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An Assessment of the Impact of Zidovudine on Rat Teeth Ontogenesis with Quantitative Laser Induced Fluorescence and an Histomorphological Analysis

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Abstract

Objectives: It is known that many conditions may impact on dental organ development at foetus or new-born stages. The most important influence in this case is caused by genetic predispositions but also by drugs delivered prenatally and postnatally to the organism. The goal of this research was to find if the applications of *Zidovudine* to mothers' organisms of rats can caused some abnormalities in dental organs of new-borns.

Methods: The study was performed on 20 Wistar female rats and their 36 new-borns bred. The group of adult pregnant rats was divided on two sub-groups: with and without *Zidovudine* receiving. The influence of *Zidovudine* administered was assessed by using the quantitative laser-induced fluorescence and compared with the group without *Zidovudine* receiving as a reference subjects.

Results: In result the dependences in *Zidovudine* receiving were obtained and the changes in tissues were detected by use the laser fluorescence method. Moreover the differences between groups with and without *Zidovudine* are obtained in morphological results. The stronger impact of drug was obtained for mothers rats than new-borns.

Conclusions: All obtained results show usefulness laser fluorescence in dental organ assessment.

Keywords: Zidowudyne; Teeth ontogenesis; Fluorescence; Drugs; Dental; Laser; AIDS

Abbreviations

NRTI: Nucleoside Reverse Transcriptase Inhibitors; AZT: Azidothymidine; AIDS: Acquired Immune Deficiency Syndrome; QLF: Quantitative Laser-Induced Fluorescence; IFZ: Root Fluorescence Index; IFD: Tooth Fluorescence Index; NADH: Reduced Nicotinamide Adenine Dinucleotide; NADPH: Reduced Form Of The Cation Dinucleotide Phosphate; BMD: Boby Mass Density; TRAP: Tartare-Resistant Acid Phosphatase Promoter

Introduction

Dental organ development is genetically determined and strictly depends on the conditions in which the foetus and, then the new-born grow. It may also be affected by drugs administered prenatally to mothers and postnatally to the growing organism. These include genotoxic medicines, namely, nucleoside reverse transcriptase inhibitors (NRTI) used for antiretroviral therapy, inter alia, in the treatment of HIV infections. One of the first NRTI derivatives is *Zidovudine*, also referred to as *Azidothymidine* (AZT).

Zidovudine is a derivative of thymidine, in which the hydroxyl group in position 3 is replaced by an azide group. It acts by blocking the process of HIV replication in infected cells. The drug, whose activity consists in blocking the HIV reverse transcriptase enzyme, inhibits DNA chain synthesis, and thus slows the progression of the effects of infection [1]. Until the early 1990s AZT was used in "monotherapy" treatment. An increase in the doses potentiated side effects with minimal improvement in efficiency. "Multidrug" therapy was introduced in the mid-1990s. Such treatment involved the simultaneous administration of no less than three antiretroviral drugs from the following three groups: nucleoside reverse transcriptase inhibitors (e.g. AZT), non-nucleoside reverse transcriptase inhibitors, and protease inhibitors.

Azidothymidine is a well-known HIV drug. It is used to treat the early stages of AIDS (Acquired Immune Deficiency Syndrome). Long-term administration of the drug prolongs the life of the infected patient, improves his comfort, and increases the number of lymphocytes. However, it causes severe side effects and is less effective in patients over time. The side effects that may occur as a result of this preparation include anaemia, bone marrow impairment with neutropenia and thrombocytopenia, as well as myopathies and skin lesions [2-4]. Long-term use results in AZT resistance, as well as in skin disorders and myopathies, expressed in the development of HIV mutants [5,6].

Until now there have been no significant reports on the impact of antiretroviral therapy on dental organ development. Only a few studies have commented on the impact of nucleoside reverse transcriptase inhibitors on the embryo/foetus in animals and humans [7,8].

This fact encouraged the authors of the following study to conduct research on antiretroviral therapy of pregnant females based on an animal model, with the aim of investigating the effects on their teeth and the dental organs of their new-borns. The aim of the study was to assess the impact of *Zidovudine* administered to rat mothers on their teeth and on the development of the dental organ during ontogenesis

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in their offspring, using quantitative laser-induced fluorescence (QLF) and the morphological method. The simultaneous application of two different assessment methods presents greater opportunities for analysing the quantitative composition and structure of particular dental organ tissues.

Material and Methods

The study was performed on 20 Wistar female rats (bodyweight 200 g-220 g) and their 36 new-borns bred at the Central Experimental Animal Farm of the Medical University of Silesia. The rats were provided with free access to food (a GLM granulated blend) and water. During the experiment the animals were housed in plastic cages at a temperature of 21 ± 2°C, with artificial light (in a light cycle-controlled room - day/night: from 8.00 hr to 20.00 hr/20.01 hr to 7.59 hr) and approximately 70% atmospheric humidity.

Prior to fertilization, the female rats were housed for seven days in a room meeting the above-mentioned conditions of lighting, temperature and humidity. Fertilization was confirmed by the presence of sperm in vaginal secretions using the May-Grünwald-Giemsa method. The fertilized females were transferred to separate cages until parturition. One unfertilized female was removed from the experiment. Pregnant female rats were grouped into the following: study group I (10 female rats) - a group in which *Zidovudine* was administered, and study group II (9 female rats), which did not receive *Zidovudine*.

Zidovudine (Retrovir, Glaxo-Wellcome) was administered orally to the female rats of study group I (*Zidovudine* group) using gastric feeding tube at a dose of 200 mg/kg in an aqueous suspension of 2 ml/kg of body weight. The solution was prepared with a drug ampoule containing 200 mg of the active substance. The drug was administered to pregnant rats once a day for 10 days between the tenth and the nineteenth day of pregnancy. Research group II female rats were treated with distilled water using a gastric feeding tube in the same volume of 2 ml/kg body weight.

The rat litters in group II were more numerous than group I. Thus, only some of the offspring of the female rats were used for further study. Eventually, 18 one-day-old rat new-borns from females that had been administered *Zidovudine* were used in the study - these formed study group III and other 18 one-day-old rat new-borns from females that had not been administered *Zidovudine* formed study group IV.

The rats involved in the experiment – both female and their progeny (group I, II, III and IV) were anesthetized with inactin and decapitated on the first day after birth. Hard tissues consisting of mandible and maxilla with teeth and tooth buds were set aside for further preparation procedures. The upper and lower incisors of adult female rats were used in the study (group I and II) together with the erupting upper and lower incisors of rat new-borns (group III and IV).

The impact of *Zidovudine* administered maternally on dental tissue was assessed both in mothers (group I) and in their progeny on their first day of life (group III) using QLF. The data obtained while assessing the teeth of female rats in group II (no *Zidovudine* administered) and the teeth of group IV new-borns (no *Zidovudine* administered) served as a reference standard.

The upper and lower incisors, which had been dissected, were cleaned with ultrasonic waves in a 5.25% sodium hypochlorite solution, and then in distilled water. The teeth were polished to remove soft tissue residue from the enamel surface, and then dried for 24 hours. A BIOSPEC fluorescence analyser based on an A/D card registered by LESA-6 software was used to measure and analyse laser-induced fluorescence.

A GaN semiconductor laser with a 407 (nm) wavelength and 10 (mW) output power was used to induce enamel and dentin fluorescence. The excitation laser beam was directed onto teeth by applying a fluorimetric sensor (catheter) to the surface of the tested tooth. The emission of the crown was examined at a distance of 3 (mm) from the surface of the crown. The emission of the root was tested at a distance of 2 (mm) from the surface of the tooth root. A QLF emission signal was collected from a circular surface of a 1.8 (mm) diameter sample and transferred using six optical fibre bundles to a monochromator with 1024 CCD detectors into the computer memory.

Crown and root fluorescence were tested ten times on each tooth. The test data were normalized to an excitation peak of λ= 407 nm.

Laser induced fluorescence was assessed by examining the maximum amplitude of the emitted light, the surface area under emission peak, and the crown and root fluorescence index (henceforth: IFZ) – respectively for rat mothers (IFZCm, IFZRm) and rat newborns (IFZCn, IFRn) according to the following formula:

$IFZCm=\frac{FcII}{FcI}$, where *FcII- fluorescence of the crown in group* II

FcI- fluorescence of the crown in group I

$IFZCn=\frac{FcIV}{FcIII}$, where *FcIV- fluorescence of the crown in group* IV

FcIII- fluorescence of the crown in group III

$IFZRm=\frac{FrII}{FrI}$, where *F rII- fluorescence of the root in group* II

F rI- fluorescence of the root in group I

$IFZRn=\frac{FrIV}{FrIII}$, where *F rIV- fluorescence of the root in group* IV

F rIII- fluorescence of the root in group III

The tooth fluorescence index (IFD) was also assessed for all study groups, i.e. group II (IFDII) group IV (IFDIV) and group I and III (Zidovudine groups) - (IFD*I*) and (IFD*III*), respectively, according to the following formulae:

$IFDII=\frac{F\,rII}{F\,cII}$, where *F rII- fluorescence of the root in group* II

F cII- fluorescence of the crown in group II

$IFDIV=\frac{F\,rIV}{F\,cIV}$, where *F rIV- fluorescence of the root in group* IV

F cIV- fluorescence of the crown in group IV

$IFDI=\frac{F\,rI}{F\,cI}$, where *F rI- fluorescence of the root in group* I

F cI- fluorescence of the crown in group I

$IFDIII=\frac{F\,rIII}{F\,cIII}$, where *F rIII- fluorescence of the root in group* III

F cIII- fluorescence of the crown in group III

Following the QLF analysis, teeth of rat new-borns in group III and group IV were decalcified together with the alveolar bone frame using the Jenkins method and were then fixed with Carnoy's solution. After decalcification the teeth were processed using the following: 96% alcohol for 3 hours, absolute alcohol for a day, and absolute alcohol and xylene for 1 hour. The dental sections were washed three times in xylene after one hour and then embedded in paraffin for 15 hours at 58°C. Paraffin blocks were cut with a rotary microtome into series of 7 µm sections, placed on protein-coated slides, and then stained with haematoxylin and eosin. Histological specimens were examined with a

Polyvar-Reichert light microscope, coupled with an Exwave Had Sony SSC-DC58AP video camera with a 24 bit RGB colour Frome-grabber (resolution 768 × 576 pixels). A 3.2 million pixel Nikon Coolpix 990 digital camera connected via a C connector to the microscope was used to obtain reference pictures. The photographs were saved to tiff format, thereby allowing for lossless compression.

The results were analysed using Statistica 5.0 software. The results presented in the tables were obtained using a separate variance Student's t-test (in a normal distribution) and the U Mann-Whitney test (in the absence of normal distribution). Approval for the study was obtained from the Ethics Committee of the Silesian Medical University (No 47/03).

Results

Laser-induced fluorescence

Fluorescence of the crowns and tooth roots for all four study groups are presented in figures and tables

The crown fluorescence emission spectrum for teeth in group III and IV ranges from 425 nm to 700 nm (Figure 1). Crown fluorescence intensity at the local maximum in group III is located near 485 nm, whereas in group IV it is close to 500 nm and amounted to 0.24 and 0.19, respectively. The difference ($p<0.01$) was statistically significant. The average value of the total light emitted by the tooth crown in group III is significantly greater than in group IV ($p<0.05$) (Table 1 and Figure 1).

The range emitted by tooth crowns in group III and IV was 425–700 nm. The maximum amplitude of the emission peak in both groups was 500 nm and amounted to 0.81 in group III, and 1.06 in group IV Figure 2). The maximum and the total volume of light emitted by group III was significantly lower compared with group IV ($p<0.001$) (Table 2 and Figure 2).

The maximum emission peak and the overall intensity of light emitted by the crowns of teeth in group I were significantly greater than in the case of group II (Table 3).

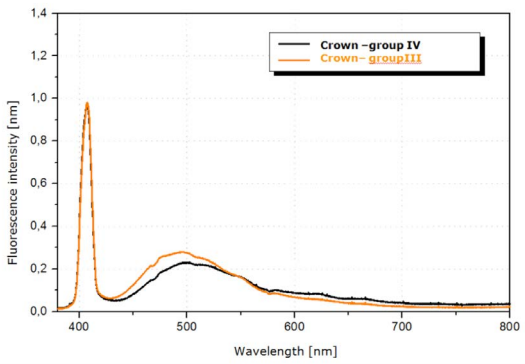

Figure 1: Fluorescence of a tooth crown in rat newborns (group III and IV).

Rat newborns		Surface under the emission peak	Maximum amplitude of the emission peak (relative unit)
		average ± SD	average ± SD
1	Group IV	19.39 ± 0.72	0.19 ± 0.012
2	Group III	22.36 ± 1.44	0.24 ± 0.014
		$p_{k\text{-}zyd}< 0.01$	$p_{k\text{-}zyd}< 0.001$

Table 1: Results of QLF of tooth crowns in rat newborns (group III and IV).

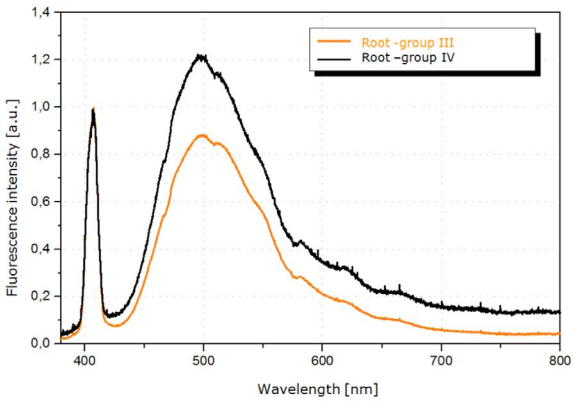

Figure 2: Fluorescence of tooth roots in rat newborns (group III and IV).

Rat newborns		Surface under the emission peak	Maximum amplitude of the emission peak (relative unit)
		average ± SD	average ± SD
1	Group IV	105.45 ± 2.57	1.06 ± 0.032
2	Group III	82.27 ± 5.35	0.81 ± 0.06
		$p_{k\text{-}zyd}< 0.001$	$p_{k\text{-}zyd}< 0.01$

Table 2: Results for QLF of tooth roots in rat newborns (group III and IV).

The range of light emitted by the two groups ranged from 420 nm to 700 nm (Figure 3).

The fluorescence of tooth roots in group I and II ranged from 416nm to 700 nm. The maximum intensity of light emitted by the teeth in group I ranged from 1.85 and was significantly lower compared to group II, as was the total fluorescence intensity (Figure 4).

The lowest Zidovudine fluorescence index / IFZ / was observed in the tooth crowns of rat newborns and their mothers, and levels were similar for all wavelengths. The fluorescence indexes of roots in both newborns and their mothers increased as the waves emitted increased. The highest coefficient indicating the greatest difference between group II and I was noted for the roots of mothers and amounted to 4 (Figure 5).

The tooth fluorescence index for group III was lower than for group IV within an emission range of waves shorter than 575 nm and longer than 650 nm. The tooth fluorescence index in group I was lower compared to group II within the entire range of emitted light spectra (Figure 6).

Morphological method

An examination of the teeth of rat newborns in group IV (no *Zidovudine* administered) revealed regular and well-structured cell systems of well-developed amelogenic, dentinogenic, and cementogenic organs. The organs were formed by cylinder shaped cells adhering to each other, with oval nuclei, located parabasally on the same level (Figure 7). The formative cells of the amelogenic and dentinogenic organs started synthesis and secretion of predentin and pre-enamel, which were regularly deposited on the secretory surfaces of these cells. In the stoma of amelogenic and dentinogenic organ, formed from numerous, polyhedral or stellate dendric cells, capillaries filled with red blood cells were visible.

In the teeth of rat new-borns from group III (administered *Zidovudine*), in all types of formative cells, in particular in a substantial number of odontoblasts, numerous bright and small vacuoles were present in the top parts the cytoplasm. The cells themselves were gradually losing their regular, structured layer system some of them had pyknotic nuclei, or an excessively eosinophilic cytoplasm. Dentin and enamel deposited on the surfaces of secretory cells repeated the disturbed system of the cells they were secreted from (Figure 8). The morphological organization of the dentinogenic and amelogenic organ did not deviate from that observed in group IV animals.

Rat newborns		Surface under emission peak	Maximum amplitude of emission peak (relative unit)
		average ± SD	average ± SD
1	Group II	62.22 ± 1.86	0.62 ± 0.023
2	Group I	123.45 ± 2.77	1.23 ± 0.062
		$p_{k\text{-}zyd}$< 0.001	$p_{k\text{-}zyd}$< 0.001

Table 3: Results for QLF of crowns in female rats (group I and II).

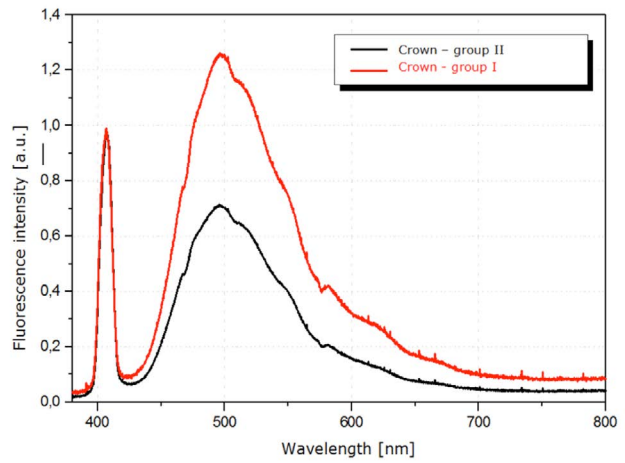

Figure 3: Fluorescence of tooth crowns in female rats (group I and II).

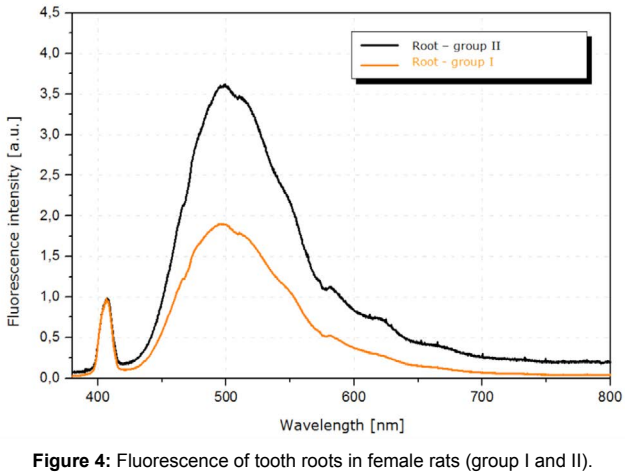

Figure 4: Fluorescence of tooth roots in female rats (group I and II).

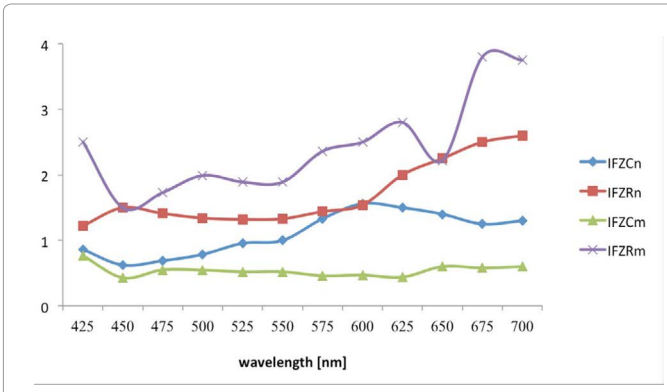

Figure 5: Zidovudine fluorescence index /IFZ/.IFZCn Zidovudine fluorescence index of the crown in newborns;IFZRn - Zidovudine fluorescence index of the root in newborns; IFZCm- Zidovudine fluorescence index of the crown in rat mothers; IFZRm- Zidovudine fluorescence index of the root in rat mothers

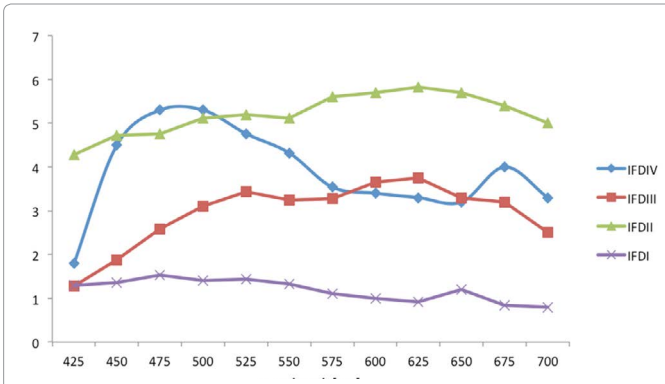

Figure 6: Tooth fluorescence index /IFD/.IFD IV-Tooth fluorescence index in group IV IFD III-Tooth fluorescence index in group III; IFD II-Tooth fluorescence index in group II; IFD I-Tooth fluorescence index in group I.

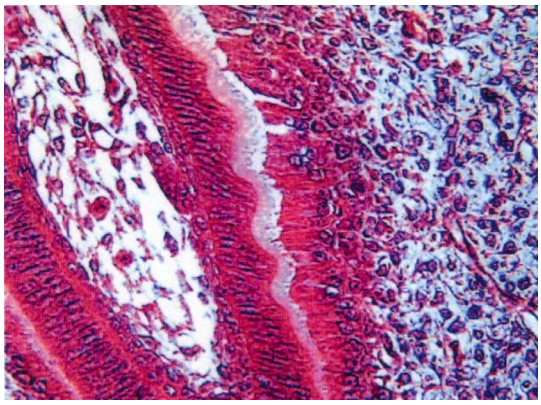

Figure 7: Microscope image of a tooth of a 1-day-old rat newborn of group IV (not receiving zidovudine); HE staining; Magnification of 400 X.

Female rats		Surface under the emission peak	Maximum amplitude of the emission peak (relative unit)
		average ± SD	average ± SD
1	Group II	36.22±1.86	3.5±0.026
2	Group I	186.08± 2.77	1.85 ± 0.012
		$p_{k\text{-}zyd}$< 0.001	$p_{k\text{-}zyd}$< 0.001

Table 4: Results for QLF of tooth roots in female rats (group I and II).

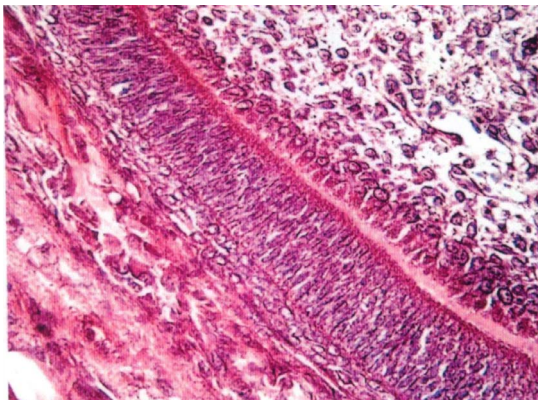

Figure 8: Microscope image of a tooth of a 1-day-old rat newborn of group III (receiving zidovudine); HE staining; Magnification of 400 X.

Discussion

The fluorescent properties of molecules present in the different tissues of living organisms were used to evaluate and monitor their actual metabolic activity, determine the redox status, oxygen concentration in tissues, mitochondrial insufficiency as well as assess the degree of bone tissue maturity, the presence of mineral compounds in teeth and detect microorganisms in living tissues [9-14]. Knowledge of the status of the above characteristics of an organism helps ensure early detection of subclinical pathologies, including early detection of premalignant and neoplastic changes [10,15-20] and differential diagnosis of different types of pathological changes [9,19]. It can also be used to assess the impact of drugs on tissues [21,22], distinguish between healthy body tissues and dysplastic lesions [19], evaluate tumour lesion demarcation [19,23,24] and provide early detection of initial caries in teeth [12,24-27]. Apart from its possible application in diagnostics, fluorescence also serves as an important guide in long-term evaluations and monitoring of spontaneous or treatment-induced changes in tissues, including as a method for monitoring the carious spot in teeth and its remineralisation [12,13,24-31], monitoring the effects of antibiotic therapy and phototherapy in bacteriological skin diseases [13], as well as in photodynamic treatment (PDT) of neoplasms [28] and precancerous conditions [20,29].

The fluorescent properties of teeth have been known since 1927, when Bommer discovered the orange and red fluorescence of teeth under a Wood's lamp. Since that time, researchers have been interested in conducting studies of the fluorescence of healthy, post-treatment, post-whitening, or decayed teeth [12,13,24-27,30,31]. However, few studies have assessed the impact of drug therapy on the dental organ and its development. According to Adeyemi, laser-induced fluorescence of teeth is more sensitive than spectrophotometry [31]. Furthermore, in contrast to histological studies, this is a non-invasive diagnostic method, which unlike histological studies allows for an objective, reproducible assessment of the examined area [19]. The study attempts to assess the impact of *Zidovudine* on crown and root fluorescence in the teeth of one-day-old rat new-borns and their mothers compared to the mothers and their offspring in the control group (Table 4).

The study indicated that the total fluorescence intensity of crowns in the rat new-borns in the research group in the first day of life was greater than in the case of the controls, whereas the maximum emission amplitude of the study group shifted approximately 20 nm towards shorter wavelengths (Table 1 and Figure 1). One of the factors affecting the nature of the emission spectrum was the kind and amount of natural fluorophores present in the test tissue [9-31]. The increase in fluorescence intensity in the range of 450-500 nm in length may originate from NADH (reduced nicotinamide adenine dinucleotide) or NADPH (reduced form of the cation dinucleotide phosphate) with an emission peak at 480 nm [9,10,13], shifted towards the blue light as compared to the control group, the maximum emission for which was 500nm. NADH / NADPH are among the indicators of tissue redox status, and the increase in their concentration may indicate a decrease in its oxygenation and thus local hypoxia. The increase in fluorescence intensity in the NADH field may be due to the role played by Zidovudine in the formation of mitochondrial dysfunctions and the occurrence of cell oxidative stress [32]. Increased tooth crown fluorescence in rat newborns in the study group compared to the controls may also result from a large number of type I collagen fibres in this tooth surface, the fluorescence range of which coincides with the results obtained in the following study [13,33,34].

A histomorphological evaluation of crowns in one-day-old rat newborns in the control group also indicated changes in the amelogenic and dentinogenic organ compared to the controls. The altered fluorescence of the area was due to the disturbed, unclear nucleus and cytoplasm structure, the presence of pyknotic nuclei and local necrosis within the dental organ (Figures 7 and 8).

The study demonstrated that the tooth roots of rat new-borns in the control group were characterised by significantly lower total and maximum fluorescence intensity compared to the control group (Tables 2 and Figure 2). The results of the histomorphological evaluation showed that cement organs in both groups triggered collagen synthesis (Figures 7 and 8). The mineralization of the basic intercellular substance began simultaneously with the formation of collagen fibres. The mineralisation of teeth and bone tissue depends inter alia on calcium-phosphate metabolism. A review of the literature indicates that bone tissue turnover is modified by reverse transcriptase inhibitors, which, among other things, reduces the BMD (Boby Mass Density) [9,10,35,36]. *Zidovudine* stimulates RANKL - dependent osteoclastogenesis by expression of TRAP (tartare-resistant acid phosphatase promoter), and thus increases osteoclast activity and bone density loss (BMD) [32]. By analysing skeletal development in rats Drzazga et al. have demonstrated a significantly lower concentration of calcium in the mandible in 7 and 28-day-old animals treated with indinavir compared to the control group [22]. According to Emami, et al. [33], the loss of tooth mineral manifests itself in a decrease in fluorescence intensity, whereas Angmar - Mansson observed that 0.15kg.m mineral loss reduces the maximum fluorescence emission by 10% [12]. The decrease in the fluorescence intensity of tooth roots in the control group compared to the controls can be explained by the smaller amount of minerals in rat new-borns treated with *Zidovudine*.

Besides the area under the peak of emission line and its maximum intensity, many researchers have determined and interpreted fluorescence indices for the characteristics of fluorescence, thereby regarding them as a decisive diagnostic indicator [10,11,19-22,34-40]. These indices can apply to various types of tissue (root-crown), different locations of the analysed area (carious spot-healthy tooth) or the intensity of the light emitted at different wavelengths / NADH / FAD /. In order to identify precisely the differences in the light spectra emitted from the crowns and tooth roots exposed to *Zidovudine* compared with the controls, the authors of the present study also used fluorescence indices – the *Zidovudine* fluorescence index (IFZ) and the tooth fluorescence index (IFD).

Tooth crowns in rat mothers had *Zidovudine* fluorescence index (IFZ) lower than one throughout the whole spectrum of emitted light, while the crowns of newborns in the spectrum of less than 550 nm. IFZ index equal or close to one was noted for tooth crowns in rat newborns within a wavelength ranging between 525-550 nm and 660-675 nm. Unlike the crowns, the tooth roots of both newborns and rat mothers had an IFZ index greater than one throughout the entire range of the emitted spectrum. The tooth roots of mothers and newborns had the highest IFZ values, i.e. 4 and 2.8 respectively, at a wavelength of 675 nm (Figure 5). It can therefore be concluded that the tooth crowns of rat newborns exhibited the smallest changes in fluorescence following *Zidovudine* treatment, whereas the biggest changes occurred t in the tooth roots of rat mothers. This result is consistent with the view of Christmas, et al., who observed that *Zidovudine* had a slight embryotoxic effect on developing rat foetuses [41]. Mitchel, et al. obtained different results in their studies. When assessing the impact of *Zidovudine* on the growth and proliferation of keratinocytes [42] they found that *Zidovudine*, even when administered in low doses and for a short time, disrupts the natural proliferation and differentiation cycle of alveolar epithelial cells. It should be emphasized that the above-cited studies were conducted in vitro. Hence their results may differ from the effects of antiretroviral drugs on the growing organism, especially when the medications affect foetal tissue after passing through the placenta [43]. This would point to the benefits of treatment involving this group of drugs in order to avoid transferring the virus to offspring despite the possible side effects that may occur in offspring following maternal administration [44-46]. Throughout the test material, the biggest difference in the *Zidovudine* fluorescence index (IFZ) was observed at a wavelength of 675 nm emitted by crowns and tooth roots both in mothers and rat new-borns (Figure 5). The result may indicate a different concentration of porphyries, or differences in the vascularization of the study area following the administration of *Zidovudine* [13].

The lowest tooth fluorescence index, (IFD), being close to one, was observed in rat mothers following the administration of *Zidovudine*. This result shows that the administration of the drug resulted in the formation of an almost identical emission spectrum of crowns and tooth roots in the analysed group. This index was clearly different from the control group of rat mothers. It can therefore be stated that *Zidovudine* increases fluorescence intensity in tooth crowns, and reduces its intensity within the root (Figure 6).

The fluorescence index following the administration of *Zidovudine* assumed a different character in the rat new-borns.. The index had values greater than one along the entire emitted spectrum, which demonstrates the greater susceptibility of the tooth roots in the control group compared to the crowns. It is important to note that in a spectrum ranging from short waves in the direction of long waves, the index increased and reached its maximum value of 3.75 at a wavelength of 633 nm (Figure 6). It can therefore be concluded that *Zidovudine* has a greater impact on roots than on tooth crowns. As is evident from the literature this impact is manifested by a change in the local environment of the tooth due to related protoporphyrin storage [13,19,24,38].

Conclusion

1. The results indicate that a quantitative method for assessing laser fluorescence can be used to determine the impact of *Zidovudine* on the developing tooth bud. Furthermore, this method makes it possible to detect such changes in tissues that are inaccessible in histomorphological evaluations.
2. *Zidovudine* has less impact on the development of the tooth tissue of rat newborns than on the tissue of rat mothers that received *Zidovudine*.
3. The different spectra of light emitted by tooth crowns and roots indicate a slight difference in the effects of *Zidovudine* on tooth tissue.
4. Further research is recommended to assess the light spectra that emitted by the dental organ in conjunction with the mechanism of drug -induced changes.

References

1. Langtry HD, Campoli-Richards DM (1989) Zidovudine. Drugs 37: 408-450.
2. Miles SA, Mitsuyasu RT, Moreno J (1991) Combined therapy with recombinant granulocyte colony-stimulating factor and erythropoietin decreases hematologic toxicity from zidovudine. Blood 77: 2109-2117.
3. Jackson GG, Paul AD, Falk AL (1988) Human immunodeficiency virus (HIV) antigenemia (p24) in the acquired immunodeficiency syndrome (AIDS) and the effect of treatment with zidovudine (AZT). Ann Intern Med 108: 175-180.
4. Chen SCA, Barker SM, Mitchell DH (1991) Concurrent zidovudine-induced myopathy and hepatoxicity in patients treated for human immunodeficiency virus (HIV) infection. Pathology 24: 109-111.
5. Larder BA, Kellam P, Kemp SD (1991) Zidovudine resistance predicted by direct detection of mutations in DNA from HIV-infected lymphocytes. Aids 5: 137-144.
6. D'Aquila RT, Johnson VA, Welles SL (1995) Zidovudine resistance and HIV-1 disease progression during antiretroviral therapy. Ann Intern Med 122: 401-408.
7. Toltzis P, Marx CM, Kleinman N, Levine EM, Schmidt EV (1991) Zidovudine-associated embryonic toxicity in mice. J Infect Dis 163: 1212-1218.
8. Sperling RS, Stratton P, O'Sullivan MJ (1992) A survey of zidovudine use in pregnant women with human immunodeficiency virus infection. N Engl J Med 326: 857-861.
9. Chung YG, Schwartz JA, Gardner CM, Sawaya RE, Jacques SL (1997) Diagnostic potential of laser-induced autofluorescence emission in brain tissue. J Korean Med Sci 12: 135-142.
10. Liu Q, Grant G, Li J (2011) Compact point-detection fluorescence spectroscopy system for quantifying intrinsic fluorescence redox ratio in brain cancer diagnostics. J Biomed Opt 16: 037004.
11. Drzazga ZK, Kluczewska-Gałka A, Michnik A, Kaszuba M, Trzeciak H (2011) Fluorescence spectroscopy as tool for bone development monitoring in newborn rats. J Fluoresc 21: 851-857.
12. Angmar-Mansson B, Bosch JJT (2001) Quantitative light-induced fluorescence (QLF): a method for assessment of incipient caries lesions. Dentomaxillofac Radiol 30: 298-307.
13. Koenig K, Schneckenburger H (1994) Laser-induced autofluorescence for medical diagnosis. J Fluoresc 4: 17-40.
14. Liu Q, Vo-Dinh T (2009) Spectral filtering modulation method for estimation of hemoglobin concentration and oxygenation based on a single fluorescence emission spectrum in tissue phantoms. Med Phys 36: 4819-4829.
15. Kapadia CR, Cutruzzola FW, O'Brien KM, Stetz ML, Enriquez R, et al. (2010) Laser-induced fluorescence spectroscopy of human colonic mucosa. Detection of adenomatous transformation. Photochem Photobiol 86: 87-95.
16. Silveira Jr L, Betiol Filho JA, Silveira FL, Zangaro RA, Pacheco MT (2008) Laser-induced fluorescence at 488 nm excitation for detecting benign and malignant lesions in stomach mucosa. J Fluoresc 18: 35-40.
17. Rodero AB, Silveira Jr L, Rodero DA, Racanicchi R, Pacheco MT (2008) Fluorescence spectroscopy for diagnostic differentiation in uteri's cervix biopsies with cervical/vaginal atypical cytology. J Fluoresc 18: 979-985.
18. Richards-Kortum R, Mitchell MF, Ramanujam N, Mahadevan A, Thomsen S (1994) In vivo fluorescence spectroscopy: potential for non-invasive, automated diagnosis of cervical intraepithelial neoplasia and use as a surrogate endpoint biomarker. J Cell Biochem Suppl 19: 111-119.
19. Khosroshahi ME, Rahmani M (2012) Detection and evaluation of normal and

malignant cells using laser-induced fluorescence spectroscopy. J Fluoresc 22: 281-288.

20. Sieroń A, Kościarz-Grzesiok A, Waśkowska J (2008) The role of autofluorescence diagnostics in the oral mucosa diseases. Photodiagn Photodyn Ther 5: 182-185.

21. Drzazga ZK, Maciejewska K, Michalik K, Kaszuba M, Nowińska B (2011) Does maternal treatment with zidovudine affect changes in mandibles of newborns? Laser induced fluorescence study. J Fluoresc 21: 883-886.

22. Drzazga ZK, Michalik K, Maciejewska K, Kaszuba M, Nowińska B, et al. (2010) Optical and X-ray fluorescence spectroscopy studies of bone and teeth in newborn rats after maternal treatment with indinavir. Photochem Photobiol 86: 87-95.

23. Wei-Chiang LA, Toms S, Motamedi M, Duco Jansen E, Mahadevan-Jansen A (2000) Brain tumour demarcation using optical spectroscopy; an in vitro study. J Biomed Opt 5: 214-220.

24. Heinrich-Weltzien R, Kühnisch J, van der Veen M, de Josselin de Jong E, et al. (2003) Quantitative light-induced fluorescence (QLF)--a potential method for the dental practitioner. Quintessence Int 34: 181-188.

25. van der Veen MH, de Josselin de Jong E (2000) Application of quantitative light-induced fluorescence for assessing early caries lesions. Monogr Oral Sci 17: 144-162.

26. de Josselin de Jong E, Sundström F, Westerling H, Tranaeus S, ten Bosch JJ, et al. (1995) A new method for in vivo quantification of changes in initial enamel caries with laser fluorescence. Caries Res 29: 2-7.

27. Tranaeus S, Al-Khateeb S, Bjorkman S, Twetman S, Angmar-Mansson B (2001) Application of quantitative light-induced fluorescence to monitor incipient lesions in caries-active children. A comparative study of remineralisation by fluoride varnish and professional cleaning. Eur J Oral Sci 109: 71-75.

28. Zhang Z, Blessington D, Li H (2004) Redox ratio of mitochondria as an indicator for the response of photodynamic therapy. J Biomed Opt 9: 772-778.

29. Kawczyk-Krupka A, Waśkowska J, Raczkowska-Siostrzonek A (2012) Comparison of cryotherapy and photodynamic therapy in treatment of oral leukoplakia. Photodiagn Photodyn Ther 9: 148-155.

30. Subhash N, Thomas SS, Mallia RJ, Jose M (2005) Tooth caries detection by curve fitting of laser-induced fluorescence emission: a comparative evaluation with reflectance spectroscopy. Lasers Surg Med 37: 320-328.

31. Adeyemi AA, Jarad FD, de Josselin de Jong E, Pender N, Higham SM (2010) The evaluation of a novel method comparing quantitative light-induced fluorescence (QLF) with spectrophotometry to assess staining and bleaching of teeth. Clin Oral Investig 14: 19-25.

32. Stone B, Dockrell D, Bowman C, McCloskey E (2010) HIV and bone disease. Arch Biochem Biophys 503: 66-77.

33. Emami Z, al-Khateeb S, de Josselin de Jong E, Sundström F, Trollsås K, et al. (1996) Mineral loss in incipient caries lesions quantified with laser fluorescence and longitudinal microradiography. A methodologic study. Acta Odontol Scand 54: 8-13.

34. Richards-Kortum R, Sevick-Muraca E (1996) Quantitative optical spectroscopy for tissue diagnosis. Annu Rev Phys Chem 47: 555-606.

35. Madeddu G, Spanu A, Solinas P (2004) Bone mass loss and vitamin D metabolism impairment in HIV patients receiving highly active antiretroviral therapy. Q J Nucl Med Mol Imaging 48: 39-48.

36. Brown TT, Qaqish RB (2006) Antiretroviral therapy and the prevalence of osteopenia and osteoporosis: a meta-analytic review. AIDS 20: 2165-2174.

37. Karlsson L, Johansson E, Tranaeus S (2009) Validity and reliability of laser-induced fluorescence measurements on carious root surfaces in vitro. Caries Res 43: 397-404.

38. SubhashN, Thomas SS, Mallia RJ, Jose M (2005) Tooth caries detection by curve fitting of laser-induced fluorescence emission: a comparative evaluation with reflectance spectroscopy. Lasers Surg Med 37: 320-328.

39. Kowada T, Kikuta J, Kubo A (2011) In vivo fluorescence imaging of bone-resorbing osteoclasts. J Am Chem Soc 133: 17772-17776.

40. Polo CF, Frisardi AL, Resnik ER, Schoua AE, Batlle AM (1988) Factors influencing fluorescence spectra of free porphyrins. Clin Chem 34: 757-760.

41. Christmas J, Little B, Knoll K, Bawdon R, Gilstrap L III (1995) Teratogenic and Embryocidal Effects of Zidovudine (AZT) in Sprague-Dawley Rats. Infect Dis Obstet Gynecol 2: 223-227.

42. Mitchell D, Israr M, Alam S, Kishel J, Dinello D, et al. (2012) Effect of the HIV nucleoside reverse transcriptase inhibitor zidovudine on the growth and differentiation of primary gingival epithelium. HIV Med 13: 276-290.

43. Sandberg JA, Slikker Jr W (1995) Developmental pharmacology and toxicology of anti-HIV therapeutic agents: dideoxynucleosides. FASEB J 9: 1157-1163.

44. Bora K (2012) Maternal HIV-1 disease progression 18-24 months postdelivery according to antiretroviral prophylaxis regimen (triple-antiretroviral prophylaxis during pregnancy and breastfeeding vs zidovudine/single-dose nevirapine prophylaxis). Clin Infect Dis 55: 449-460.

45. Bora K (2011) Safety and effectiveness of antiretroviral drugs during pregnancy, delivery and breastfeeding for prevention of mother-to-child transmission of HIV-1: the Kesho Bora Multicentre Collaborative Study rationale, design, and implementation challenges. Contemp Clin Trials 32: 74-85.

46. Bora K, de Vincenzi I (2011) Triple antiretroviral compared with zidovudine and single-dose nevirapine prophylaxis during pregnancy and breastfeeding for prevention of mother-to-child transmission of HIV-1. Lancet Infect Dis 11: 171-180.