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Biopharmaceutical characterization of some synthetic purine drugs

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Hydrophilic-lipophilic properties (water solubility, n-octanol/water partition coefficient, transport across membranes) of some mercaptopurines (6-MP, 6-TG, AZA and a new AZA derivative – metazathioprine (MAZA) were determined. MAZA is the most lipophilic compound due to low aqueous solubility and high *n*-octanol/water partition coefficient. The fluxes from the donor medium into the membrane and from the membrane into the acceptor medium are highest for MAZA as well. The partition coefficients of the other purines decrease in the order: AZA > 6-TG > 6-MP.

1. Introduction

The purine derivatives 6-mercaptopurine (6-MP), 6-thioguanine (6-TG) and azathioprine (AZA) have immunosuppresive and anticancer activities [1, 2]. Some are used in the treatment of leukemia (6-MP, 6-TG), as immunosuppressants and for treatment of diseases of auto-immunological origin (AZA) [3, 4]. Side effects of AZA are considerable and can limit therapeutic use. However, AZA has been employed for many years after kidney transplantation. Moreover, new AZA derivatives are under clinical investigation [5]. A methylated AZA derivative called MAZA is among those investigational drugs having a patented synthesis [6]. The kinetics of AZA and MAZA metabolism in human blood [7] as well as their mercaptolysis in presence of physiological thiols such as glutathione and cysteine [8] have been examined.

Biopharmaceutical characterisation of potential new drugs is crucial for predicting pharmacokinetic properties. Along this line it is necessary to characterise the drugs with respect to hydrophilicity/lipophilicity. Important parameters are water solubility, partition coefficient and membrane transport.

This article reports the water solubility, partition coefficients and transport across artificial membranes for some purine derivatives in order to understand the pharmacokinetic properties of these compounds. The literature contains only few data concerning the physical chemical properties of the title purine derivatives.

2. Investigations, results and discussion

As shown in Table 1 a solubility equilibrium was reached for the substances in buffered solutions within five hours. Furthermore, it could be observed that the solubility of all purine derivatives in an aqueous buffer solution at pH 7.4 is slightly higher than at pH 5.7. The pk_a values of 6-MP and AZA (7.70 and 7.90, respectively) favour a better solubility in buffer of pH 7.4 compared to the aqueous medium at pH 5.7. Therefore, 6-MP displays the best so-

Table 1: Solubility (S, $g \cdot l^{-1}$) of some purine derivatives in phosphate buffer (pH 5.7 and 7.4) at different equilibrium time (ET)

Purine derivative	$S \pm SD^*$ ($g \cdot l^{-1}$) Coefficient of variation (C.V.) (%)			
	pH 5.7		pH 7.4	
	ET (h)		ET (h)	
	5	24	5	24
6-MP	0.195 ± 0.007 (3.59)	0.196 ± 0.005 (2.55)	0.287 ± 0.012 (4.18)	0.355 ± 0.008 (2.25)
6-TG	0.109 ± 0.003 (2.75)	0.098 ± 0.002 (2.04)	0.137 ± 0.005 (3.65)	0.146 ± 0.003 (2.05)
AZA	0.217 ± 0.006 (2.76)	0.212 ± 0.006 (2.83)	0.271 ± 0.004 (1.47)	0.274 ± 0.006 (2.19)
MAZA	0.088 ± 0.003 (3.41)	0.092 ± 0.001 (1.09)	0.105 ± 0.001 (0.95)	0.108 ± 0.002 (1.85)

* n = 3

Table 2: Partition coefficient (P) in octanol-buffer system at 37 °C for some purine derivatives

Purine derivative	P ± SD* Coefficient of variation (C.V.) (%)	
	pH 5.7	pH 7.4
6-MP	0.677 ± 0.010 (1.48)	0.376 ± 0.006 (1.59)
6-TG	0.552 ± 0.005 (0.90)	0.446 ± 0.014 (3.14)
AZA	1.544 ± 0.046 (2.98)	1.007 ± 0.023 (2.28)
MAZA	1.740 ± 0.021 (1.21)	1.178 ± 0.042 (3.56)

* n = 10

Table 4: Molar absorptivity coefficient (ε) of some purine derivatives at λ_{max} in phosphate buffer at pH 5.7 (1) and 7.4 (2)

Purine derivative	λ_{max} (nm)	ε ± SD ($cm^{-1} \cdot l \cdot mol^{-1}$)	Coefficient of variation C.V. (%)	Coefficient of correlation (s)
6-MP	(1) 323.4	22362 ± 184*	0.82	0.9998
	(2) 321.3	20287 ± 32	0.16	0.9999
6-TG	(1) 342.0	22321 ± 172	0.77	0.9999
	(2) 341.1	20147 ± 47	0.23	0.9999
AZA	(1) 278.7	16795 ± 36	0.21	0.9999
	(2) 280.3	16387 ± 25	0.15	0.9999
MAZA	(1) 280.6	16469 ± 38	0.23	0.9999
	(2) 281.8	15672 ± 96	0.61	0.9999

* n = 6

lubility of all purines in the buffer pH 7.4, whereas the aqueous solubility of MAZA is 2.5-fold lower compared to AZA.

As shown in Table 2 differences in the partition coefficients were apparent when different buffer solutions were used. At higher pH (7.4) all purines showed lower partition coefficients in comparison to the buffer solution at pH 5.7 because of acidic dissociation.

As indicated by the partition coefficient, MAZA is the most lipophilic compound among the purines followed by AZA. As shown in Table 2 6-MP has a higher partition coefficient at pH 5.7 compared to 6-TG. In contrast 6-TG is more lipophilic at pH 7.4 than 6-MP. This is because 6-TG possesses a primary amine group, which is less dissociated at pH 7.4 than at pH 5.7. In the case of 6-MP there is the other possibility due to dissociation at pH 7.4, the solubility of 6-MP at pH 7.4 is greater than of 6-TG (Table 1).

As shown in Table 3 the most lipophilic purine derivative (MAZA) showed the highest flux both from the donor into the membrane and from the membrane into the acceptor. Due to different lipophilicity, the fluxes were different as well. More MAZA is able to penetrate into the membrane from the acceptor side. Therefore, MAZA has the highest membrane content.

AZA showed the second highest fluxes, followed by 6-MP. In contrast, 6-MP has a higher membrane content than AZA because the flux of 6-MP from the membrane into the acceptor compartment is lower than the flux of AZA.

On the other hand, no 6-TG could be detected in the acceptor compartment indicating that no 6-TG permeates from the donor across the membrane into the acceptor compartment.

Table 3: Flux and membrane content of some purine derivatives using dodecanol-collodion membranes

Purine derivative	Flux ± SD ($\mu g/cm^2 \cdot min$)		Membrane content ± SD (%)
	DM*	MA**	
6-MP	0.064 ± 0.008	0.012 ± 0.001	7.47 ± 1.13
6-TG	–	–***	–
AZA	0.099 ± 0.017	0.043 ± 0.003	4.98 ± 1.10
MAZA	0.274 ± 0.037	0.057 ± 0.009	18.58 ± 2.54

n = 4,
* Flux from donor compartment into the membrane,
** Flux from the membrane into acceptor compartment,
*** No 6-TG in the acceptor compartment could be detected

In conclusion, the following statements can be made:

Among the purines studied, 6-MP demonstrated the highest solubility in a phosphate buffer (0.196 ± 0.005) g l^{-1} and (0.371 ± 0.008) g l^{-1}, at pH = 5.7 and pH = 7.4, respectively. The solubility of MAZA was approximately 2.5-fold lower than that of AZA and amounted to (0.092 ± 0.001) g l^{-1} and (0.108 ± 0.002) g l^{-1}, at pH = 5.7 and pH = 7.4, respectively.

Introduction of the additional methyl group to imidazole ring of AZA augmented the more lipophilic property of MAZA. This was apparent by the highest n-octanol/phosphate buffer (pH = 7.4) partition coefficient (1.178 ± 0.042). Partition coefficients of the other purines followed the order: AZA > 6-TG > 6-MP at pH = 7.4.

6-TG possess a higher partition coefficient than 6-MP at pH 7.4. However, at pH 5.7 the opposite relationship exsists. The above phenomenon can be explained by the presence of the primary amine group in 6-TG but not in 6-MP.

MAZA has the highest membrane content because it has the highest flux both from the donor into the membrane and from the membrane into the acceptor.

3. Experimental

3.1. Materials

6-Mercaptopurine (6-MP), 6-thioguanine (6-TG) and azathioprine (AZA) were purchased from Carl Roth GmbH & Co., Karlsruhe, Germany; ether, ethanol, hydrochloric acid, phosphoric acid, NaOH, dodecanol were from Riedel-de-Haen AG, Germany. Collodion solution 4% w/w ether/ethanol was from Caelo, Hilden, Germany. MAZA was synthesized at the Department Organic Chemistry of the Faculty of Pharmacy in Poznań, Poland.

Stock solutions of the above compounds were prepared in a volumetric flask 50 ml of 0.01 g each dissolved in 5 ml 0.1 M NaOH and made up to the volume with distilled water. The stock solutions were diluted with the phosphate buffer of pH either 5.7 and/or 7.4 to obtain the final concentration of 3.525×10^{-5} M. The UV spectra of those solutions were recorded at the range 220–400 nm against a suitable phosphate buffer blank. The λ_{max} values were read (Table 4).

3.2. Molar absorptivity coefficients (ε)

The standard solutions of the above compounds were prepared from the above stock solutions at the concentration range from 3.525×10^{-5} to 0.325×10^{-5} M in the phosphate buffer pH 5.7 and/or 7.4. Those experiments were repeated twice and 5 standard solutions were always used (3 of each for every series). Standard curves were calculated for averaged results according to Beer's law.

The molar absorptivity coefficient (ε) was calculated as the slope of the above equation by means of the least square method and the data obtained are presented in Table 4.

3.3. Solubility

Solubilities of 6-MP, 6-TG, AZA and MAZA were measured in phosphate buffer pH 5.7 and 7.4 at 37 °C as a function of the equilibrium time at 5 h

and 24 h. Ten milligrams of the compound were weighed into screwed cap tubes and 5 ml of the above phosphate buffer were added. The tubes were placed in a shaking water bath at 37 °C. The above tubes were centrifuged 5 h and 24 h later at 3000 × g for 5 min and a suitable volume of a supernatant was withdrawn, diluted with the phosphate buffer of pH either 5.7 or 7.4 and the absorbances were recorded at λ_{max} of the appropriate compound against a suitable phosphate buffer blank. The concentrations of the above compounds were calculated from the Beer's law equation. The solubilities were calculated in $g \cdot l^{-1}$ from an average of 3 experiments done with each compound (Table 1).

3.4. n-Octanol/phosphate buffer partition coefficient

The partition coefficients of the above compounds were determined for n-octanol/phosphate buffer pH 5.7 and 7.4 at 37 °C. In screwed cap tubes were placed 4 ml of solutions of the above purines in the phosphate buffer of pH 5.7 or 7.4 of their concentration 3.525×10^{-5} M or 1.766×10^{-5} M and 4 ml of n-octanol. The tubes were placed in a shaking water bath at 37 °C. At the equilibrium time (5 h) the tubes were centrifuged. The n-octanol layer was separated and discarded and the absorbances were read for the aqueous phase at a suitable λ_{max} against the blank of the phosphate buffer of a suitable pH. The partition coefficients were calculated from 10 determinations each (5 samples for 3 concentrations of each purine) (Table 2).

3.5. Membrane transport

Transport across artificial membranes was measured with the permeation apparatus developed by Neubert and Fürst [9, 10]. The permeation cell consisted of 2 polyacryl (Piacryl® Piesteritz, Germany) cells (donor and acceptor cell) with a volume of 20 ml each separated by a collodion-dodecanol-membrane (for membrane preparation see [9, 10]). The permeation area of the membrane was 15.8 cm^2. The pH of the donor and of the acceptor media were 7.4. At selected time intervals (30, 60, 90, 120, 150, 180 min) samples were taken from the acceptor cell and the concentration was determined spectrophotometrically at 280 (AZA, MAZA), 320 (6-MP) and 340 nm (6-TG) using a Spectrophotometer M 40 (Carl Zeiss, Jena, Germany). The phosphate buffer solutions were placed in the cells maintained at 37 °C. When the experiment was finished the concentration in the donor compartment was measured in order to estimate the amount of the purine remaining in the lipid membrane. The flux was calculated according to eq. (1):

$$\text{Flux} = \frac{C_{AC} V_{AC}}{Ft} \,. \qquad (2)$$

Where are
C_{AC}: concentration in the acceptor cell,
V_{AC}: volume of the acceptor cell,
F: permeation area of the membrane and
t: time.

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