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How important is the oxidative degradation of spermine?: Minireview article

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Summary. Spermine is a constituent of most eucaryotic cells, however, it is not of vital importance for the vertebrate organism, as is demonstrated by the existence of transgenic (Gy) mice that lack spermine and spermine synthase. In contrast its degradation appears to be of vital importance, since mice die after chronic administration of N^1,N^4-bis(2,3-butadienyl)-1,4-butanediamine (MDL 72517). Under this condition spermine accumulates in red blood cells and blood plasma. Lethal toxicity can be avoided by intervals of MDL 72527-free periods. During these periods spermine appears to be directly degraded to spermidine without an intermediary acetylation step within the red blood cells. Since this reaction is of enormous physiological significance, it will be important to characterise the red blood cell spermine oxidase, and it will be particularly important to determine whether this oxidase is identical with the FAD-dependent polyamine oxidase that is considered to be involved in the polyamine interconversion sequence, or whether it is one of the recently characterised spermine oxidase isoenzymes.

Keywords: Spermine – Polyamine oxidase – MDL 72527 – AcetylCoA:spermidine N^1-acetyltransferase (SAT) – Red blood cells

Since spermine (Spm) is a general constituent of eucaryotes, but not of procaryotes, the search for specific Spm functions in nuclei-containing cells was, and still is a serious concern. It is now accepted that Spm has no direct role in cell growth. The observations that DFMO-treated cells with normal, or even elevated Spm concentrations stop growing as soon as the spermidine (Spd) pools are depleted (Mamont et al., 1978), and that the depletion of Spm pools by selective and potent inhibitors of spermine synthase (Spmsynth) has no significant effect on cell growth (Pegg et al., 1995), contributed to this notion. Moreover it has been shown that exogenous Spm supports the growth of Spd-depleted cells only after its degradation into Spd (Kramer, 1996). More recently we learned that Spm has no vitally important function. This became evident from the existence of transgenic mice, which lack a Spmsynth gene (together with the phosphate regulating phex gene) (Gy mice) (Lorenz et al., 1998), and consequently do not contain Spm in detectable amounts in tissues and body fluids. The pools of putrescine (Put) and Spd in tissues of Gy mice, and in Spm-lacking transgenic cells are elevated to an extent that the total polyamine concentration remains unchanged, and cell growth rates are the same as those of the parental cells (Korhonen et al., 2001). The higher sensitivity of the Spm-lacking cells to polyamine biosynthesis inhibitors may be interpreted as a role of Spm as precursor of Spd. The small size of Gy mice was considered as a consequence of Spm deficiency.

In situ substrate specificity determinations on rat liver, kidney and duodenum support the view that Spm is mainly degraded by polyamine oxidase (PAO), since hydrogen peroxide formation was observed only by using N^1acSpm, but not with Spm as substrate (van den Munckhof et al., 1995). In view of this and related observations, and in view of the non-vital role of Spm in vertebrates, one may wonder why vertebrate cells have several FAD-dependent oxidases, which split Spm into Spd and 3-aminopropanal, as has recently been shown by several groups (Wang et al., 2001; Vujcic et al., 2002; Murray-Stewart et al., 2002; Cervelli et al., 2003). Since the polyamine oxidase (PAO) that is responsible for polyamine interconversion has also been cloned from mammalian cells (Vujcic et al., 2003; Wu et al., 2003) a clarification of

specific functions of the different FAD-dependent polyamine oxidases can be expected from the distribution and precise localisation of these oxidases. The inducibility of SMO isoenzymes (Wang et al., 2001; Vujcic et al., 2002) will presumably be of importance for their functions.

There is evidence in favour of the idea that the formation of Spd from Spm plays a role in early embryonic development (Fozard, 1987), and the contragestational effect of MDL 72527 (N^1,N^4-bis-(2,3-butadienyl)-1,4-butanediamin) (Mehrotra et al., 1998), the well-known inactivator of PAO (Seiler et al., 2002), may be interpreted as the prevention of Spd formation from Spm. Since, however, high concentrations of MDL 72527 are cytotoxic (Dai et al., 1999), other interpretations of the interruption of pregnancy by this compound are possible.

Very little is known at present about the direct oxidation of Spm. Indirect evidence was obtained from studies on polyamine turnover in brain. Administration of MDL 72527 produced a linear increase of N^1-acetylspermidine concentration in the brains of experimental animals. The rate of increase corresponded to the turnover rate of spermidine, as obtained by a different method. This finding is in agreement with the notion that the acetylation step is obligatory for the degradation of Spd to Put. Cells devoid of spermidine N^1-acetyltransferase (SAT) are unable to form Put from Spd (Niiranen et al., 2002). In contrast, the increase of N^1-acetylspermine concentration in the brains of MDL 72527-treated mice was by far to slow as to correspond with its turnover. This was taken as evidence for pathways of Spm elimination, which are independent of N^1-acetylation by SAT (Seiler and Bolkenius, 1985). In this context it is interesting to remember that an inducible oxidase, which generates 3-aminopropanal in ischaemic brain has been described (Ivanova et al., 1998). While high acute doses of MDL 72527 had no obvious toxic effect, long-term treatment with this compound at an oral dose, which produced a 90–96% inactivation of PAO in kidney and liver caused lethal toxicity in mice. This effect was particularly impressive in situations of enhanced cell death (e.g. in the presence of a tumour, or the administration of DFMO). A reason for the toxicity of the PAO inactivator was found in the accumulation of Spm in blood to concentrations $>100\,\mu$M (Sarhan et al., 1991). Discontinuation of MDL 72527 administration allowed the degradation of Spm, and Spd appeared concomitantly with the decrease of Spm within the red blood cells (without an indication of N^1-acetylspermine formation), and the mice survived.

It can not be excluded that after the removal of MDL 72527 the Spm present in plasma and red blood cells is degraded in various PAO containing organs. However the decrease of Spm and the concomitant increase of Spd within the red blood cells suggests the direct transformation of Spm into Spd within these organelles. If this interpretation is correct it follows that the oxidase of red blood cells, although of low activity, has a vital role, and we face the somewhat paradoxical situation that the degradation of an ubiquitous cell constituent is more important for survival than its formation. Urinary excretion of Spm together with its oxidative deamination by diamine oxidase to N^8-(2-carboxyethyl)spermidine and spermic acid (N^1,N^4-bis(2-carboxyethyl)-1,4-butanediamine (both compounds are detectable in urine after [^{14}C]Spm administration (Noto et al., 1978)) are obviously inadequate for substituting oxidative deaminations by FAD-dependent spermine oxidases. Other reactions, such as the transformation of Spm into Spd by serum amine oxidase (Bachrach and Bar-Or, 1960; Houen, 1999) appear to play no role in vertebrates, with the possible exception of ruminants with their particular digestive tract.

The recently cloned SMO isoenzymes have been shown to be relatively insensitive to MDL 72527 (Wang et al., 2001; Vujcic et al., 2002), while a daily oral dose of 60–70 mg/kg of the drug was sufficient to prevent Spm degradation. This is in favour of the assumption that the red blood cell oxidase is similar to, or identical with the PAO of the interconversion cycle. In view of the presumed physiological importance of the red blood cell oxidase, its characterisation is an eminent task. It will contribute to our understanding of a basic role of polyamine catabolism in the physiology of higher organisms.

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