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Partition coefficients of some purine derivatives and its application to pharmacokinetics

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Metazathioprine (MAZA), a methylated derivative of azathioprine (AZA), demonstrated the greatest values of apparent and specific partition coefficients in n-octanol/phosphate buffer at pH 5.7 and pH 7.4 among purine derivatives such as 6-mercaptopurine (6-MP), 6-thioguanine (6-TG) and AZA. Introduction of a methyl group into the imidazole ring of AZA increases lipophilic properties of MAZA compared to AZA. Mass balance of purine derivatives in n-octanol and in phosphate buffer indicated their chemical stability in those media.

1. Introduction

Purine derivatives, 6-mercaptopurine (6-MP), 6-thioguanine (6-TG) and azathioprine (AZA), possess anticancer and immunosuppressive activities (Elion 1989; Sahasranaman et al. 2008). The therapeutic use of AZA is limited due to its side effects in some patients with genetic predisposition to high-risk myelosuppression during therapy (Boonsrirat et al. 2008; Czaja and Carpenter 2006). Therefore, it is crucial to search for new compounds or to modify the structure of parental drugs for their therapeutic use (Crafword et al. 1996). An example is metazathioprine (MAZA), a methylated derivative of AZA, whose synthesis is protected by a patent (Bałoniak et al. 1986). Kinetic studies of MAZA metabolism in blood under *in vitro* conditions and mercaptolysis MAZA to 6-MP in phosphate buffer in presence of physiological thiols, glutathione and cysteine, demonstrated significantly greater rate constants for MAZA than for AZA (Chrzanowska et al. 1985; Hoffmann et al. 2001; Chrzanowska et al. 2003a). *In vitro* studies in human cancer cells as well as *in vivo* studies in mice with transplanted cancer cells showed greater anticancer activity of MAZA than AZA (Hładoń et al. 1989).

Properly balanced hydrophilic-hydrophobic properties determine the ability of a drug to transport through cell membrane and the interaction between a drug and a receptor. Earlier studies (Chrzanowska et al. 2003b) indicated that chemical modification of AZA increased the lipophilicity of a new derivative - MAZA. Thus, solubility, partition coefficient in n-octanol/phosphate buffer at pH 5.7 and pH 7.4 as well as studies of transport through dodecanol membrane were analyzed. The previously calculated partition coefficients of MAZA, AZA, 6-MP and 6-TG were apparent but not specific, because pKa values for MAZA and 6-TG were not available.

The aim of this study was to determine specific partition coefficients in n-octanol/phosphate buffer for thiopurines mentioned as well as to check their mass balance in both phases. Furthermore, pKa values of the above thiopurines were supposed to be determined.

2. Investigations, results and discussion

In this study specific partition coefficients in n-octanol/phosphate buffer for thiopurine drugs such as 6-MP, 6-TG and AZA as well as a methylated derivative of AZA, MAZA, not used so far in therapeutics, were determined. Their mass balance was checked in both phases. The values of pKa for thiopurines mentioned above were calculated prior to the study. UV spectra of these purine derivatives demonstrated a batochromic shift with an increase in pH. This change in UV spectrum allowed to determine pKa values from the absorbance records at the same concentration of a given compound but at different pH value. The pKa values calculated for 6-MP and AZA were in accordance with literature data (Budavari et al. 1996). However, the values for 6-TG and MAZA were not available in the literature. The apparent partition coefficients in n-octanol/phosphate buffer at pH 7.4 increased according to the following order: 6-MP, 6-TG, AZA and MAZA (Fig. 1). The data obtained were in accordance with previously presented values of apparent partition coefficients of thiopurine derivatives at both pH 7.4 and pH 5.7 (Table 1) (Chrzanowska et al. 2003b). Taking into consideration the determined pKa values, it was proved that except for 6-MP the fractions of unionized species were close to unity in phosphate buffer at pH 7.4 and/or at pH 5.7. Assuming that the purine derivatives do not dissociate in n-octanol it has been shown that MAZA demonstrated the greatest apparent as well as specific partition coefficient. For the other purine derivatives, it increased as follows: 6-MP > 6-TG > AZA (Figs. 1 and 2). No significant differences between partition coefficients values for MAZA and AZA were found. This can be explained by similar pKa values for both compounds.

An introduction of methyl group into the imidazole ring of AZA resulted in an increased lipophilicity of MAZA and greater affinity of MAZA to transport through dodecanol membranes compared to AZA (Chrzanowska et al. 2003b). This was also confirmed by the results of quantum-chemical calculations with the Solvation Model SM5/PM3 method (Hoffmann et al. 2005). In that study values of free energy for solution the compounds

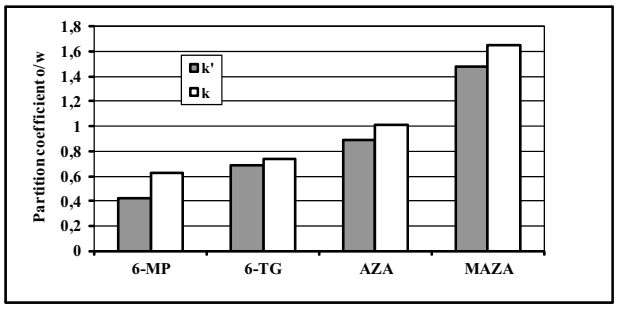

Fig. 1: Apparent (k′) and specific (k) partition coefficients n-octanol/phosphate buffer pH 7.4 for thiopurines

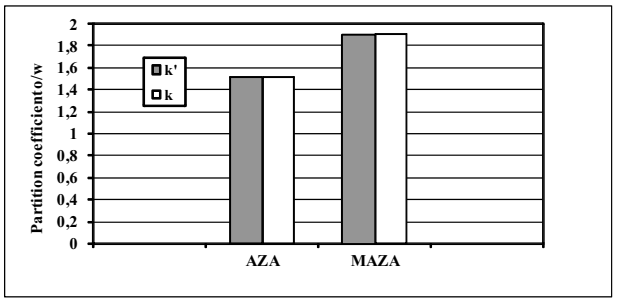

Fig. 2: Apparent (k′) and specific (k) partition coefficients n-octanol/phosphate buffer pH 5.7 for thiopurines

in water and in n-octanol as well as transitions of the compounds from water to n-octanol were compared. It was stated that 6-MP and 6-TG were better soluble in water than in n-octanol in contrast to MAZA and AZA, which were better soluble in n-octanol than in water. The values of free enthalpy of the compounds for transition from water to n-octanol were 16.7; 7.7; -2.1 and -6.3 kJ/mol for 6-TG, 6-MP, AZA and MAZA, respectively. The results of quantum-chemical calculations were consistent with the experimental values of their solubility in phosphate buffer and showed the greatest affinity of MAZA to the lipophilic phase (Chrzanowska et al. 2003b).

The mass recovery for all compounds ranged from 92.6 ± 1.5 to 106.5 ± 3.3 % (Table 2).

The results of individual studies along with the previous studies (Chrzanowska et al. 2003a, b; Hoffmann et al. 2005) allow to state that MAZA is the most lipophilic compound among the purine derivatives. Its mass recovery amounted to 99.7 ± 0.6 and 104.9 ± 1.7 % for partition coefficient n-octanol/phosphate buffer at pH 7.4 and 5.7, respectively and proved the stability of MAZA under the experimental conditions (Table 2).

The higher lipophilicity of MAZA compared to the others purine derivatives, the considerably greater rate of mercaptolysis from MAZA to 6-MP under the influence of physiological thiols, and the greater anticancer activity of MAZA compared to AZA (Hładoń et al. 1989) may support the idea to conduct preclinical trials for this compound.

3. Experimental

3.1. Materials

Metazathioprine, synthesized in the Department of Organic Chemistry, University of Medical Sciences at Poznań, Poland; 6-MP monohydrate (Sigma-Aldrich); 6-TG (Sigma-Aldrich); AZA, the Wellcome Foundation Ltd.; disodium hydrogen orthophosphate (Merck, Darmstadt, Germany); potassium dihydrogen orthophosphate (Merck, Darmstadt, Germany); n-octanol, 99 % (Sigma-Aldrich); water deionized, Seradest USF 1900 (USF Seral, Germany).

3.2. Apparatus

Spectrophotometer UV-VIS Specord M-40 (Carl Zeiss, Jena, Germany) and software, Medson, Poznań; pH-meter, CyberScan, Singapore; analytical balance BP 110S, Sartorius (Germany); vortex mixer type 358S (Elpan, Lubawa, Poland) automatic pipettes 20-5000 µl, Eppendorf (Germany).

3.3. Methods

3.3.1. Spectrophotometric determination of dissociation constants

Saturated thiopurines solutions in deionized water at 37 °C were prepared. First, 10 mg of each compound were weighed into centrifuged test tubes, and subsequently 5 ml of water were added. The tubes were vortexed for 24 h. The solutions were filtered and on the basis of previously determined solubilities in phosphate buffer at pH 7.4 approximate concentrations of thiopurines solutions were calculated (Chrzanowska et al. 2003b). For each compound approximately 20 samples were prepared. Each contained the thiopurine at the same concentration but at different pH and constant ionic strength ($\mu = 0.2$). According to UV spectra the absorbances at λ_{max} for MAZA, AZA, 6-MP and 6-TG at 37 °C, were plotted as a function of pH. The absorbances of unionized (A_{HA}) and ionized species (A_{A^-}) were calculated from the function A = f(pH).

The Henderson-Hasselbalch equation was used for calculation of pKa values:

$$\log \frac{[HA]}{[A^-]} = pKa - pH \qquad (1)$$

where: [HA] and [A^-] denote concentrations of unionized and ionized species of a relevant thiopurine, respectively.

The concentrations of appropriate thiopurines in Eq. (1) may be substituted with suitable absorbance differences:

$$[HA] = (A_{A^-} - A)$$
$$\left[A^-\right] = (A - A_{HA})$$

and resulted in Eq. (2)

$$\log \frac{(A_{A^-} - A)}{(A - A_{HA})} = pKa - pH \qquad (2)$$

The plot of Eq. (2) demonstrates a straight line with the slope equal -1. If the slope is different than 1, it should be included in the equation (Hermann 1974).

Table 1: Apparent and specific partition coefficients o/w for some thiopurines

Purine derivatives	pKa ± SD*	Partition coefficients ± SD** (CV, %)			
		Apparent (c_o/c_{buf})		Specific ($[HA]_o/[HA]_{buf}$)	
		pH		pH	
		5.7	7.4	5.7	7.4
6-MP	7.71 ± 0.05	–	0.424 ± 0.012 (2.83)	–	0.632 ± 0.018 (2.85)
6-TG	8.52 ± 0.01	–	0.688 ± 0.019 (2.76)	–	0.740 ± 0.020 (2.7)
AZA	8.26 ± 0.04	1.514 ± 0.092 (6.08)	0.892 ± 0.001 (0.11)	1.518 ± 0.092 (6.1)	1.015 ± 0.001 (0.1)
MAZA	8.33 ± 0.05	1.896 ± 0.075 (3.95)	1.473 ± 0.044 (2.98)	1.906 ± 0.078 (4.1)	1.647 ± 0.049 (2.97)

* n = 2
** n = 8

Table 2: Mass balance of purine derivatives at pH 7.4 and 5.7

Purine derivatives	pH	Initial mass [10^5 g]	Mass in both solvents [10^5 g] $\bar{m} \pm$ SD*; (C.V., %)		Mass balance [10^5 g] $\bar{m} \pm$ SD*; (C.V., %)	Mass recovery (%) (C.V., %)
			Phosphate buffer	n-Octanol		
MAZA	7.4	4.107	1.648 ± 0.020 (1.21)	2.429 ± 0.064 (2.63)	4.076 ± 0.069 (1.69)	99.7 ± 0.6 (0.6)
	5.7	4.110	1.480 ± 0.003 (0.20)	2.877 ± 0.07 (2.54)	4.357 ± 0.071 (1.63)	104.9 ± 1.7 (1.6)
AZA	7.4	3.909	1.930 ± 0.005 (0.26)	1.731 ± 0.014 (0.80)	3.662 ± 0.012 (0.33)	92.6 ± 1.5 (1.6)
	5.7	3.910	1.518 ± 0.073 (4.81)	2.249 ± 0.019 (0.84)	3.763 ± 0.082 (2.18)	95.8 ± 0.8 (0.8)
6-MP	7.4	2.400	1.606 ± 0.002 (0.16)	0.670 ± 0.008 (1.24)	2.275 ± 0.009 (0.41)	93.9 ± 1.2 (1.3)
6-TG	7.4	2.358	1.513 ± 0.010 (0.63)	1.063 ± 0.021 (1.97)	2.577 ± 0.028 (1.09)	106.5 ± 3.5 (3.3)

* n = 4

3.3.2. Molar absorption coefficient in phosphate buffer

Weighed amounts containing 0.01 g of 6-MP, 6-TG, AZA and MAZA were dissolved in 5 ml of 0.1 mol l^{-1} NaOH and subsequently made up to 50 ml with deionized water. The solutions were diluted with phosphate buffer of pH 7.4 or pH 5.7 in order to obtain concentrations within 3.525×10^{-5} mol l^{-1} to 0.352×10^{-5} mol l^{-1} range. The absorbances of five standard solutions (two separate samples at each concentration) were read at λ_{max} for each compound against the blank of phosphate buffer of pH either 7.4 or 5.7. Calibration curves were calculated from averaged absorbance values. Molar absorption coefficient ε was calculated from the slope of linear equation $A = \varepsilon \cdot c$.

3.3.3. Molar absorption coefficient in n-octanol

Weighed amounts containing 0.0038 g and 0.0037 g of 6-MP and 6-TG, respectively, were dissolved in 200 ml of n-octanol saturated with phosphate buffer, pH 7.4. Weighed amounts containing 0.0044 g and 0.0048 g of AZA and MAZA, respectively, were dissolved in 100 ml of n-octanol saturated with phosphate buffer pH 7.4. Subsequent procedures were as previously described.
Molar absorption coefficients in n-octanol saturated with phosphate buffer, pH = 5.7, were also calculated for MAZA and AZA. Weighed amounts of 0.0042 g AZA and 0.0044 g of MAZA were dissolved in 100 ml of n-octanol saturated with phosphate buffer. Subsequent procedures were as previously described.

3.3.4. n-Octanol/phosphate buffer partition coefficient of 6-MP, 6-TG, AZA and MAZA

Detailed conditions for procedures were described elsewhere (Chrzanowska et al. 2003b). For each compound the absorbance at λ_{max} against appropriate blank (phosphate buffer or n-octanol saturated with phosphate buffer) was recorded in both phases.

3.3.5. Apparent partition coefficients n-octanol/phosphate buffer

The concentrations of purine derivatives in aqueous and octanol phases were calculated from the Lambert-Beer law using determined molar absorption coefficients (ε) and recorded absorbances of purine derivatives in a suitable phase. Apparent partition coefficient, k′, was calculated from purines concentrations determined in phosphate buffer and in n-octanol phases.

3.3.6. Fraction of unionized species concentration (f_{HA})

A suitable purine f_{HA} fraction was calculated from Eq. (3):

$$f_{HA} = \frac{[H^+]}{[H^+] + K_a} \tag{3}$$

3.3.7. Specific partition coefficient n-octanol/phosphate buffer

Concentrations of unionized form in phosphate buffer ($[HA]_{buf}$) were calculated from Eq. (4):

$$[HA]_{buf} = ([HA]_{buf}) + [A^-]_{buf}) \cdot f_{HA} \tag{4}$$

using previously determined fraction f_{HA}.

Specific partition coefficient was calculated from Eq. (5):

$$k = \frac{[HA]_o}{[HA]_{buf}} \tag{5}$$

assuming the purine does neither dissociate nor associate in the octanol phase.

3.3.8. Mass balance

Initial masses of 6-MP, 6-TG, AZA, MAZA as well as their actual masses in n-octanol and phosphate buffer after partition were calculated from their molar concentration, molar mass and known volumes of phases.

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