LASER-INDUCED FLUORESCENCE OF RAT ENAMEL AFTER MATERNAL ADMINISTRATION OF ANTIRETROVIRAL DRUGS – *IN VITRO* STUDY

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Abstract: The teeth from female rats, treated with *cyclophosphamide* and *indinavir* during pregnancy, and their 1- and 15-days old newborns were studied by means of the laser-induced fluorescence using the 407nm excitation source. The samples were also examined by morphological and histological. It follows from our studies that *cyclophosphamide* treatment is more damaging for teeth ontogenesis than treatment with *indinavire.* The results of laser-induced fluorescence measurements revealed compatibility with histological estimations improving usefulness this method in the analysis of rat dental tissue after maternal administration of antiretroviral and anti-neoplastic drugs.

Introduction

Acquired Immunodeficiency Syndrom (AIDS) is caused by Human Immunodeficiency Virus (HIV) infection. In therapy of HIV-positive patients antiretroviral drugs such as *indinavir* and *azidothyminide* are used [1,2]. Efficiency of treatment depends on systematic therapy which is recommended also to pregnant women. However pharmacotherapy is never free from side-effects. It is known that antiretroviral drugs have toxic influence on different internal organs [3-8]. Thus an experiment estimating influence of antiviral treatment during pregnancy on tooth development of newborn rats has been performed. Changes of development within the dental organ and alveolar process bones in rats treated with *indinavir* are compared to alterations observed after application of *cyklophosphamide* – standard anti-neoplastic drug.

The aim of this work is *in-vitro* studies of changes in enamel and dentin after maternal administration of antiretroviral and antineoplactic drugs samples by means of the laser induced fluorescence (LIF) [9-15]. LIF method permits to detect endogenous fluorophores which concentration and kind depends on tissue states. Therefore spectrofluorometry can give diagnostic information often unobtainable with standard morphological and histological methods. Fluorescence of endogenous as well as exogenous dyes are applied in photodynamic diagnosis (PDD) an photodynamic therapy (PDT).

Materials and Methods

The study was performed in Wistar female rats (body weight ±200g). Animals came from Central Experimental Animal Quarters of Silesian Academy of Medicine. After fertilization rats were administered antiviral drug – *indinavir* (per os, using intragastric probe, 200mg/kg) in two series: on the 10^{th}, 11^{th} and 12^{th} day and on the 16^{th}, 17^{th} and 18^{th} day of pregnancy. Antineoplastic drug – *cyklophosphamide* was administered in a single dose of 5mg/kg on the 10^{th} day of pregnancy. Rats from control group were administered distilled water in volume of 5ml/kg using intragastric probe.

The teeth were sampled from female rats, treated with *cyclophosphamide* and *indinavir* during pregnancy, and their 1 and 15 days old newborns. The teeth were mechanically cleaned from soft tissues before ultrasonical cleaning in filtered and purified water. After their preparation, samples were air-dried for 24 h before fluorescence measurements. All samples were spilled into groups as regards kind of treated drugs, and age of newborn rats.

Laser-induced fluorescence of the enamel and dentin in upper incisors was *in vitro* measured. Studies of LIF in particular samples were performed using the measurement system presented in Figure 1. Fiberoptical fluorescence analyzer was constructed basing on BIOSPEC technology and LESA 6 software. To induce the emission of endogenous fluorophores contained in dental tissues the semiconductive GaN laser (wavelength 407 nm, initial energy 10 mW) was used [14]. The fluorescence measurements were performed putting optical fibre to inner surface of the upper incisor. The fluorescence spectra were recorded within the range of 400-800nm. Each tooth enamel and dentine fluorescence was measured 10 times and obtained results were averaged.

The measurements were extended by morphological and histological examinations which were based on

ISSN: 1727-1983

microscopic estimation and comparison of slides previously stained with standard H-E (hematoxilin-eosin) technique.

Ethical approval was obtained from the Ethical Committee of the Silesian Medical University (No 47/03).

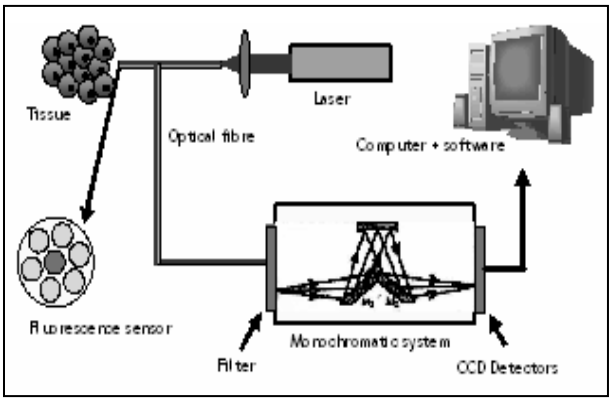


Figure 1: The diagram of measurement system.

Results

Figures 2 - 6 present results of laser-induced fluorescence from study samples (the excitation peak at 407nm is thought to be reference signal). One can see that after activation of dental tissues with laser light 407nm emission spectrum with peak located near 500nm maximum is observed.

It follows from Figure 2 that intensity of the dentin emission is several times higher than enamel one. The dentin emission spectra for samples from control group is about 6 times higher than maximum of enamel emission spectra. There are no significant differences between emission spectra of 1- and 15-days old newborn rats for enamel as well as dentin.

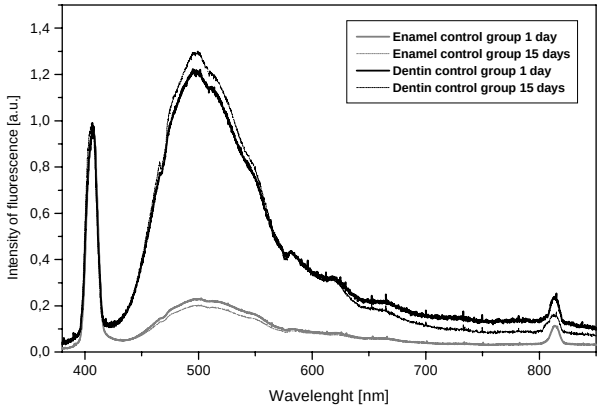


Figure 2: Enamel and dentine spectral characteristic of 1 and 15 days old newborn rats from control group**.**

Influence of maternal administration of *indinavir* and *cyclophosphamide* on dental tissues is manifested in Figure 3 and 4. The fluorescence intensity of dentin increased several times under *cyclophosphamide* while the administration of *indinavir* caused some decrease of fluorescence (Figures 3). The drug effect on the fluorescence spectra of enamel are markedly smaller and in the case of *indinavir* even negligible (Figures 4). However the fluorescence measurements performed for dental tissues of mothers treated with *cyclophosphamide* showed significant changes in dentin as well as enamel (Figure 5).

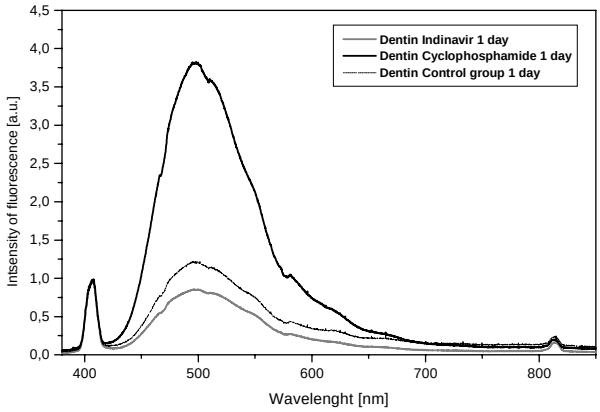


Figure 3: Dentin spectral characteristic of newborn rats taken from mothers treated with *cyclophosphamide* and *indinavir.*

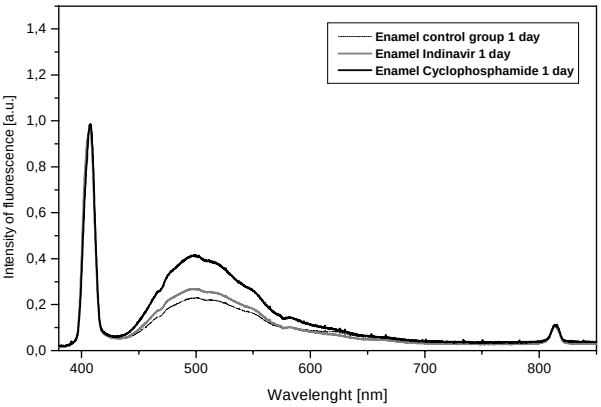
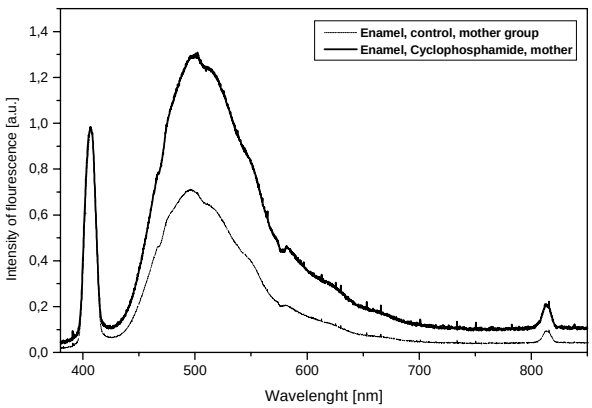


Figure 4: Enamel spectral characteristic of newborn rats taken from mothers treated with *cyclophosphamide* and *indinavir.*



ISSN: 1727-1983

Figure 5: Enamel spectral characteristic mother rats treated with *cyclophosphamide* and control group.

It should be noted from Figure 6 that fluorescence intensity of dentin for 15-days in comparison with 1-daysold newborn rats after maternal administration of cyclophophamide decreases. This behaviour of fluorescence spectra seems to indicate some regeneration of dentin tissue.

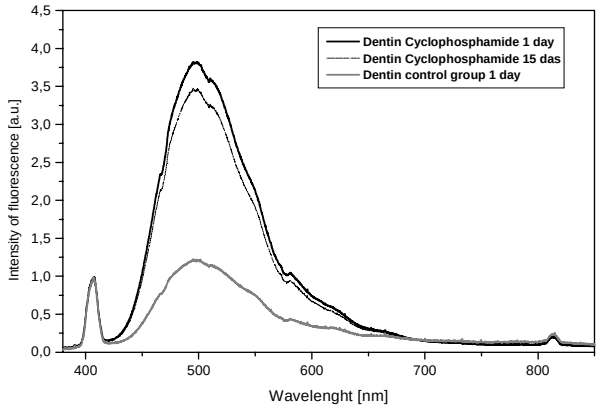


Figure 6: Dentin spectral characteristic of control group and 1- and 15-days old newborn rats taken from mothers treated with *cyclophosphamide*.

To better insight into influence of administrated drugs on rat teeth ontogenesis the morphological studies were done. The microscopic images of the dental tissues after maternal administration of indinavir and cyclophospamide in comparison to control group are shown in Figures 7 - 9.

The classical morphological status of rat teeth ontogenesis is presented in Figure 7.

The histological estimation of dental tissues after maternal administration of indinavir reveals odonto- and enameloblasts, within supranuclear areas of mentioned cells' cytoplasm microalveolar (Figure 8). Despite local vacuolization of some cells generating dentin and enamel, no changes in predentin and dentin regularity were noticed. In the 15 days old rat group no changes during development from the tooth bud to the finished inscisors dentition were observed.

Figure 9 shows that the changes in dental tissues under the cyclophosphamide are the biggest. Within the all time ranges, in blastic cells – odontoblasts, enameloblasts and cementoblasts (in smaller extent) alterations of regular cell location/placement were observed, and some of them presented necrotic or apoptotic death. In all blastic cells, especially in enamloblasts and odontoblasts sampled from 15-day-old rats, strong micro- and mesoalveolar cytoplasm vacuolisation was observed. Layers of deposited predentin, dentin as well as ceement were definetly thinner than in parallel control groups. The system of dentin channels was not regular.

Discussion

It is interesting to note that results of laser-induced fluorescence measurements are compatible with histological estimations.

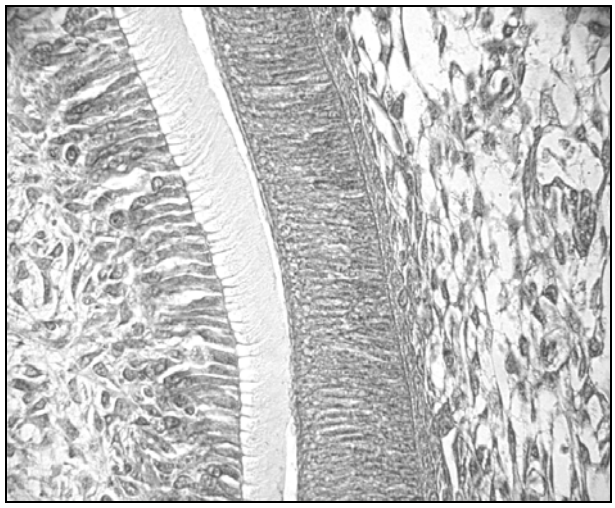


Figure 7: Microscopic image of dental tissues newborn rats (control group).

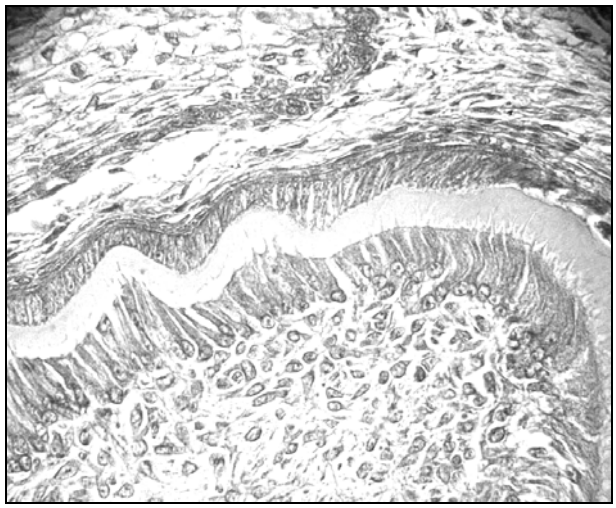


Figure 8: Microscopic image of dental tissues newborn rats taken from mother treated to *indinavir*.

Fluorescence spectra of dental tissues for newborn rats taken from mothers treated with *indinavir* show small changes, especially for the enamel. The same result can be derived from microscopic images. *Indinavir* does not cause significant alterations in dental ontogenesis which could be observed in light microscope with classical H-E staining.

However *Cyklophosphamide* causes obvious changes in structure of enamelo- and odontoblasts cytoplasm during the whole experiment. Those changes influence efficiency and quality of both dentin and cement synthesis. The laser-induced fluorescence is sensitive for changes in this dental tissue. Abnormal increase of fluorescence intensity confirms negative

ISSN: 1727-1983

effect of administration anti-neoplastic drug on the rat teeth ontogenesis.

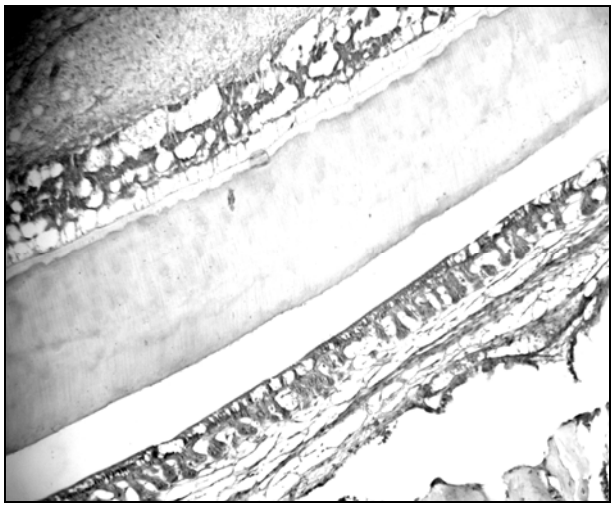


Figure 9: Microscopic image of dental tissues newborn rats taken from mother treated to *cyclophosphamid.*

The examination of antiviral and anti-neoplastic drug influence on the tooth and enamel development seems to be interesting not only because of it's scientific aspects, but is also giving us the possibility to observe the side-effects of the AIDS therapy during pregnancy.

Conclusions

Our initial quantitative analysis showed usefulness of laser-induce fluorescence method in the analysis of rat dental tissue after maternal administration of antiretroviral and anti-neoplastic drugs.

It follows from our results that progeny mothers treatment with *cyclophosphamide* is more damaging for teeth ontogenesis than treatment with *indinavire.*

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ISSN: 1727-1983