INDUCED SENSIBILIZED FLUORESCENCE OF NORMAL AND ATYPICAL CELLS AND TISSUES

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The problem of selective accumulation of fluorescent dyes by neoplastic formations is actual and has not been solved so far. Classic luminescent histo- and cytochemistry employs various fluochromes, including well-known sodium fluorescein. This dye presents a great interest due to its high fluorescence quantum output and increased accumulation by atypical cells in vitro et in vivo(I).

This article presents the studying of accumulation of sodium fluorescein(FLNa) in cells, organs and tumours of mice employing fluorescent analysis. The experiments were carried out on mice series C57Black and F1 with replanted Erlich’s tumour(solid form) and the WUT-5. A number of series of experiments were carried out, with 10 mice used in each group. 0.2 ml of FLNa were injected into retroorbital sinus(7.5 mg/kg and 75 mg/kg of animal weight).

The fluorescence intensity was evaluated in kidneys, liver, spleen, lungs and tumour 30 min., 1, 2, 3, 4, 6, 12, 24 hours after FLNa was injected.

The characteristic form of spectrum and fluorescence intensity of the same objects without FLNa injection served as reference sample.

The fluorescence spectra were registered by the fluorescence spectrophotometre at $\lambda_{ex}=441$ nm, fluorescence intensity was measured at $\lambda_{f1}=520$ nm.

The pieces of tissues to be researched were placed into screen window, which permitted to maintain the same standard dimensions of samples. Fluorescence intensity was expressed in relative units. The obtained data was processed according to the variational statistics methods.
The results of researches showed that with FlNa being injected at a dose of 7.5 mg/kg or less the luminescence of some tissues (including tumours) happened to be comparable with the tissues' own characteristic fluorescence in the fluorescein spectrum area.

The fig. I shows results of determining of relative fluorescence intensity of various organs and Erlich's tumour upon the time of animals' injection with 75 mg/kg of FlNa, as well as the area of fluorescence of organs themselves and of various tumours (10 - 120 rel. un.).

The intensity of the tumour fluorescence exceeded that of the organs excepting kidney - organ, that takes FlNa out of organism. Kidney is followed by the liver, lungs, spleen by their stage of dye accumulation. That's why in the later experiments the stage of FlNa accumulation by the tumour was evaluated in comparison with the liver.

The intensity of tumour fluorescence in 1, 2, and 3 hours after the dye was injected exceeded that of the liver 13, 11 and 9 times accordingly.

The analysis of spectra shows that 1 hour after the FlNa injection is an optimal time for observation of dye selective accumulation by mice' tumours.

Similar character of FlNa accumulation was noticed with the WUT-5, a tumour of different genesis compared with the Erlich's. In 1 hour after FlNa injection the fluorescence intensity of the WUT-5 and that of animal's liver were: 4726 and 543 rel. un. respectively, that of Erlich's tumour and liver - 3376 and 240 rel. un. respectively.

Evidently there's such a moment when the fluorescence intensity of tumour is maximum. Most probably that these parameters are related to the type of tumour, its localization, character of the organism's metabolism, etc. There's some information about the importance of membrane contacts' state between cells when FlNa is being accumulated or extracted by cells: in case slot contacts are defected, the dye between cells is not transported (2).

Cell suspensions in cultural medium were used for the analysis of dye accumulation by atypical cells of the above-mentioned tumours. The cells' concentration and their viability were determined before the experiment started with thripane-blue dye. FlNa solution was injected into the medium with cells in portion of 0.1 ml of 0.05% - solution per 50 mg of medium.
The cells were kept in thermostat for 30 min. with further sedimentation at 1000 rev/min and were washed off the dye then with Chenx's solution. The fluorescence intensity of the dye in cells in the area of about 520 nm was measured with photometric attachment on luminescent microscope, with the He-Cd lazer irradiation of $\lambda = 441.6$ nm being an exciting light.

When estimating the cell fluorescence of various lines, it was discovered that each type of atypical cells is characterized by its own parameters of FlNa accumulation and considerably exceeds sensibilized fluorescence of normal cells.

However, mechanism of selective accumulation of dye by atypical cells and tumour tissue still doesn't present a completely clear picture.

So we make a conclusion that at a dose of 75 mg/kg of FlNa in 1 hour after mice were injected the tumours' fluorescence intensity becomes an order of magnitude as much as that of other organs (liver, lungs, spleen), which permits to differentiate the tumour from tissues of other organs.

References

Dependence of relative fluorescence intensity of various organs and Erlich's tumour upon the time after FlNa injection to the animals: kidney(x), tumour(circle), liver (o), lung(■), spleen(▲), boundary of maximum organs' own fluorescence and various tumours is marked with dotted line.