Ontogenetic View on Lipid Peroxidation in Bone in Liver Osteopathy

O. Tagadiuc[1], A. Saulea[2], V. Gudumac[1]

Biochemistry Laboratoty[1], Department of Physiology and Medical Rehabilitation[2]
Nicolae Testemitanu State Medical and Pharmaceutical University

Aspecte ontogenetice ale oxidării peroxidice a lipidelor în ţesutul osos în osteopatia hepatică

Scopul cercetării a fost studiul intensităţii proceselor de oxidare peroxidică a lipidelor în ţesutul osos în osteopatia hepatică, la diferite etape ale ontogeniei postnatale. Experienţele au fost efectuate pe şobolanii tineri (2 luni), adulţi (6 luni) şi bătrâni, postmenopauzali (18 luni), care au fost divizaţi în două subgrupuri – martor şi cu osteopatie hepatică, modelată prin administrarea de durată a tetraclorurii de carbon (sol. de 50%). Intensitatea oxidării peroxidice a lipidelor a fost apreciată prin determinarea în pulberea de os femural a activităţii oxidante totale, a produşilor iniţiali ai oxidării peroxidice a lipidelor–hidroperoxizilor lipidici, a conjugatelor cetodienice şi a compuşilor carbonilici de tib baze Schiff, în fazele hexanică şi hidroalcoolică, precum şi a dialdehidei malonice. Administrarea de durată a tetracloruruii de carbon nu induce modificări statistic veridice ale activităţii oxidante totale în ţesutul osos al animalelor experimentale, indiferent de vârstă. Se atestă creşterea semnificativă a produşilor iniţiali ai oxidării peroxidice a lipidelor în faza hidroalcoolică, la masculi în toate loturile, cele mai mari valori fiind depistate la masculii bătrâni. La femele s-a înregistrat doar creşterea nivelului compuşilor carbonilici la animalele adulte în ambele faze. Cantitatea dialdehidei malonice nu variază semnificativ în loturile experimentale cercetate, relevându-se doar o tendinţă de descreştere la animalele bătrâne de ambele sexe. Astfel, administrarea de durată a tetraclorurii de carbon induce o amplificare mai pronunţată a oxidării peroxidice a lipidelor la masculi indifernent de vârstă în faza hidroalcoolică, care posibil relevă o sensibilitate mai mare a lipidelor polare ale ţesutului osos, la acţiunea prooxidantă a tetraclorurii de carbon. Numărul şi amploarea minimă a modificărilor oxidării peroxidice a lipidelor la femele se explică, probabil, prin capacitatea şi eficienţa mai înaltă a sistemului antioxidant al ţesutului osos la femele.

Cuvinte-cheie: ţesut osos, osteopatie hepatică, oxidare peroxidică a lipidelor.

Перекисное окисление липидов костной ткани при печеночной остеопатии

Целью работы было исследование перекисного окисления липидов в костной ткани при печеночной остеопатии на разных этапах постнатального онтогенеза. Опыты били проведены на молодых (2 месяца), взрослых (6 месяцев) и старых, постменопаузальных (18 месяцев) крысах, которые были разделены на две группы: контрольная и животные с печеночной остеопатией, индуцированной длительным введением четыреххлористого углерода (50% раствор). Интенсивность перекисного окисления липидов оценивали в пудре бедренной кости по общей окислительной активности, содержанию первичных продуктов перекисного окисления липидов (гидроперекисей липидов, диеновых кетоконъюгатов и карбонильных продуктов типа Шиффовых оснований), а также малонового диальдегида. Длительное введение четыреххлористого углерода не ведет к статистически значимым измененим общей оксидантной активности в костной ткани экспериментальных животных независимо от их возраста. Отмечается значительное увеличение количества первичных продуктов перекисного окисления липидов в гидроалкогольной фазе у самцов всех групп. Самый высокий уровень был зарегистрирован у старых самцов. У самок отмечается повышение только карбонильных соединений. Содержание малонового диальдегида не изменялось (со статистической достоверностью) ни в одной экспериментальной группе, однако имело тенденцию к снижению у старых крыс. Таким образом, длительное введение четыреххлористого углерода вызывает более интенсивное идуцирование перекисного окисления липидов у самцов в гидроалкогольной фазе независимо от возраста животных, что, возможно, обусловлено более высокой чувствительностью полярных липидов костной ткани к перекисному воздействию четыреххлористого углерода. Минимальное количество и незначительная выраженность изменений перекисного окисления липидов у самок, возможно, объясняется более эффективной и мощной антиоксидантной системой их костной ткан.

Ключевые слова: костная ткань, печеночная остеопатия, перекисное окисление липидов.

Introduction

Bone and joint diseases are acute and important problems in modern medicine and medico-biological sciences. WHO declared 2000-2010 The Bone and Joint Decade to highlight the necessity to continue and enhance research into normal bone metabolism and its disturbances in different diseases in order to develop new efficient diagnostic and treatment methods [2].

Numerous studies confirm the involvement of oxygen free radicals, lipid peroxidation (POL) and antioxidant system in the pathogenesis of bone and joint diseases (primary and secondary osteoporosis, osteoarthritis, rheumatoid arthritis, etc.) [1, 4]. POL is well studied in blood and different tissues, but there is no data about POL intensity and POL product levels in bone and other mineralized tissues, especially in bone and joint diseases [3].

The aim of the study was to investigate the intensity of POL in bone in liver osteopathy at different ontogenetic stages.

Materials and Methods

The experiments were performed on 90 white rats of different age, divided into three groups:

- Ist group – young rats, before the age of reproduction (2 months),
- IInd group – adult rats, able to reproduce (6 months),
- IIIrd group –old, postmenopausal rats (18 months).

The rats from each group were divided in four subgroups: control male rats (1) and control female rats (2) – that received olive oil, twice a week for 8 weeks; experimental male rats (3) and experimental female rats (4) with liver osteopathy – that received 50% carbon tetrachloride solution in olive oil, twice a week for 8 weeks.

The rats were terminated 24 hours after the last administration. At termination, the femurs were carefully removed, stripped of all soft tissue and the bone marrow washed out. The femurs were homogenized to bone powder in liquid nitrogen.

POL intensity was appreciated in femur bone powder by measuring the quantity of initial POL products - lipid hydroperoxides (LHP), dienic ketoconjugates (DKC) and carbonyl compounds Schiff-base type (CC), total oxidant activity (TOA) and final the POL product - malonic dialdehyde (MDA).

The quantity of initial POL products - LHP, DKC and CC (conventional units/g bone), were determined in hexane and hydroalcoholic phases by the procedure described by Livovskaia E.I. et al. (1991).

TOA (%) was appreciated by MDA accumulation in a modelling system described by Galaktionova L.P. et al. (1998), that contains Tween-80 as substrate and bone as the initiator of the reaction.

MDA was determined by the classic tiobarbituric method (μM/g bone powder) according to the procedure described by Galaktionova L.P. et al. (1998).

Statistical analysis was carried out using the Stat Direct (2001) program. Non-parametric tests (U Mann-Whitney) were used for comparisons due to a skewed distribution of data. Correlations were tested using Spearman's correlation. Results were considered significant at $p < 0,05$.

The study was approved by the Committee for biomedical research ethics at the State University of Medicine and Pharmacy "N.Testemitanu".

Results and Discussions

Our data revealed a very high intensity of POL in the bone of control rats (tab. 1). The quantities of the individual POL products in animals of all ages were similar in both phases – LHP were in the highest amount and the CC in the lowest. The same relationship was revealed in senile rats in our previous research [5]. The quantity of the POL products is higher in the hexane phase than in the hydroalcoholic phase in young and old control rats of both sexes. The most important differences were determined in old control rats. Only in adult control rats, the amount of POL products is significantly higher in the hydroalcoholic phase compared with the hexane one. This data suggests that in the bone of young and old intact rats, POL is more intensive in the hydrophobic compartment, whereby non-polar lipids are more sensitive to POL. In comparison, in the bone of the adult rats, peroxidation of polar fats, extracted in the hydroalcoholic phase, is more important.

There are no statistical significant differences between the amounts of POL products in male and female rats from groups I and II. Levels of all POL products are higher in old female then in old male rats, except CC in the hydroalcoholic phase which are 56% lower ($p < 0,05$).

In young male and female rats, there is a correlation between the amounts of LHP and DKC, LHP and CC, DKC and CC in both phases. In young male rats there is uniform correlation between all the POL products, whereas in females, the correlations are close, but less significant between DKC and CC. In adult and old rats, there are fewer correlations between the POL products. The correlations mentioned above reveal the chain nature of the lipid peroxidation in the bone of control rats of both sexes at different ages.

Important quantitative and qualitative changes of POL occurred in the rats bone after long-term CCl4 administration (tab. 2). All POL products significantly increased in the hydroalcoholic phase in the bone of male rats from all experimental groups. In males from the group I (young rats) LHP increased by 33%, DKC – by 30 % and CC – by 49% ($p < 0,05$, for all cases), in group II DKC increase by 111% ($p < 0,05$) and CC by 70% ($p < 0,01$) and in group III, LHP increased by 369%, DC - by 215% and CC - by 42% ($p < 0,05$ for all cases). There was no statistically significant change in the quantities of POL products in female rats.

Qualitative changes consisted of the modification in the relationships between the concentrations of POL products in both phases. In intact animals, the amount of LHP was higher than DKC and DKC higher than of CC (LHP > DKC

Table 1

Quantities of lipid hydroperoxides (LHP), dienic ketoconjugates (DKC) and carbonyl compounds (CC) in the bone of control rats of different age

Gr	Sex	Hexane phase			Hydroalcoholic phase		
		LHP	DKC	CC	LHP	DKC	CC
I	M	43,9±4,7	34,1±3,6	2,9±0,7	33,2±3,2	17,7±2,0****	5,5±0,6*
	F	48,4±11,0	38,9±8,2	5,5±0,97#	34,5±2,7	18,7±1,6**	6,7±0,5
II	M	3,2±0,7	1,6±0,4	0,9±0,3	48,8±4,6***	37,2±3,5***	2,4±0,6*
	F	3,9±0,7	4,0±1,6	0,8±0,08	49,7±6,8***	34,9±4,7***	1,6±0,3*
III	M	104,5±17,2	80,3±14,8	5,6±1,1	6,0±3,3**	4,8±2,1**	3,2±0,9
	F	205,1±25,0#	153,3±18,8##	16,2±1,9##	21,9±3,2**;#	15,6±2,1**;##	1,3±0,4**

a) Each value represents M±m, conventional units/g bone.
b) Statistical significance between the POL products quantities in hexane and hydroalcoholic phases according to U Mann-Whitney: * $p < 0,05$; ** $p < 0,01$; *** $p < 0,001$; **** $p < 0,0001$.
c) Statistical significance between the LPO products quantities in male and female rats according to U Mann-Whitney: # $p < 0,05$; ## $p < 0,01$.

Table 2

Quantities of lipid hydroperoxides (LHP), dienic conjugates (DC) and carbonyl compounds (CC) in the bone of rats with liver osteopathy of different age

Gr.	Sex	Hexane phase			Hydroalcoholic phase		
		LHP	DC	CC	LHP	DC	CC
I	M	27,3±4,7*	32,1±4,8	2,8±0,6**	44,1±3,7#	23,1±1,9#	8,2±0,7#
	F	27,7±4,3	28,8±5,2	2,5±0,6#	33,2±1,4	17,5±0,9	5,8±0,5****
II	M	3,6±0,7****	2,5±0,8****	1,2±0,3	57,9±15,0	78,3±19,8#	4,0±1,7###
	F	3,6±0,7*	2,6±0,9****	1,1±0,2	36,6±9,9	49,5±14,1	2,5±1,4
III	M	78,9±16,0*	90,3±20,8*	9,5±2,8	28,0±6,6#	15,0±4,8##	4,6±1,1
	F	140,7±25,8***	157,0±33,6***	16,0±3,9***	9,9±2,7#	8,1±2,9	1,4±0,3

a) Each value represents M±m, conventional units/g bone.
b) Statistical significance between the POL products quantities in hexane and hydroalcoholic phases according to U Mann-Whitney: * p < 0,05; ** p < 0,01; *** p < 0,005; **** p < 0,0005.
c) Statistical significance between the POL products quantities in control rats and rats with liver osteopathy according to U Mann-Whitney: # p < 0,05; ## p < 0,01, ### p < 0,001.

> CC) in all cases. After prolonged administration of CCl4 the relationship changed to DKC > LHP > CC in the hexane phase in young and old animals and in hydroalcoholic phase in adult animals regardless of their sex.

The correlations between the POL products also changed. The most important changes were registered in female rats. All correlations between POL products disappeared in both phases in young females, except the correlation between LHP and DKC in hexane phase (r = 0,86, p < 0,005). In adult females the correlation between LHP and DKC in hexane phase was conserved and new correlations appeared between all POL products in both phases. In old female rats, the correlations between LHP and DKC in hexane phase and LHP and CC in both phases disappeared. Almost all correlations were preserved in male rats. Only the correlations between DKC and CC in both phases in young rats and between LHP and DKC in hydroalcoholic phase in adult animals disappeared.

CCl4 induced some changes in the relationships between POL products in different phases. In young rats with liver osteopathy the levels of POL products were lower in hexane phase than in the hydroalcoholic, while in control rats of the same age the relationships were opposite. In the hexane phase the amount of LHP were 1,6 times (p < 0,05) and CC – 3 times (p < 0,001) lower in young males and in young females LHP were 1,2 times and CC – 2,3 times (p < 0,005) lower. Only DKC contents were higher in the hexane phase in animals of both sexes, like in control rats. The phenomena revealed a higher intensity of POL in the hydroalcoholic phase in young experimental rats versus control young animals and experimental adult and old rats. Later, POL intensification was more important in the hexane phase, like in control rats of the same age.

The quantitative prevalence of POL products was conserved in the hydroalcoholic phase versus the hexane phase in adult rats and in hexane phase versus the hydroalcoholic phase in the old animals. Both in adult male and female rats the amounts of LHP, DKC and CC in the hydroalcoholic phase were significantly higher than in the hexane phase. LHP levels were 14 times (p < 0,0005), DKC 31 times (p < 0,0005) and CC – 3 times higher in the hydroalcoholic phase than in the hexane phase in male rats with liver osteopathy, in female rats –correspondingly, 10 times (p < 0,0005), 14 times (p < 0,0005) and 3 times higher.

In old male rats with liver osteopathy the amount of LHP in the hexane phase was 1,8 times higher than in the hydroalcoholic (p < 0,05), DC – 5 times (p < 0,01) and CC by 106%. In female rats from the same group the differences were even greater– the quantity of LHP was 14 times, DKC – 20 times and CC – 12 times higher in the hexane phase versus the hydroalcoholic phase (p < 0,005, for all cases).

There are no sex or age dependent differences of TOA in control rats. Long-term CCl4 administration did not amplify the TOA in the bone of rats of different sex and age; therefore, it did not influence the prooxidant state of bone (fig. 1).

MDA quantity was significantly higher only in old female control rats versus males from the same group (24%, p < 0,05). There were no sex differences in other groups (fig. 2).

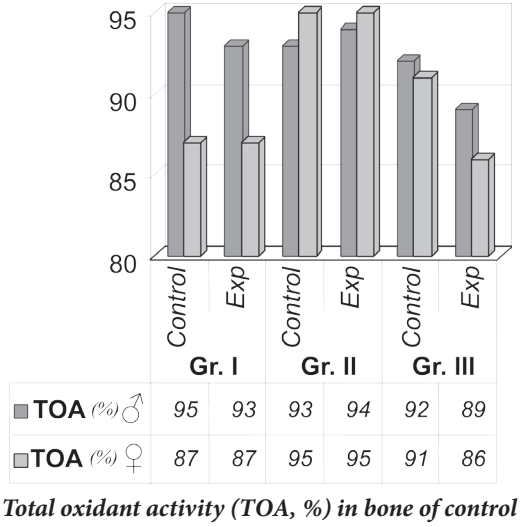

Fig. 1. Total oxidant activity (TOA, %) in bone of control rats and with liver osteopathy at different ontogenetic stages.

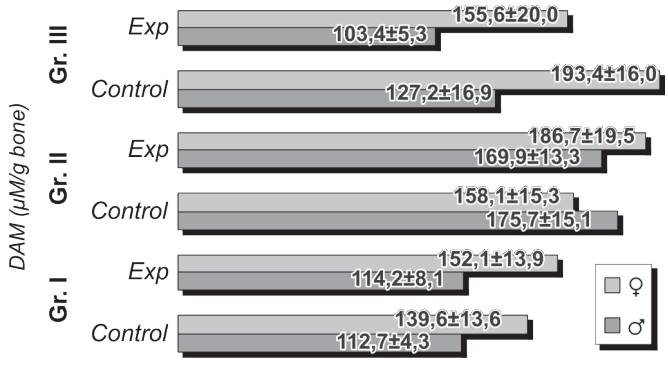

Fig. 2. Amount of malonic dialdehyde (MDA) in bone of control and experimental rats of different ages (μM/g bone).

Age differences were determined between the MDA amounts in young and adult male rats (56%, $p < 0,0001$) and between the MDA amounts in young and old female rats (39%, $p < 0,05$) of the control group.

There were no significant changes of MDA amount in bone of the animals with liver osteopathy induced by CCl4 administration, but MDA exhibited a tendency to decrease in old animals of both sexes. Lack of MDA accumulation after long-term administration of CCl4 can indicate a disturbance in the chain of POL with primary accumulation of the initial products of LPO (LHP, DC and CC) in bone.

Conclusions

1. Lipid peroxidation had a high intensity in bone of control rats and an obvious chain character, revealed by the relationships between the lipid peroxidation products – lipid peroxides were in higher concentration than dienic ketoconjugates which in turn were higher than the Schiff-base like carbonyl compounds, and the correlations between them.

2. Non-polar lipids, extracted in the hexane phase, were more sensitive to lipid peroxidation in young and old control rats, while in the adult rats – the polar lipids from the hydroalcoholic phase were more sensitive.

3. Old rats revealed sex determined differences of lipid peroxidation intensity which were more pronounced in females.

4. Liver osteopathy, caused by prolonged administration of CCl4, induced statistically significant quantitative changes in LPO in the hydroalcoholic phase in male rats of all ages.

5. Qualitative changes consisted of modifications in the relationship between lipid peroxidation products in the same phase, between different phases and the correlations between these products.

6. Administration of CCl4 did not change the TOA and MDA in all investigated groups.

References

1. Cuzzocrea S. Role of nitric oxide and reactive oxygen species in arthritis. Curr. Pharm. Des., 2006; 12 (27): 3551-3570.
2. International Osteoporosis Foundation. Osteoporosis review of evidence for prevention, diagnosis, treatment and cost-effectiveness analysis. Osteop. Int., 1999; 8 (suppl. 4): 51-88.
3. Ozgocmen S., Kaya H., Fadillioglu E., Aydogan R., Yilmaz Z. Role of antioxidant systems, lipid peroxidation, and nitric oxide in postmenopausal osteoporosis. Mol. Cell. Biochem., 2007; 295 (1-2): 45-52.
4. Surapaneni K. M., Venkataramana G. Status of lipid peroxidation, glutathione, ascorbic acid, vitamin E and antioxidant enzymes in patients with osteoarthritis. Indian. J. Med. Sci., 2007; 61 (1): 9-14.
5. Tagadiuc O. Modulator effects of carnosine and carnosine-Zn complex on lipid peroxidation in bone and blood serum. Archives of the Balkan Medical Union, 2008; 43 (3 suppl.): 383-385;
6. Галактионова Л. П., Молчанов А. В., Ельчанинова С. А., Варшавский Б. Я. Состояние перекисного окисления у больных с язвенной болезнью желудка и двенадцатиперстной кишки. Клин. Лаб. Диагн., 1998, 6:10-14.
7. Львовская Е. И., Волчегорский И. А., Шемяков С. Е., Лифшиц Р. И. Спектрофотометрическое определение продуктов перекисного окисления липидов. Вопр. Мед. Химии, 1991, 31 (4): 92-93.

Olga Tagadiuc, *M.D., Ph.D., senior researcher*
Biochemistry Laboratory
Nicolae Testemitanu State Medical and Pharmaceutical University
165, Stefan cel Mare Blvd, Chisinau-2004
Republic of Moldova
Tel.: 205136
E-mail: tagadiuc_olga@yahoo.co.uk

***Recepționat** 24.11.2008*