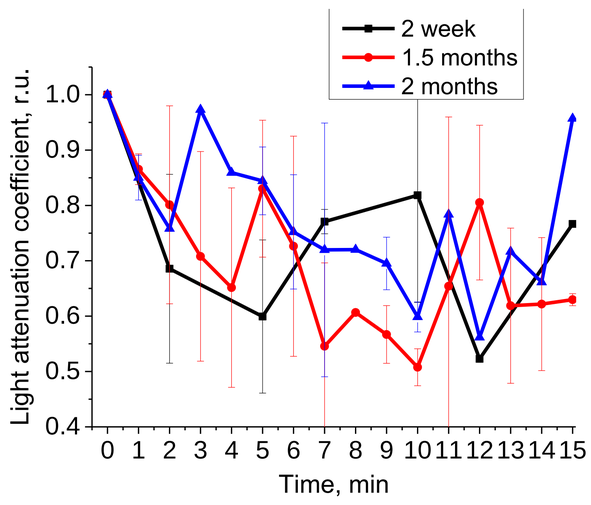
where $\mu_t^{teor}(D,t_i)$ is the calculated light attenuation coefficient in the skin model at a given value of D at time t, sec; $\mu_t^{exp}(t_i)$ - experimentally measured light attenuation coefficient in skin at time t, sec; N_t - number of experimental points in the kinetics of light attenuation coefficient in skin.

RESULTS

OCT tomograms of rat skin *in vivo* before (a) and after 5 minutes (b) of exposure to an aqueous 70% glycerol solution



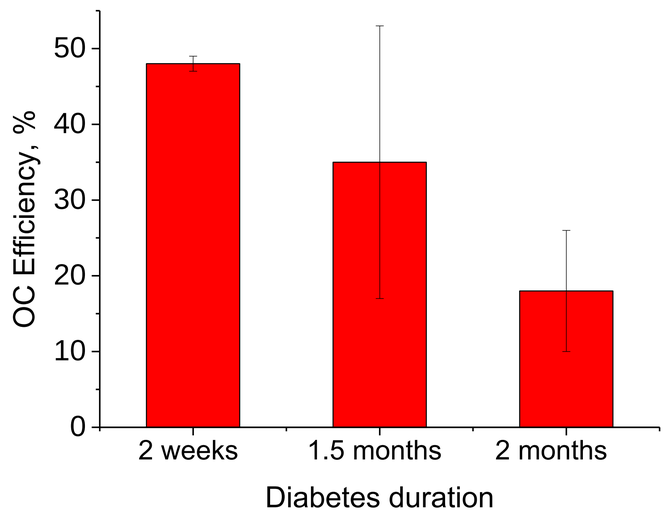
a b



Dependence of the light attenuation coefficient in the skin of rats with diabetes of varying duration on the exposure time to 70% glycerol solution

Values of glycerol diffusion coefficient in rat skin *in vivo* (D)and efficiency (OC_{eff}) of optical clearing of rat skin *in vivo*

Diabetes duration	2 weeks	1.5 months	2 months
D, cm^2/s	$(1.47±0.22)×10^{-6}$	$(1.09±0.72)×10^{-6}$	$(0.41±0.29)×10^{-6}$
OC_{eff}, %	48±1	35±18	18±8



CONCLUSION

The studies demonstrate a decrease in the rate of optical clearing of rat skin and a reduction in the efficiency of *in vivo* skin clearing as the duration of alloxan diabetes increased. The data suggest that with longer diabetes duration, the reverse effect (increased scattering) occurs more rapidly. However, the clearing process subsequently resumes because the solution on the skin surface continues to exert an osmotic effect; notably, the second stage of optical clearing is more effective in each case.

Acknowledgements

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