CONDITION OF PRO- AND ANTIOXIDANT SYSTEM OF ANIMALS UNDER SALMONELLA INFECTION

Kulmanova M.U., Sabirova R.A., Sayfullayeva S.A.
Tashkent Medical Academy, Tashkent, e-mail: asirifiamoako@yahoo.com

Understanding of mechanisms of emergence and development of salmonella intoxication, one of the most terrible displays of salmonella infection, requires a comprehensive study of the condition of membrane structures in tissue. Disturbances in lipid, protein metabolism, neutralization of toxic junctions that take place in tissue of mucous membranes of stomach and bowel can be a reason of development of heavy irreversible alterations that can lead to chronic pathological processes and even death of patients with salmonella infection [6, 7]. Every year bout 40000 precedents of salmonella are registered in the USA [9]. And considering the unregistered cases (a weaker strain or not-diagnosed intoxication) the index can be several times higher. Salmonella infection is registered in average of 15 to 18 precedents per 100000 of people a year in republic Uzbekistan [20]. Among factors that have unfavourable impact upon the development of salmonella infection in a patient's organism a significant part belongs to hypoxia [8]. Intensification of processes of peroxide oxidation of lipids (POL), disbalance in activity of ferments of antioxidant system (AOS) [3]. Disturbance in balance of ferments of POL-AOS leads to expressed damages in structural-functional organization of biologic membranes [4, 5]. An important factor of hypoxia development and intensification of POL is an emergence of surplus toxic products of interstitial metabolism, toxins, caused by infection in tissue. Markers of endogenous intoxication (EI) can be represented as peptides of low and average molecular mass (AMP) [1, 2]. A high biologic activity of AMP is expressed in increase and penetrability of cellular membranes, decrease in tone of vessels, disturbance in activity of heart, lungs, it has a direct toxic effect on liver, kidney, central nervous system, immune system, blood production. All that makes the disease course and recovery even more complicated.

Keywords: salmonella, intoxication, membrane structures, antioxidant system

As we know, malonicdialdehyde (MDA) is one of products of POL. According to the majority of authors [12, 13], increase in contents of MDA in tissue is a result of impact of hydrogen peroxide (H_2O_2) and other active forms of oxygen (AFO), such as O_2^-; OH; NO; NO_2; NO_3; $ONOO^-$, etc.

AFO affect membrane lipids, proteins, ferments, cellular membranes, nucleic acids, etc., thus disturbing their structure, metabolic processes, and function of cells. Impact of AFO increases due to ineffective activity of antioxidant system [14]. Important ferments of AOS are superoxide dismutase (SOD) and catalase (CT). SOD takes part in dismutation of radical of superoxide O_2^- before hydrogen peroxide (H_2O_2) forms, and ferment CT metabolizes $H_2O_2$to water and oxygen [10]. Besides, significance of processes POL-AOS in interaction with processes EI in pathogenesis of salmonella intoxication is not studied completely, and it defines the urgency of this problem and the necessity to carry out purposeful research on this topic.

The objective of this research is to study processes of POL-AOS in dynamics, expression of EI in tissue of mucous membrane of stomach and bowel, and blood serum among rats with salmonella infection.

Materials and methods of research

In this work we have used 60 white pedigreeless male rats of mass about 100–120 g. The animals were placed in terms of free movement, general ratio. All painful procedures were carried out according to Helsinki declaration of human attitude towards animals. In order to reproduce the model of salmonella infection, we have endogastrically introduced strain of S. typhimurium of 1 billion microbal bodies per 100 g of an animal mass. The animals were slain under Rausch narcosis via method of instantaneous decapitation after day 1, 4, 7, 10 after their infection. In 0,1 ml of blood serum, as well as homogenates of mucous membrane and thin bowel in solved 1:5 tissue (grams)/ environment of discharge (HCl 0,05M, KCl 1/15 M, pH 7,4), we have defined contents of MDA (nmole/ml), activity of SOD (Units/min/ml), CT (mcat/min/l) according to the method of L.I. Andreeva and co-authors [18], E.E. Dubinnina and co-authors [15], M.A. Korolyuk and co-authors [19]. Accordingly, in blood serum and in 9000 g of over-sediment liquid, we have studied concentration of AMP spectrophotometrically on SF-46 (Russia) with waves length of 260 and 286 nm (17). The number of products of peptide metabolism was calculated with coefficients of re-calculating according to the formula of Kalkar for the indicated wave length – 1,45 and 0,74 [16]. AMP was calculated according to the formula:

$$AMP = (E_{286}\ 1{,}45 - E_{260}\ 10\ (g/l)),$$

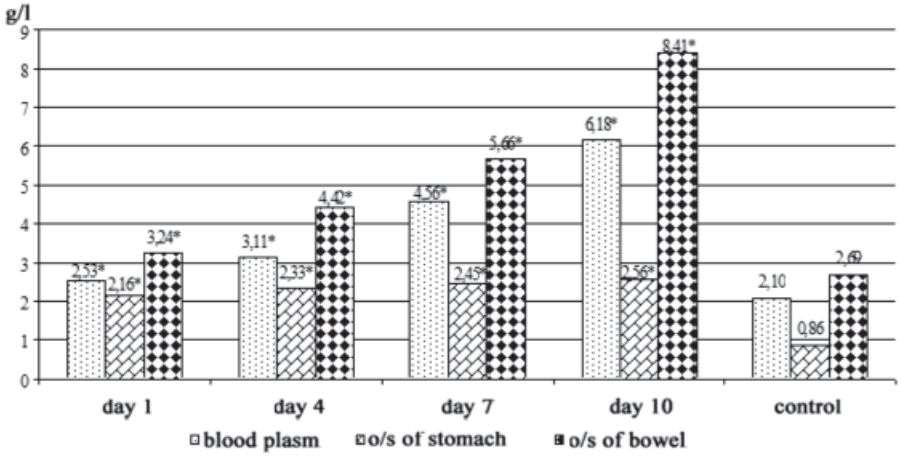
while AMP is a number average molecules in tissue (g/l), E_{286} and E_{260} are values of optical density of solution for the indicated wave length. The data was processed with variative-statistic method according to the programme Statistica V.6 with *t*-Student criterion and coefficient of linear correlation (r) of Pirson. Reliability of differences were calculated under $p < 0{,}05$.

Results of research and their discussion

It has been established that in blood serum and in 9000 g of over-sediment liquid of homogenates of tissue of stomach mucous membrane among animals after a day of intoxication with salmonella infection high level of AMP, compared to the control, is registered (Figure). This index grows dynamically in the studied probes along with an increase in observation time. We can suppose that an in-

crease in AMP in blood serum is defined by destructive processes in mucous membranes of stomach and bowel due to the developed pathogenic processes. An important part in this process, obviously, is linked to the destruction of membranes, strengthen in their cellular metabolism. It is proved by the data of progressing increase in MDA concentration in blood serum and in 9000 g over-sediment liquid of stomach and bowel according to the dynamics of observation period (table). Besides, we should outline that in 1 and 4 days after the experiment we have registered a high activity of ferments AOS-SOD and CT along with expression of MDA level in blood serum and in mucous membranes of stomach and bowel. After 7 and days of the experiment in blood serum, and after 4, 7, and 10 days in mucous tissues of stomach and bowel activity of these ferments decreased significantly in comparison to the control. An increase in activity of ferments AOS-SOD and CT in blood serum after 1 and 4 days of the experiment we treat as a protective mechanism of the organism against the introduced infection and presence of MDA in the system blood flow from the damaged organs. Alongwith increase in the experiment period potential reserves of ferments AOS – SOD and CT wear out due to an increasing level of MDA against the development of endogenous toxic products in this tissue. In order to testify the suggested concept we have taken a correlation analysis between the indexes of AMP and levels of MDA, SOD, and CT in tissue, and also between indexes of MDA, SOD, and CT. The results have shown that there is a clear direct correlation between indexes of expression in tissue of AMP and MDA that grows along with the period of salmonella intoxication in animals' organisms. In blood serum index of correlation (r) between AMP and MDA equaled $r = 0{,}73$ ($P < 0{,}05$); 0,76 ($P < 0{,}01$); 0,81, and 0,88 ($P < 0{,}001$) after 1, 4, 7, and 10 days of experiment, in 9000 g of over-sediment liquid, discharged from mucous membranes of stomach $r = 0{,}76$ ($P < 0{,}01$); 0,82 ($P < 0{,}01$); 0,86, and 0,90 ($P < 0{,}001$), in 9000g of over-sediment liquid, discharged from mucous tissue of small bowel $r = 0{,}79$ ($P < 0{,}01$); 0,86, 0,93, and 0,97 ($P < 0{,}001$) correspondingly to the terms of observation over animals. While estimating correlation between indexes of AMP and ferments AOS, we have established that increase in level of EI before 4 days of experiment was directly and statistically dependent on increase in activity of ferments SOD and CT. After 1 and 4 days of experiment in blood serum correlation equaled: between AMP and SOD $r = 0{,}77$ and 0,79 ($P < 0{,}01$), between AMP and CT – $r = 0{,}73$ and 0,77 ($P < 0{,}01$); in 9000 g of over-sediment liquid of stomach $r = 0{,}70$ and 0,72 – 0,74 and 0,76 ($P < 0{,}05$); in 9000 g of over-sediment of small and large bowel $r = 0{,}76$ и 0,78 – 0,77 – 0,79 ($P < 0{,}01$) correspondingly. After 7 and 10 days of salmonella intoxication increase in index of AMP correlated clearly with decrease in ferments of AOS-SOD and CT in studied tissues. However, this dependence had an indirect characteristic. After 7 and 10 days of experiment between high index of AMP and parameters if low activity of SOD in blood serum $r = -0{,}81$ and 0,89 ($P < 0{,}001$), between AMPand CT $r = -0{,}88$ and 0,91 ($P < 0{,}001$), in 9000 g of over-sediment liquid of stomach $r = -0{,}83$ и 0,92–0,87 и 0,95 ($P < 0{,}001$), in 900 g of over-sediment liquid of bowel $r = -0{,}87$ и 0,96–0,91 и 0,95 ($P < 0{,}001$) correspondingly. Results, similar in values and direction, were received while studying this index between parameters MDA, SOT, CT. In blood serum, 9000 g of over-sediment liquid of stomach and bowel it had a direct dependence, and after 7 and 10 days of experiment a strong indirect dependence ($P < 0{,}001$). The significance of the relations between indexes of EI and processes of POL intensification in mechanisms of decrease in potential capacity of AOS ferments is testified by data of alteration in coefficient (C) for correlation EI + POL/AOS. With a steady balance of this index it equaled $1{,}43 \pm 0{,}047$ in blood serum of the control animals. After 1, 4, 7, abd 10 day of salmonella intoxication this index equaled $1{,}38 \pm 0{,}061$ ($P < 0{,}001$), $2{,}14 \pm 0{,}081$ ($P < 0{,}001$), $9{,}36 \pm 0{,}440$ ($P < 0{,}001$) and $12{,}61 \pm 0{,}366$ ($P < 0{,}001$). In 9000 g of over-sediment liquid of stomach mucous membrane (for control – $0{,}65 \pm 0{,}023$) after 1, 4, 7, and 10 days of experiment it equaled $0{,}82 \pm 0{,}025$; $1{,}38 \pm 0{,}057$; $2{,}0 \pm 0{,}078$ and $2{,}74 \pm 0{,}081$ ($P < 0{,}001$) correspondingly. At the same time in over-sediment liquid of mucous membrane of small and large bowel index C equaled $0{,}61 \pm 0{,}021$, and after 1, 4, 7, and 10 days correspondingly – $0{,}57 \pm 0{,}018$ ($P < 0{,}05$), $1{,}40 \pm 0{,}053$; $2{,}53 \pm 0{,}074$ and $4{,}58 \pm 0{,}188$ ($P < 0{,}001$) among control animals. Therefore, development of salmonella intoxication is characterized bya significant disturbance in dynamic balance between systems EI and POL, and also an intensity of EI, POL, and activity of ferments of AOS. Before days 1 and 4 of experiment a suppressive factor of development of EI and intensification of POL is represented by activation of AOS ferments – SOD and CT. It is testified by the data of decrease in homogenates of mucous membranes of stomach and bowel in blood serum and over-sediment liquid. However, expression of EI and POL show the tension of these

processes against a high activity of ferments of AOS-SOD and CT in response to an increasing salmonella intoxication. Further, in blood serum and in tissue of stomach and bowel decrease in index C after 7 and 10 days of experiment testify the prevalence of EI and POL over activity of AOS ferments that leads to the destruction of membrane structures in mucous membranes of stomach and bowel, penetration of toxic products into system blood flow. Thesefactorsdefine the degree of increasing EI and complication of clinic disease, disturbance of mucous membranes of stomach and bowel, development of pathologic process in them.



N o t e : * – $P < 0,05$ compared to the control.

Dynamics in AMP contents in blood plasm and 9000 g of over-sediment liquid of homogenates in mucous membranes of stomach and bowel among rats under salmonella intoxication

Dynamics of indexes of system AOS-POL in blood serum, 9000 g of over-sediment liquid (n/o) of homogenates in stomach and bowel among animals with salmonella intoxication, M ± m

Group and terms	Blood serum			9000 g in the stomach			9000 g in the intestine		
	MDA, Nmole/ml	SOD, Units/min/ml	CT, Mcat/ml	MDA, Nmole/ml	SOD, Units/min/ml	CT, Mcat/ml	MDA, Nmole/ml	SOD, Units/min/ml	CT, Mcat/ml
Control (intact)	0,87 ± 0,051	2,21 ± 0,120	19,45 ± 0,841	2,08 ± 0,062	5,20 ± 0,213	35,74 ± 1,501	1,07 ± 0,034	7,23 ± 0,297	44,03 ± 1,564
Day 1	1,41 ± 0,072 *	3,28 ± 0,161*	32,73 ± 1,276*	2,46 ± 0,085*	6,43 ± 0,226*	43,59 ± 1,775*	1,51 ± 0,108*	9,56 ± 0,249*	58,15 ± 2,022*
Day 4	1,88 ± 0,112 *	2,60 ± 0,144*	22,84 ± 0,798*	3,41 ± 0,092*	4,37 ± 0,214*	39,61 ± 1,657*	2,45 ± 0,179*	5,69 ± 0,295*	36,43 ± 2,539*
Day 7	3,29 ± 0,151*	0,91 ± 0,043*	10,87 ± 0,442*	4,23 ± 0,101*	3,76 ± 0,205*	30,27 ± 0,926*	3,81 ± 0,178*	4,25 ± 0,180*	31,24 ± 1,56*
Day 10	3,77 ± 0,142 *	0,86 ± 0,051*	9,83 ± 0,491*	5,15 ± 0,113*	3,13 ± 0,174*	27,49 ± 1,209*	4,34 ± 0,149*	3,11 ± 0,208*	26,24 ± 1,077*

N o t e : * – $P < 0,05$ compared to the control.

Thus, we can claim that clinical significance of EI in mechanisms of disturbance in balance between POL and AOS and importance of evaluating these systems to predict development of salmonella infection is obvious. We suppose that pathogenetic impact of EI and POL is mainly expressed in cell membranes and mucous membranes of stomach and bowel, and their impact of suppressing activity of AOS ferments aggravates the severity of endogenous intoxication and provides for a progress of salmonella intoxication. According to the received correlation analysis and index of correlation between EI + POL/AOS that alter in dynamics of salmonella intoxication, we can conclude that these processed are interrelated, mutually-dependent and are important links in development of pathogenesis of salmonella infection in a patient's organism.

References

1. Karyakina E.V., Belova S.V. Molecules of average mass as an integral index of metabolic disturbances // Clinical laboratory diagnostics. – 2004. – № 3. – P. 3–7.

2. Sheyanov G.G., Mazankova L.N., Mukhina Y.G., Kurokhtina I.S. Clinical-laboratory evaluation of severity of syndrome of endogenous intoxication against acute bowel inections among children // Russian messenger of peritonitology and pediatry. – 2006. – № 3. – P. 53–60.

3. Motoyama T., Okamoto K., Kukita J. et al. Possble of increased oxidant stress in multipl organ failure after systemietuflammatory response syndrome // Crit Care Med. – 2003. – Vol. 31, № 4. – P. 1048–1052.

4. Stocker R., Keaney J.F. Role of oxidative modifications in atherosclerosis // Physiol. Rev. – 2004. – Vol. 84. – P. 1381–1478.

5. Guzik T., Harrison D. Vaseular NADPH oxidases as drugtargest for novel antioxidant strategis // Drug Discor. Today. – 2006. – Vol. 11. – P. 524–533.

6. Kumar H., Kawai T., Akira S. Pathogen recognition in the innate immune response // Biochem. J. – 2009. – Vol. 420. – P. 1–16.

7. Layton A.N., Galyov E.E. Salmonella-induced enteritis: molecular pathogenesis and therapevtic implications // Expert Re.Mol.Med. – 2007. – Vol. 9. – P. 1–17.

8. Patel I.K., Hueffer K., Lam T.T., Golan I.E. Diversification of a salmonella virulence protein function by ubiqtin-dependent differential localization // Cell. – 2009. – Vol. 137. – P. 283–294.

9. Sun l., Hobert M.E., Kao A.S. Bacterial activation of beta-catenin signaling in human epithelia // Am. J. Physiol.gastrointest. Liver Physiol. – 2004. – Vol. 287. – P. G220–G227.

10. Lukyanova L.D. Role of bioenergetics disturbance in pathogenesis of hypoxia // Pathologic physiology. – 2004. – № 2. – P. 2–11.

11. ZintuckV.V. Disfunction of endothelium and oxygen-linking characteristics of haemoglobin // Cardiology. – 2009. – № 7-8. – P. 81–89.

12. Lukyanova L.D., Dudchenko A.M., Unbina T.A., Germanova E.L. Regulative part of mitochondrial disfunction under hypoxia and its interaction with transcriptional activity // Messenger of RAMS. – 2007. – № 2. – P. 3–13.

13. Lyachovich V.V., Vavilin V.A., Zenkov N.K., Menshikova E.B. Activated oxygen metabolites in mono-oxygenase reactions // Bulletin of RAMS. – 2005. – № 4. – P. 7–12.

14. Manuchina E.B., Douney H.F., Mallet R.T., Manshev I.Y. Protective and damaging effects of periodic hypoxia: role of nitrogen oxide // Messenger of RAMS. – 2007. – № 2. – P. 25–33.

15. Dubinina E.E., Salnikova L.A., Efimova L.F. Activity and iso-ferment spectre of superoxide dismutase of erythrocytes and plasm of a man // Laboratory business. – 1983. – № 10. – P. 30–33.

16. Kovtunova M.E., Pankov V.N., Perevalova N.N. Ceruloplasmin and average-molecular peptides as criterions of the flow of acute mieloblast leucosis // Clinical laboratory diagnostics. – 2003. – № 5. – P. 52–54.

17. Gabriyelyan N.I., Lipatova V.I. Experience of using index of average molecules in blood to diagnose nephrological diseases among children // Laboratory business. – 1984. – № 3. – P. 138–140.

18. Andreyeva A.I., Kozhemyakin L.A., Kishkun A.A. Modification of method of defining lipid peroxides in test with tiobarbituric acid // Laboratory business. – 1989. – № 7. – P. 41–49.

19. Method of defining catalase activity / M.A. Korolyuk, L.I. Ivanova, I.G. Mayorova, V.E. Tokareva // Laboratory business. – 1988. – № 1. – P. 12–15.

20. Rustamova K.E., Babajanov A.S., Stozharova N.K., Ebosheva K.U. Retrospective analysis of disease rate among population of Uzbekistan // Messenger of Tashkent Medical Academy. – 2011. – № 3. – P. 97–100.