J Fluoresc (2011) 21:883–886
DOI 10.1007/s10895-009-0591-7

ORIGINAL PAPER

Does Maternal Treatment with Zidovudine Affect Changes in Mandibles of Newborns? Laser Induced Fluorescence Study

Zofia Krystyna Drzazga · Karina Maciejewska · Katarzyna Michalik · Michał Kaszuba · Barbara Nowińska

Received: 30 October 2009 / Accepted: 22 December 2009 / Published online: 19 January 2010

Abstract The influence of antiretroviral drug *zidovudine* treatment during pregnancy on mandible development in newborn rats was studied. The fluorescence of mandibles from 7-, 14- and 28-days old individuals was measured by means of fiber-optical fluorescence analyzer with 407 nm laser excitation. Obtained results revealed disturbing effect of maternal *zidovudine* administration on mandible fluorescence intensity which should decrease with bone development. Small changes in fluorescence of porphyrin forms are maintaining in the first month of newborns life while the changes observed in 440–585 nm range disappear.

Keywords Laser (407 nm)—induced fluorescence · Zidovudine · Mandible

Introduction

Fluorescence spectroscopy as a sensitive, non-invasive method, which measures the emission intensity of the sample at very low concentrations of fluorophores in biological systems plays an important role in medical diagnostic [1–3]. It was shown that excitation wavelengths producing the highest contrasts are between 400 and 480 nm with a peak at 405 nm [4, 5]. There are a number of papers dealing with fluorescence as a diagnostic tool for cancer tissue [6, 7]. However, to our knowledge, no systematic study has conducted the clinical value of non-neoplastic structures fluorescence, especially bones [8].

Human immunodeficiency virus (HIV) and acquired immune deficiency syndrome (AIDS) has become a major contributor to global morbidity and mortality. Unfortunately the antiretroviral medicaments administered to patients often affect many organs during and after therapy, what has been widely reported [9, 10]. One of such drug is *zidovudine*, a nucleoside reverse transcriptase inhibitor (NRTI) which activity consists in blocking the reverse transcriptase enzyme which changes HIV's genetic material (RNA) into the form of DNA. It has been reported that *zidovudine* is incorporated into nuclear and mitochondrial DNA in cultured mammalian cells, in animal models, in human adults and newborn infants and it inhibits DNA synthesis in bone tissue [11]. This drug is used in antiretroviral therapy also to pregnant women because it prevents mother-to-child transmission of HIV virus [12]. However there are relatively small reports concerning influence of antiretroviral treatment of pregnant women on development of their offspring. This study is undertaken to determine an influence of antiretroviral drug *zidovudine* treatment during pregnancy on mandible development in newborn rats.

Material and method

A good approximation to investigating influence of drug administration on human body is using an animal model,

Z. K. Drzazga (✉) · K. Maciejewska · K. Michalik
Chełkowski's Institute of Physics,
Department of Medical Physics, University of Silesia,
40 007 Katowice, ul. Uniwersytecka 4,
Poland
e-mail: Zofia.Drzazga@us.edu.pl

M. Kaszuba
Institute of Stomatology,
40-534 Katowice, ul. Łabędzia 2,
Poland

B. Nowińska
Faculty of Pharmacy, Department of Pharmacology,
Medical University of Silesia,
41 200 Sosnowiec, ul. Jagiellońska 4,
Poland

which enables to estimate changes in living organism without affecting humans. Therefore experiment was carried out on flat bones (mandibles) from 7-, 14- and 28-days old newborn rats. The bones were divided into two groups. First one named *zidovudine* group, contained bones of rats which mothers were treated with antiretroviral drug *zidovudine* (GlaxoSmithKline) in a dose 200 mg/kg po once a day for 10 days between the tenth and nineteenth day of pregnancy [13, 14]. The second—control group included bones of rats after maternal application of 0.9% NaCl. The experiment was performed on bones from 6 to 7 newborns from each age control group and 8–13 newborns from *zidovudine* group. The female rats were breed in Centre of Experimental Medicine of the Medical University of Silesia. The procedure of the experiments on animals was approved by the Local Ethics Commission in Katowice, Poland.

Emission spectra of mandibles were registered from two areas on the compact surface: A on the ramus and B on the body as shown in Fig. 1. The spectra were taken from three points among each region of interest.

The measurements were performed using fiber-optical fluorescence analyzer with laser 407 nm excitation source and registered by LESA-6 software. Radiation at the excitation wavelength of 407 nm was generated by a semiconductive laser (GaNd) with the initial power of 10 mW corresponding to 3.5 mW mm at the bone surface. The emission spectra were collected at a given site by means of the fiber bundle of 1.8 mm diameter placed in gentle contact with the tissue at a fixed distance. Each data collection set of ten raw spectra was considered to be an independent measurement. The raw spectra were recorded in the range 407–814 nm and were normalized to the excitation peak at 407 nm considered as the reference signal. A linear background was defined by the signal 407 and 814 nm and subtracted from each measurement. The spectra were averaged when there were no significant differences between measured areas in mandible.

The results were analyzed using Origin 7.5 and Statistica 8.1 software. Differences with significance level $p<0.05$ were regarded as statistically significant.

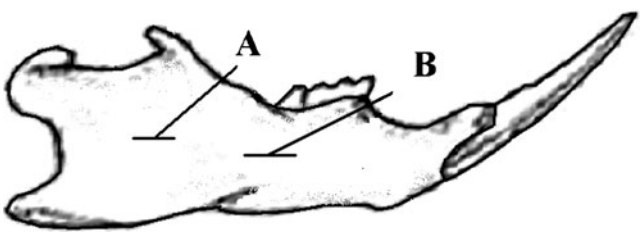

Fig. 1 The regions of interest on the surfaces of mandibles (A - body, B - ramus)

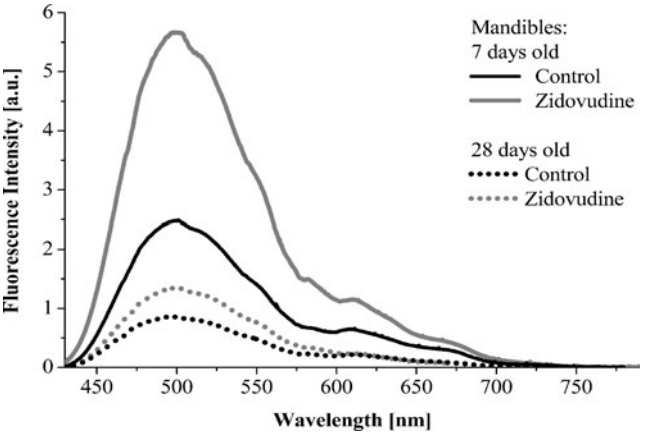

Fig. 2 The emission spectra of mandible from newborn rats of control and *zidovudine* (200 mg/kg po) group from 7- and 28-days old rats

Results

The characteristic raw autofluorescence spectra of mandible with the broad band located near 500 nm maximum together with the strengthened longer wavelength shoulder are presented in Fig. 2. The main band in the range about 440–585 nm originates from lipids, metabolic coenzymes (flavins: FAD, FMN) and vitamins similarly what was reported also in [15].

It should be noted that fluorescence intensity of spectra decreases with increasing age of newborns for control as well as *zidovudine* group. This tendency is also confirmed by statistical analysis of integral area (Fig. 3). It seems to be connected with bone mineralization [15] but the dynamics of observed changes is different between studied bone

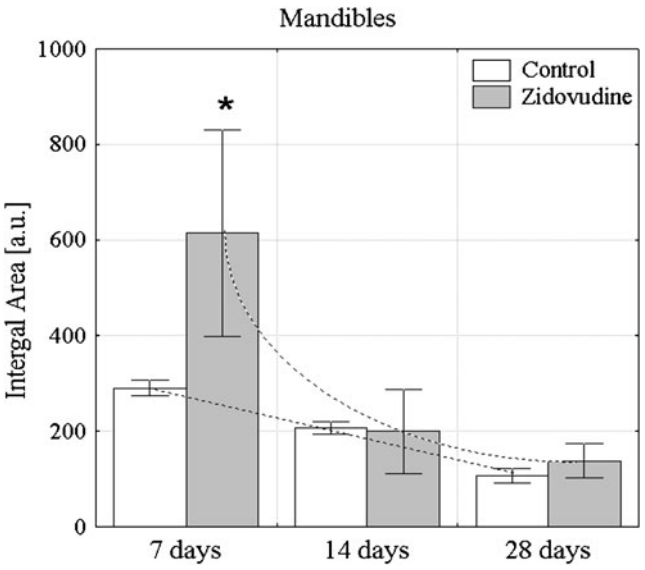

Fig. 3 Comparison of integral fluorescence for mandible of 7-, 14- and 28-days old newborns whose mothers were treated with *zidovudine* (200 mg/kg po) with control (* - p<0.05)

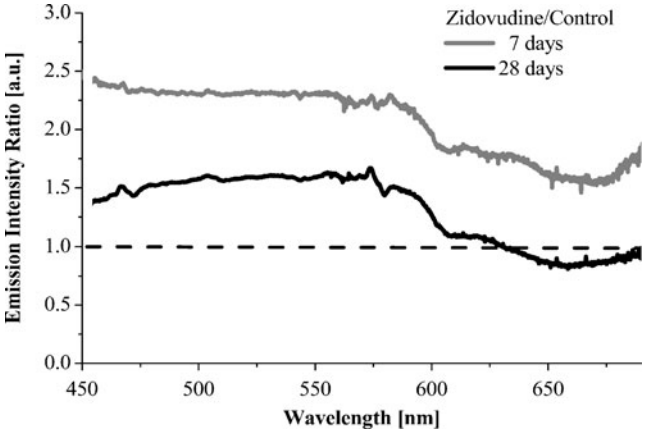

Fig. 4 The plots of *zidovudine*/control emission ratio in the range of studied wavelength for the mandible (7- and 28-days newborns)

groups, what is marked by trend lines in Fig. 3. The maternal administration of *zidovudine* caused an increase of fluorescence emission from 7-days old newborn's mandibles while for bones from older rats the differences are in the range of error (Fig. 3).

In order to deeper insight into fluorescence changes, plots of *zidovudine*/control emission ratio have been performed what is depicted in Fig. 4. This ratio shows an enhancement of fluorescence intensity in the range 440–585 nm and a lowering in the long-wavelength range. To emphasize the changes in porphyrin region the spectra have been normalized to main peak maximum what is illustrated in Fig. 5. One can see that fluorescence intensities from mandibles of rats after maternal treatment of *zidovudine* are distinctly lower when compared to control. The differences in fluorescence intensity at local maximum (about 611 nm) between *zidovudine* and control group were statistically significant.

Discussion

The emission spectra could be analyzed in two regions: more intense in the range 440–585 nm and less intense above 585 nm.

The first range reveals changes in intensity of fluorescence originating from lipids, metabolic coenzymes (flavins: FAD, FMN) and vitamins as a function of newborns age as well as drug administration. Obtained results indicate the decrease of mentioned emission signals from mandibles with age of newborns which can be perturbated by maternal *zidovudine* administration. Decrease of emission in 440–585 nm range could be connected with following mineralization of bone [15], since the enhancement of mandible fluorescence of 7-days old newborns after maternal *zidovudine* treatment could suggest a weakness of bone microarchitecture.

The changes in autofluorescence of porphyrin forms are clearly visible in spectra which are normalized to main peak maximum. It follows from Fig. 5 that fluorescence intensity in red range is nearly independent on rat age during the first month of life. However maternal *zidovudine* treatment decreases fluorescence of porphyrins what implies a destroying effect of examined drug on bone marrow and blood cells as was reported in [16].

The obtained results suggest that biochemical processes connected with lipids, metabolic coenzymes and vitamins aim to normal state of bone tissue with age of newborns (Figs. 3 and 4) while small changes in fluorescence of porphyrin forms maintain on statistically significant level up to 28th day of rat life.

Conclusion

Our studies show that laser induced fluorescence is an effective tool to determine influence of maternal administration of *zidovudine* on bone ontogenesis.

Decrease of mandible fluorescence intensity with age is disturbed by maternal *zidovudine* administration. The emission changes observed in 440–585 nm range disappear during first month of newborns life unlike those observed in the porphyrin range. It follows that maternal *zidovudine* treatment can have long term adverse effect on bone marrow and blood cells. However antiretroviral therapy with use of zidovudine give chance to HIV positive mothers to have offspring despite of some its health risk. Since it is important to look for less toxic but simultaneously effective new generation of nucleoside reverse transcriptase inhibitors.

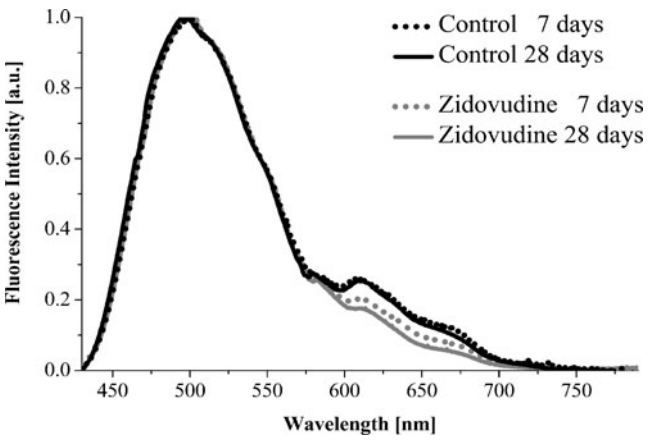

Fig. 5 Normalized to main peak maximum spectra of mandibles of 7- and 28- days old newborns after administration of *zidovudine* (200 mg/kg po) in comparison with control

Acknowledgement Karina Maciejewska, a co-author of this work is a scholarship holder of UPGOW Project.

References

1. Wagnieres G, Star W, Wilson B (1998) In vivo fluorescence spectroscopy and imaging for oncological applications. Photochem Photobiol 68(5):603–632
2. Zuluaga AF, Utzinger U, Durkin A, Fuchs H, Gillenwater A, Jacob R, Kemp B, Fan J, Richards-Kortum R (1999) Fluorescence excitation emission matrices of human tissue: a system for in vivo measurement and method of data analysis. Appl Spectrosc 53(3):302–311
3. Ramanujam N (2000) Fluorescence spectroscopy of neoplastic and non-neoplastic tissues. Neoplasia 2:89–117
4. Zellweger M, Grosjean P, Goujon D, Monnier P, van der Bergh H, Wagnières G (2001) In vivo autofluorescence spectroscopy of human bronchial tissue to optimize the detection and imaging of early cancer. J Biomed Opt 6:41–51
5. Buchalla W (2005) Comparative fluorescence spectroscopy shows differences in noncavitated enamel lesions. Caries Res 39:150–156
6. Li B-H, Xie S-S (2005) Autofluorescence excitation-emission matrices for diagnosis of colonic cancer. World J Gastroenterol 11 (25):3931–3934
7. Lualdi M, Colombo A, Leo E, Morelli D, Vannelli A, Battaglia L, Poiasina E, Marchesini R (2007) Natural fluorescence spectroscopy of human blood plasma in the diagnosis of colorectal cancer: feasibility study and preliminary results. Tumori 93(6):567–571
8. Prentice AID (1967) Autofluorescence of bone tissues. J Clin Path 20:717–719
9. Meng Q, Walker DM, Olivero OA, Shi X, Antiochos BB, Poirier MC, Walker VE (2000) Zidovudine–didanosine coexposure potentiates DNA incorporation of zidovudine and mutagenesis in human cells. Proc Natl Acad Sci USA 97(23):12667–12671
10. Volberding PA, Hughes M, Jacobson MA, Fischl M, Gundacker H (1996) Zidovudine side effects as reported by black, Hispanic and white non-Hispanic patients with early HIV disease: combined analysis of two multicenter placebo-controlled trials. J Acquir Immune Defic Syndr Human Retrovirol 11(1):45–52
11. Staal A, French MH, Watson LM, Sasseville VG, Feyen JHM (2001) Effects of nucleoside analogue reverse transcriptase inhibitors and protease inhibitors on bone explants in vitro. Antivir Ther 6(4):79–80
12. Nishimura T, Seki Y, Sato K, Chishu T, Kose N, Terasaki T, Kang Y-S, Sai Y, Nakashima E (2008) Enhancement of zidovudine uptake by dehydroepiandrosterone sulfate in rat syncytiotrophoblast cell Line TR-TBT 18d-1. Drug Metab Dispos 36(10):2080–2085
13. Greene JA, Mayers K, de Miranda P, Tucker WEJr (1990) Postnatal survival in Wistar rats following oral dosage with zidovudine on gestation 10. Fundam Appl Toxicol 15:201–206
14. Mamede JAV, Simoes MDJ, Novo NF, Juliano Y, Oliveira-Filho RM, Kulay LJr (1995) Chronic effects of azidothymidine and acyclovir on pregnant rats. Gen Pharmac 26:523–526
15. Drzazga Z, Michalik K, Maciejewska K, Kaszuba M, Nowińska B (2009) Optical and X-ray fluorescence spectroscopy studies of bone and teeth in newborn rats after maternal treatment with indinavir. Photochem Photobiol, in press
16. Agur Z, Arnon R, Sandak B, Schechter B (1991) Zidovudine toxicity to murine bone marrow may be affected by the exact frequency of drug administration. Exp Hematol 19(5):364–368