

Original Scientific Article

CONCENTRATION OF POLYAMINES IN THE RAT LIVER DURING POSTNATAL PERIOD

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Abstract. Polyamines, spermine, spermidine, and putrescine are ubiquitous in living cells. Polyamines play important roles in cell growth, proliferation, and survival. Our aim was to determine the concentration of the spermine, spermidine, and putrescine in the rat liver during the first 6 months of postnatal life. A total of 45 albino Wistar rats, maintained under controlled temperature ($20\pm 2^\circ\text{C}$) in the animal room facilities were included in this study. On the day 1, 3.5 months, and 6 months of postnatal life, rats were sacrificed by cervical dislocation. The liver was removed and washed in the 0.9% solution of sodium chloride. Concentrations of spermine, spermidine, and putrescine were determined. Concentration of polyamines in the liver tissue of the Wistar albino rats aged 1 day, 3.5 months, and 6 months was respectively: spermine (Sp) (33.81 ± 3.04 ; 128.15 ± 6.62 ; 74.34 ± 1.12 $\mu\text{g/g}$ of wet weight); spermidine (Spd) (121.92 ± 6.23 ; 53.34 ± 3.31 ; 56.32 ± 1.41 $\mu\text{g/g}$ wet weight); and putrescine (Put) (8.92 ± 0.98 ; 9.37 ± 0.98 ; 20.93 ± 1.15 $\mu\text{g/g}$ wet weight). Polyamine concentration in the rat liver fluctuated during postnatal life. The concentration of the spermidine was highest in the rat liver on the first postnatal day, much higher than spermine, this ratio inverted in 3.5 months old rats. The concentrations of the spermidine and spermine were almost equal at the 6 months of the postnatal life. The concentration of putrescine steadily increases during postnatal life.

Key words: spermin, spermidin, putrescine, rat liver, postnatal life.

Background

Polyamines spermine, spermidine, and putrescine are commonly ubiquitous in nature. They exist in millimolar concentrations in all living cells. The triamine spermidine and the tetraamine spermine are the most abundant polyamines in eukaryotic cells while the diamine putrescine and the triamine spermidine are found in prokaryotic cells such as bacteria, plants, and fungi [1–3]. Polyamines are involved in the regulation of a range of vital cellular processes including cell proliferation, differentiation, signal transduction, membrane stabilization, regulation of ion channels, and apoptosis [4–6]. They are also involved in the regulation of gene expression and translation [7]. Polyamines could act as antioxidants as they have been shown to inhibit lipid peroxidation [8]. It was found that spermidine, that is present in the nucleus in millimolar concentration, directly acts as a scavenger of reactive oxygen species (ROS), while spermine is able to protect DNA from ROS [9–11]. On the contrary, excessive polyamine catabolism is a prominent source of oxidative stress thought to be involved in infective, neurological, and malignant diseases [8]. Cancers cells contain a great amount of polyamines [12–14].

Recently, the relation between polyamines, especially spermidine and ageing was studied. The concentration of polyamines in body tissues declines with increasing age. Polyamine spermidine can prolong the lifespan by controlling the aging process [15, 16]. Therefore, spermidine may act as a universal anti-aging drug.

Our aim was to determine the concentration of polyamines (spermine, spermidine, and putrescine) in rat liver during postnatal development

Materials and Methods

A total 45 albino Wistar rats weighting 180 to 230 g, maintained under controlled temperature ($20\pm 2^\circ\text{C}$) in the animal room facilities were included in this study. Animals were divided into three groups: The first experimental group consisted of the youngest animals, the first day after birth ($n = 3$); the second experimental group consisted of 3.5 months old rats ($n = 15$); and the third experimental group consisted of 6 months old rats ($n = 27$). Rats were sacrificed by cervical dislocation. The liver was removed quickly and washed in the 0.9% solution of sodium chloride. Excess blood was removed by bloating and rinsing with ice-cold saline. Concentrations of spermine, spermidine, and putrescine were determined with butanol extraction followed by electrophoresis. Separated polyamines were identified by ninhydrin and quantified by the spectrophotometric method. For the statistical analyses, we used Sigma Stat 4.0 (SPSS Inc, Chicago, Ill). The obtained results were expressed as means \pm standard deviation.

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Results

The collected particles were assigned to one of 5 major Concentration of polyamines in the liver tissue of the Wistar albino rats old 1 day, 3.5 months, and 6 months was respectively: spermine (Sp) (33.81 ± 3.04 ; 128.15 ± 6.62 ; 74.34 ± 1.12 $\mu\text{g/g}$ of wet weight); spermidine (Spd) (121.92 ± 6.23 ; 53.34 ± 3.31 ; 56.32 ± 1.41 $\mu\text{g/g}$ wet weight); and putrescine (Put) (8.92 ± 0.98 ; 9.37 ± 0.98 ; 20.93 ± 1.15 $\mu\text{g/g}$ wet weight).

Polyamine concentration in the rat liver fluctuated during postnatal life (Figure 1). While the concentration of the spermidine was highest in the rat liver on the first postnatal day, 3.6 times higher than spermine, this ratio inverted in 3.5 months old rats when the concentration of the spermine was 2.4 times higher than spermidine. The concentrations of the spermidine and spermine were almost equal at the 6 months of the postnatal life. The concentration of the putrescine steadily increases during postnatal life (Figure 1).

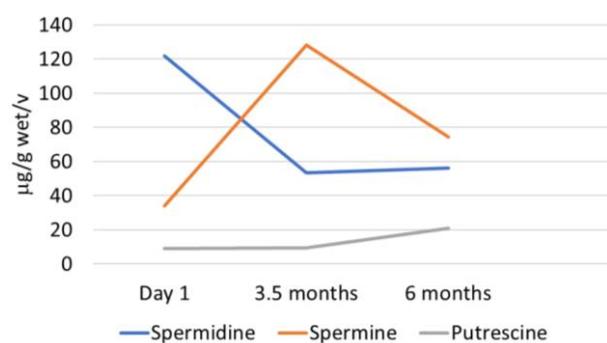


Fig. 1 Polyamine levels in the rat liver during first 6 months of postnatal life.

Discussion

Our study showed fluctuations of concentration of polyamines in the rat liver during postnatal life.

Our results are in agreement with the results of other studies. The concentration of polyamines is highest in fast-growing tissues [17–19]. Examination of polyamine metabolism in the rat liver during the development found that the concentration of spermidine was highest in the fetal liver followed by spermine level. The putrescine level was lowest [20, 21]. Jänne J et al. [22] found that the polyamine contents decreased with development and aging. This decrease is highest in the first month of life. Spermine concentration increased during the first month of postnatal life and then had the same concentration or decreased [22]. The increases in the putrescine concentration may be explained by the action of spermine/spermidine N1 acetyl transferase [23]. Our result,

which pointed out the highest spermidine concentration in the liver of the youngest animals (on the first day after birth) is in agreement with the results of other studies [24]. Sturman et al. [24] analysed polyamine biosynthesis in the human fetal liver. They found three- to four-fold greater concentration of spermine, spermidine, and putrescine in fetal human liver than in mature human liver [24].

A recent study suggested that polyamine spermidine may increase lifespan and maybe used as a universal drug against aging [25]. Administration of spermidine, a natural polyamine whose intracellular concentration declines during human ageing, markedly extended the lifespan of the human body. The inclusion of spermidine in the supramolecular complex with different polymers has optimal effects on the regenerative processes [26].

Autophagy is the major lysosomal degradation pathway for recycling damaged and potentially harmful cellular material [27]. It is thought that autophagy may be essential for healthy ageing and longevity. The basal level of autophagy assures maintenance of cell homeostasis. Recently, the correlation between polyamines, especially spermidine, autophagy, and aging was studied [28–30]. Eisenberg et al. [31] examined the involvement of spermidine in autophagy. Spermidine enhanced autophagy in a number of cell types including human cells. It was suggested that spermidine-induced autophagy increased lifespan by decreasing histone acetylating. Acetylating has a great function in autophagy and longevity. Genetics and functional examination showed that spermidine inhibits the process of histone acetylating. Hypoacetylation has the main role in process of the autophagy control and regulation of longevity [31].

Our study showed fluctuations of the polyamines in the rat liver during postnatal life. While the concentration of the spermidine was highest in the rat liver on the first postnatal day, significantly higher than spermine, this ratio inverted in 3.5 months old rats. The concentration of spermidine and spermine were almost equal at the 6 months of the postnatal life. The concentration of the putrescine steadily increased during postnatal life.

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References

- Cohen SS. A guide to the polyamines New York: Oxford University Press; 1998 [Available from: <http://catalog.hathitrust.org/api/volumes/oclc/35174769.html>].
- Handa AK, Fatima T, Mattoo AK. Polyamines: bio-molecules with diverse functions in plant and human health and disease. *Front Chem*. 2018; 6(10). doi: 10.3389/fchem.2018.00010
- Tabor CW, Tabor H. Polyamines. *Annu Rev Biochem* 1984; 53(1):749–790.
- Agostinelli E. Biochemical and pathophysiological properties of polyamines. *Amino Acids* 2020; 52(2):111–117.
- Pegg AE. Functions of polyamines in mammals. *J Biol Chem* 2016; 291(29): 14904–14912.
- Pegg AE. Introduction to the thematic minireview series: sixty plus years of polyamine research. *J Biol Chem* 2018; 293(48):18681–18692.
- Childs AC, Mehta DJ, Gerner EW. Polyamine-dependent gene expression. *Cell Mol Life Sci* 2003; 60(7):1394–1406.
- Hussain T, Tan B, Ren, et al. Polyamines: therapeutic perspectives in oxidative stress and inflammatory diseases. *Amino Acids* 2017; 49(9):1457–1468.
- Fariol M, Segovia-Silvestre T, Venereo Y, Orta X. Antioxidant effect of polyamines on erythrocyte cell membrane lipoperoxidation after free-radical damage. *Phytother Res* 2003;17(1):44–47.
- Fujisawa S, Kadoma Y. Kinetic evaluation of polyamines as radical scavengers. *Anticancer Res* 2005; 25(2A):965–969.
- Ha HC, Sirisoma NS, Kuppusamy P, Zweier JL, Woster PM, Casero RA. The natural polyamine spermine functions directly as a free radical scavenger. *Proceedings of the National Academy of Sciences*. 1998; 95(19):11140–11145.
- Green DR. Polyamines and aging: A CLEAR connection? *Mol Cell* 2019; 76(1):5–7.
- Madeo F, Eisenberg T, Pietrocola F, Kroemer G. Spermidine in health and disease. *Science* 2018;359(6374):eaan2788.
- Wallace HM. The polyamines: past, present and future. *Essay Biochem* 2009; 46:1–10.
- Madeo F, Carmona-Gutierrez D, Kepp O, Kroemer G. Spermidine delays aging in humans. *Aging* 2018; 10(8):2209–2211.
- Minois N. Molecular basis of the 'anti-aging' effect of spermidine and other natural polyamines - a mini-review. *Gerontology*. 2014; 60(4): 319–326.
- Sagar NA, Tarafdar S, Agarwal S, Tarafdar A, Sharma S. Polyamines: functions, metabolism, and role in human disease management. *Med Sci* 2021; 9(2):44.
- Nakanishi S, Cleveland JL. Polyamine homeostasis in development and disease. *Med Sci* 2021; 9(2):28.
- Bae D-H, Lane DJR, Jansson PJ, Richardson DR. The old and new biochemistry of polyamines. *Biochim Biophys Acta Gen Subj* 2018; 1862(9):2053–2068.
- Eguchi K, Yonezawa M, Ohmoto H, Mitsui Y, Hiramatsu Y. Development changes of polyamine biosynthesis in rat liver. *Biol Neonate* 1992; 62(6):402–408.
- Russell DH, Medina VJ, Snyder SH. The dynamics of synthesis and degradation of polyamines in normal and regenerating rat liver and brain. *J Biol Chem* 1970; 245(24):6732–6738.
- Jänne J, Raina A, Siimes M. Spermidine and spermine in rat tissues at different ages. *Acta Physiol Scand* 1964; 62(4):352–358.
- Cuervo AM, Bergamini E, Brunk UT, Dörge W, French M, Terman A. Autophagy and aging: the importance of maintaining "clean" cells. *Autophagy*. 2005; 1(3):131–140.
- Sturman JA, Gaull GE. Polyamine biosynthesis in human fetal liver and brain. *Pediatr Res* 1974; 8(4):231–237.
- Eisenberg T, Abdellatif M, Schroeder S, et al. Cardioprotection and lifespan extension by the natural polyamine spermidine. *Nat Med* 2016; 22(12):1428–1438.
- Madeo F, Bauer MA, Carmona-Gutierrez D, Kroemer G. Spermidine: a physiological autophagy inducer acting as an anti-aging vitamin in humans? *Autophagy* 2019; 15(1):165–168.
- Vellai T, Bicsák B, Tóth ML, Takács-Vellai K, Kovács AL. Regulation of cell growth by autophagy. *Autophagy* 2008; 4(4):507–509.
- Komatsu M, Ichimura Y. Selective autophagy regulates various cellular functions. *Genes Cells* 2010; 15(9):923–933.
- Morselli E, Galluzzi L, Kepp O, et al. Autophagy mediates pharmacological lifespan extension by spermidine and resveratrol. *Aging* 2009; 1(12):961–970.
- Yorimitsu T, Klionsky DJ. Autophagy: molecular machinery for self-eating. *Cell Death Differ* 2005; 12(2):1542–1552.
- Eisenberg T, Knauer H, Schauer A, et al. Induction of autophagy by spermidine promotes longevity. *Nat Cell Biol* 2009; 11(11): 1305–1314.