Azathioprine and 6-Mercaptopurine Alter the Nucleotide Balance in Endothelial Cells

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Abstract

Graft vascular disease after solid organ transplantation is a main complication that limits long-term survival of the graft. An injury of the endothelium and subsequent vascular response is considered to be responsible for smooth muscle cell hyperplasia with resulting luminal narrowing. What is less certain are the precise steps leading to endothelial injury and subsequent vessel disease. Since the immunosuppressive drug azathioprine is in clinical use due to its antiproliferative effect on lymphocytes, we were interested in how far it exerts effects on the vascular endothelium. Azathioprine and its metabolite 6-mercaptopurine, a potent inhibitor of purine salvage pathway enzymes, dose dependently led to decreased endothelial cell proliferation as well as to decreases in intracellular purine nucleotides adenosine-triphosphate and guanosine-triphosphate. By increasing the formation of the pyrimidine nucleotide uridine-triphosphate within 24 hours, azathioprine and its metabolite altered the endothelial nucleotide balance. Since not only the formation of toxic thio- and methylthiopurines (thio-guanosine-monophosphate, methyl-thio-inosine-monophosphate) was measured, the activity of the enzyme thiopurinemethyltransferase was induced (3.21±2.04 U per 10^9 cells, mean±SD). These findings indicate that the vascular endothelium plays an active role in the metabolization of the established immunosuppressant azathioprine that then exerts specific toxic effects on endothelial cells. © 1999 Elsevier Science Ltd. All rights reserved.

Key Words: Azathioprine; 6-mercaptopurine; Endothelial injury; Purine nucleotides

Abbreviations: GMP, guanosine-monophosphate; IMP, inosine monophosphate; GMP-S, GMP-synthetase; GMP-RED, GMP-reductase; IMP-DH, IMP dehydrogenase; HGPRT, hypoxanthine-guanine phosphoribosyltransferase; APRT, adenine phosphoribosyltransferase; PRPP, phosphoribosylpyrophosphate; TPMT, thiopurine methyltransferase; PNP, purine nucleotide phosphorylase; 5' NT, 5' nucleotidase; AK, adenosine kinase; 6-Me-thio-IMP, 6-methyl-thio-IMP; 6-MeMP, 6-methyl-mercaptopurine; GSH, glutathione; SAM, S-adenosyl-methionine; 6-MP, 6-mercaptopurine; 6-MPR, 6-MP ribonucleoside; 6-MPR, 6-methyl-mercaptopurine ribonucleoside.

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Azathioprine (AZA) is used as antileukemic and immunosuppressive drug. It is nonenzymatically converted to 6-mercaptopurine (6-MP), which displays a high immunosuppressive activity [1–3]. By interfering with lymphoid cell mitosis, azathioprine affects the division of activated B and T lymphocytes. Although the thiopurines formed during drug metabolization are reported to exert a selectivity in their functions, the actions of AZA are rather crude and responsible for the occurrence of myelotoxic side effects during therapy. AZA is first cleaved to 6-MP by reacting with SH-groups that are donated by glutathione.
(Figure 1). 6-MP is then taken up by the cell and converted into thio-inosinemonophosphate (thio-IMP) by the enzyme hypoxanthine-guanine-phosphoribosyltransferase (HGPRT) with phosphoribosyl-pyrophosphate (PRPP) as co-substrate [1, 4]. Thio-IMP can be converted into thio-guanine nucleotides by the enzymes inosine-5’-monophosphate dehydrogenase (IMP-DH) and guanosine-monophosphate-synthetase (GMP-S). The thionucleotides formed can be incorporated into RNA and DNA. Their incorporation into DNA results in delayed cytotoxicity [5–7] by inhibition of transcription of DNA and by induction of DNA damage. On the other hand, 6-MP can be methylated by the enzyme thiopurine methyltransferase and converted by adenine-phosphoribosyltransferase (APRT) to methyl-thio IMP. Another possibility for the formation of methyl-thio-IMP is the conversion of 6-MP via the enzymes purine-nucleotidase-phosphorylase (PNP), 5’-nucleotidase (5’NT) and adenosine kinase (AK). Methyl-thio-IMP is—by blocking the phosphoribosylpyrophosphate-aminotransferase—a strong inhibitor of the first step in the purine de novo synthesis [1,8]. This results in a diminished formation of purine nucleotides for DNA and RNA synthesis, causing cytotoxicity [9–12].

Graft vascular disease, a major problem after cardiac transplantation, is associated with atherosclerotic lesions after endothelial injury. Since AZA is in clinical use together with cyclosporine A and steroids for immunosuppression, we were interested whether AZA or 6-MP exert any cytotoxic effects on the vascular endothelium thereby possibly contributing to the development of graft sclerosis. Since humans have more endothelial cells (approximately $10^9$) than mononuclear cells (approximately $10^6$), the effects of AZA and 6-MP on the nucleotide content of human umbilical vein endothelial cells (HUVEC) were investigated with respect to metabolism and incorporation of these substances into endothelial nucleotide pools. A pharmacologically active role of the endothelium seems likely since it was shown that endothelial cells possess the enzymes for the salvage pathway, which are necessary for metabolizing 6-MP.

1. Materials and Methods

1.1. Cell Culture

HUVEC cultures were prepared using a modified standard technique [13]. Briefly, fresh human umbilical veins were filled with 0.1% collagenase solution and incubated at 37°C for 5 minutes. Thereafter the veins were perfused with M199 medium (Sigma, St. Louis, MO, USA) containing 20% fetal bovine serum (FBS; pH 7.4) (Gibco, Paisley, UK). Cells were collected from the perfusate by centrifugation at $200 \times g$ for 5 minutes and seeded into culture T-75 flasks precoated with human fibronectin (Upstate Biotechnology, Lake Placid, NY, USA). Cells were cultured in M199 medium (pH 7.4) containing 20% FBS, 100,000 U/L penicillin (Gibco), 100,000 µg/L streptomycin (Gibco), 100,000 U/L low molecular weight heparin (Sigma, Germany) and 30 mg/L bovine hypothalamic growth factor (Upstate Biotechnology). The confluent primary monolayers were washed twice with Dulbecco’s phosphate buffered saline (DPBS; Gibco). Cells were harvested by gentle scraping, centrifuged at $200 \times g$ and resuspended. The cell suspensions were transferred into each well of a 6-well
culture plate and cultivated for 6 days in a humidified incubator (37°C, 5% CO₂). Only cells from these first subcultures were used for the experiments described below. The cells were identified as endothelial cells by the typical contact-inhibited morphology, otherwise known as cobblestone [14] and by factor VIII (F VIII: vWF) staining [15]. No contamination by myocytes or fibroblasts was detected in the first subculture.

1.2. Incubation Experiments

For the incubation experiments, the culture medium was removed and the cell layers were washed gently with prewarmed (37°C) DPBS and equilibrated with 900 μl prewarmed (37°C) serum-free RPMI 1640 medium (without growth factors) for 1 hour at 37°C. The cells were then incubated for 4 up to 48 hours by adding 100 μl of a solution containing AZA (Sigma, St. Louis, MO, USA) or 6-MP (Sigma). AZA and 6-MP stock solutions were prepared using a DL-dithiothreitol [DTT] solution (Sigma) and 4 mol/L KOH (Merck, Darmstadt, Germany). The pH was adjusted with H₃PO₄; further dilutions were performed with RPMI 1640 medium, and the final concentrations yielded in the experiments were 0.0, 0.1, 0.5, 1.0, 2.0, 10.0, or 20 μmol/L. At the end of the incubation period supernatants were withdrawn and cell counts were determined by crystal violet staining or cell lysates (for the measurement of purine nucleotides) were prepared as described below.

1.3. Sample Preparation and Determination of Endogenous Nucleotides

At the end of the incubation period the supernatants were withdrawn. The incubation was stopped by the addition of 0.42 mol/L HClO₄ and rapid freezing. After thawing, the cell lysate was neutralized with 4 mol/L K₂HPO₄, centrifuged at 4°C at 3000×g and the supernatant analyzed by means of HPLC. Intracellular adenosine-triphosphate (ATP), adenosine-diphosphate (ADP), guanosine-triphosphate (GTP), guanosine-diphosphate (GDP), and uridine-triphosphate (UTP) were separated injecting 100 μl of the neutralized supernatant onto a Partisil 10 SAX column (Whatman, Maldstone, UK) by using a KH₂PO₄ gradient. Buffer A consisted of 0.05 mol/L KH₂PO₄ (pH 3.25) containing 3% acetonitrile and 0.1% sodium azide, buffer B of 3% acetonitrile, and 0.1% sodium azide, buffer C of 0.5 mol/L KH₂PO₄ (pH 5.25) containing 1.5% acetonitrile and 0.1% sodium azide. All buffers were preconditioned on a Chelex 100 column (Bio-Rad, Hertfordshire, UK) to remove any heavy metal traces. A gradient rising from 10% A, 90% B, 0% C to 44% A, 44% B, 12% C, and back to 10% A, 90% B, 0% C was used with a total run time of 78 minutes and an equilibrium delay of 10 minutes. The flow rate was 1.3 ml/min. The nucleotide concentrations were determined at a wavelength of 254 nm.

1.4. Sample Preparation and Determination of 6-methyl-thio-IMP and thio-GMP

HUVEC were incubated with 2 μmol/L AZA for 24 and 48 hours. At the end of the incubation period, the supernatants were withdrawn. Cells were washed with DPBS containing 0.005% (w/v) DTT, scraped on ice, transferred into cryo-tubes, frozen in liquid nitrogen, and lyophilized. After lyophilization the cells were resuspended with 100 μl 0.005% DDT and mixed with 150 μl 55 mmol/L EDTA (pH 10.5). After addition of 100 μl methanol and vortexing, 500 μl dichlormethane was added. Samples were centrifuged for 3 minutes at 4°C at 3000×g. 100 μl of the hydrous phase were mixed with 10 μl 1 mol/L NaHCO₃ (pH 10) and vortexed. After the addition of 25 μl 2% KMnO₄ samples were incubated at room temperature for 10 minutes. Thereafter, samples were treated with 10 μl 30% hydrogen peroxide for 5 minutes, centrifuged at 4°C and 3000×g for further 5 minutes and the supernatant analyzed by means of HPLC as described above. Methyl-thio purines and thio-purines were determined by fluorescence detection with an excitation wavelength of 329 nm and an emission wavelength of 410 nm.

1.5. Determination of Reduced (GSH) and Oxidized (GSSG) Glutathione Content in HUVEC

For the incubation experiments, the culture medium was removed and the cell layers were washed gently with prewarmed (37°C) DPBS and equilibrated with 900 μl prewarmed (37°C) serum-free RPMI 1640 medium (without growth factors) for
1 hour at 37°C. The cells were then incubated for 4 hours by adding 100 μl of a solution containing AZA or 6-MP. At the end of the incubation period supernatants were withdrawn and 750 μl 0.5 mol/L HClO₄ was added to the cells followed by rapid freezing. After thawing, the cells were scraped and 2000 μl of the pooled lysate was neutralized with 500 μl of a solution containing 0.5 mol/L triethanolamin and 2 mol/L K₂CO₃, centrifuged at 4°C at 3000×g and analyzed spectrophotometrically. For the measurement of GSH 2000 μl of the neutralized cell extract was mixed with 1000 μl 0.1% bovine serum albumine. Measurements of GSH were performed at 240 nm. A glyoxalase-1 solution (10 μl; Sigma) and 20 μl methylglyoxal (Sigma) were added (=A1). Again, absorption was measured after 12 minutes (=A2). Another 20 μl methylglyoxal was added, and the increase in absorption was measured after further 5 minutes (=A3). After the addition of 50 μl 11 mmol/L NADPH constant absorption was measured at 340 nm (=A4). Glutathione-reductase (10 μl) was added and the decrease in absorption was measured after 10 minutes (=A5). The molar coefficients of absorption for S-lactyl-GSH and NADPH together with the changes in absorption (A2-A1, A3-A2 = δ GSH, A4-A5 = δ GSSG) were used for calculating the levels of GSH and GSSG [16].

1.6. Determination of Thiopurine Methyltransferase (TPMT) Activity

Approximately 5×10⁶ cells in 0.05 mol/L MgCl₂ (Merck, Germany) dissolved in 0.5 mol/L Tris (hydroxymethyl)aminomethan/HCl (Merck, Germany) were incubated with 23 μmol/L S-adenosyl-L-¹⁴C-methionine (Amersham, Buckinghamshire, UK), 15 mmol/L GSH (Sigma), 50 μmol/L allopurinol (Sigma), 7.7 mmol/L 6-MP or dimethylsulfoxide (Sigma) and 24 mmol/L KH₂PO₄ for 90 minutes at 37 °C. At the end of the incubation the reaction was stopped by the addition of 0.5 mol/L sodium borate (pH 10). Thereafter the reaction mixture was filled up with 2.5 ml isoamyl alcohol (20% isoamyl alcohol in toluene), vortexed, and centrifuged at 700×g for 5 minutes. 1.5 ml of the organic phase was transferred into a liquid scintillation vial containing 0.5 ml alcohol and counted on a liquid scintillation counter (Rackbeta, LKB, Wallac, Turku, Finland).

Fig. 2. Line plots showing the influence of different concentrations of AZA (A) or 6-MP (B) on endothelial cell count. Asterisks indicate statistically significant (p<0.01) differences compared with untreated controls. Data points represent the mean percentages of 0 hour control cell count (n=5).

1.7. Statistical Analysis

Data are given as mean±SD. For determination of significance the results were subjected to a matched pairs t-test. All calculations were conducted using the statistical software package SAS/STAT (SAS Institute Inc., USA). A p value of less than 0.01 was considered statistically significant.

2. Results

2.1. Cell Proliferation

The influence of AZA and 6-MP on endothelial cell proliferation is given in Figures 2A and 2B. The treatment of the cells with increasing concen-
approximately 10% of the activity detectable in lymphocytes and comparable to that reported in platelets [17]. These results suggest that endothelial cells are able to methylate thio-IMP.

### 2.4. Effects of AZA and 6-MP on Endothelial Nucleotide Content

Intracellular ATP levels started to statistically significantly decline within 24 hours in presence of either 0.5 μmol/L AZA or 6-MP (Table 1). After 48 hours, even a dose of 0.1 μmol/L AZA decreased the ATP content significantly (Table 2). Concentrations ≥2.0 μmol/L AZA led to statistically significant decreases of intracellular GTP within 24 hours, whereas in presence of 6-MP this effect was first observed at doses ≥10.0 μmol/L. After 48 hours of treatment, however, both decreased GTP levels significantly using doses ≥2.0 μmol/L (Table 2). UTP levels increased during 24 hours in presence of 2–10 μmol/L AZA or 6-MP (Table 1). This effect could not be observed after 48 hours of incubation. Intracellular ADP of untreated HUVEC (0.3 ±0.1 nmol/10⁶ cells) was increased in presence of 2 μmol/L AZA after 24 and 48 hours (0.6 ±0.3 and 1.0 ±0.4 nmol/10⁶ cells, respectively), whereas GDP levels remained unchanged.

### 2.5. Formation of Thio-Nucleotides and Methyl-Thio Nucleotides

A formation of methyl-thio-IMP could be detected first after 24 hours upon incubation with either AZA or 6-MP. Thio-GMP production was also measured

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**Table 1. Influence of increasing concentrations of AZA or 6-MP (μmol/L) on endothelial nucleotide content during 24 hours**

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>AZA 0.1</th>
<th>AZA 0.5</th>
<th>AZA 1.0</th>
<th>AZA 2.0</th>
<th>AZA 10.0</th>
<th>AZA 20.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>15.7±0.9</td>
<td>14.7±0.8</td>
<td>14.0±0.7</td>
<td>13.3±0.5</td>
<td>9.3±0.6</td>
<td>9.5±0.6</td>
<td>10.0±0.7</td>
</tr>
<tr>
<td>GTP</td>
<td>2.1±0.3</td>
<td>2.0±0.4</td>
<td>2.2±0.1</td>
<td>1.7±0.4</td>
<td>1.6±0.2</td>
<td>1.0±0.1</td>
<td>1.1±0.3</td>
</tr>
<tr>
<td>UTP</td>
<td>1.7±0.2</td>
<td>1.7±0.3</td>
<td>1.5±0.2</td>
<td>1.6±0.3</td>
<td>2.2±0.2</td>
<td>2.3±0.2</td>
<td>1.9±0.3</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>6-MP 0.1</th>
<th>6-MP 0.5</th>
<th>6-MP 1.0</th>
<th>6-MP 2.0</th>
<th>6-MP 10.0</th>
<th>6-MP 20.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>15.7±0.9</td>
<td>14.4±1.0</td>
<td>13.3±1.2</td>
<td>13.4±0.9</td>
<td>13.2±1.0</td>
<td>11.3±0.8</td>
<td>11.1±0.6</td>
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<tr>
<td>GTP</td>
<td>2.1±0.3</td>
<td>2.2±0.2</td>
<td>2.2±0.4</td>
<td>1.8±0.1</td>
<td>2.3±0.3</td>
<td>1.5±0.1</td>
<td>1.2±0.1</td>
</tr>
<tr>
<td>UTP</td>
<td>1.7±0.2</td>
<td>1.6±0.3</td>
<td>1.3±0.3</td>
<td>1.7±0.2</td>
<td>2.1±0.1</td>
<td>2.2±0.1</td>
<td>2.0±0.2</td>
</tr>
</tbody>
</table>

Data are given as mean±SD in mmol/10⁶ cells (n=5).

* Statistically significant (p<0.01) compared with controls.
Table 2. Influence of increasing concentrations of AZA or 6-MP (µmol/L) on endothelial nucleotide content during 48 hours

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>AZA 0.1</th>
<th>AZA 0.5</th>
<th>AZA 1.0</th>
<th>AZA 2.0</th>
<th>AZA 10.0</th>
<th>AZA 20.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP (nmol/10^6 cells)</td>
<td>15.5±1.1</td>
<td>13.6±0.7a</td>
<td>12.1±0.7a</td>
<td>12.0±0.6a</td>
<td>9.7±1.1a</td>
<td>9.7±1.8a</td>
<td>10.9±1.2a</td>
</tr>
<tr>
<td>GTP (nmol/10^6 cells)</td>
<td>2.1±0.3</td>
<td>1.9±0.5</td>
<td>1.8±0.1</td>
<td>1.7±0.1</td>
<td>1.5±0.2a</td>
<td>1.2±0.1a</td>
<td>1.1±0.3a</td>
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<tr>
<td>UTP (nmol/10^6 cells)</td>
<td>1.7±0.1</td>
<td>1.7±0.2</td>
<td>1.6±0.1</td>
<td>1.6±0.3</td>
<td>1.9±0.1</td>
<td>1.6±0.1</td>
<td>1.6±0.2</td>
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<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>6-MP 0.1</th>
<th>6-MP 0.5</th>
<th>6-MP 1.0</th>
<th>6-MP 2.0</th>
<th>6-MP 10.0</th>
<th>6-MP 20.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP (nmol/10^6 cells)</td>
<td>15.5±1.1</td>
<td>14.9±1.2</td>
<td>12.2±1.5a</td>
<td>11.7±0.9a</td>
<td>9.9±1.5a</td>
<td>10.2±0.7a</td>
<td>10.4±0.5a</td>
</tr>
<tr>
<td>GTP (nmol/10^6 cells)</td>
<td>2.1±0.3</td>
<td>1.8±0.4</td>
<td>1.9±0.2</td>
<td>1.7±0.1</td>
<td>1.6±0.1a</td>
<td>1.5±0.1a</td>
<td>1.4±0.2a</td>
</tr>
<tr>
<td>UTP (nmol/10^6 cells)</td>
<td>1.7±0.1</td>
<td>1.8±0.2</td>
<td>1.8±0.1</td>
<td>1.6±0.2</td>
<td>1.8±0.1</td>
<td>1.7±0.1</td>
<td>1.6±0.3</td>
</tr>
</tbody>
</table>

Data are given as mean±SD in nmol/10^6 cells (n=5).
* Statistically significant (p<0.01) compared with controls.

after 24 hours, which was more pronounced during the incubation with AZA (Table 3).

3. Discussion

To study the influence of AZA and 6-MP on cell proliferation, endothelial nucleotide content and the formation of thio- and methylthiopurines, human endothelial cells were incubated with increasing concentrations of AZA and 6-MP over various time periods.

We were able to demonstrate that doses as low as 0.1 µmol/L AZA and 6-MP lead to decreased cell proliferation as well as to reduced intracellular ATP levels, whereas GTP levels were decreased after 24 hours by using a concentration of 2 µmol/L AZA or 10 µmol/L 6-MP. Higher concentrations in the incubation medium and/or longer incubation times led to a general decrease in purine nucleotides. These results indicate that both substances may actively be metabolized and incorporated into HUVEC. Since we were able to show in the present study that HUVEC possess the enzyme TPMT, it is obvious that 6-MP is not only converted to thio-IMP and thio-GMP but also methylated to 6-methylmercaptopurine ribonucleoside (6-methyl-MPR), which is further metabolized to methyl-thio IMP via AK. The active role of the endothelium in AZA metabolism is further supported by our finding that significant amounts of methyl-thio IMP beside thio GMP are formed. In leukocytes, 6-MP—as analogue of hypoxanthine—and 6-methyl-MPR are reported to act as substrates for HGPRT and APRT, key-enzymes of the salvage pathway, thereby leading to enzyme inhibition. Since in human macrovascular endothelial cells the salvage pathway predominates the de novo synthesis, inhibition of HGPRT and APRT by 6-MP and 6-methyl MPR [18] might be one explanation for the depletion of the adenine and guanine nucleotide pools. The more pronounced decrease of adenine nucleotides compared to guanine nucleotides could be due to an additional competitive inhibition of AK by 6-methyl-MPR. As a result of this, conversion of adenosine

Table 3. Influence of 2 µmol/L AZA or 6-MP on the formation of t-GMP and me-t-IMP

<table>
<thead>
<tr>
<th>pmol/10^6 cells</th>
<th>AZA: 0–6 hours</th>
<th>AZA: 24 hours</th>
<th>AZA: 48 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>t-GMP</td>
<td>0</td>
<td>99±21a</td>
<td>1010±223a</td>
</tr>
<tr>
<td>me-t-IMP</td>
<td>0</td>
<td>57±10a</td>
<td>48±9a</td>
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</table>

<table>
<thead>
<tr>
<th>pmol/10^6 cells</th>
<th>6-MP: 0–6 hours</th>
<th>6-MP: 24 hours</th>
<th>6-MP: 48 hours</th>
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<tbody>
<tr>
<td>t-GMP</td>
<td>0</td>
<td>171±33a</td>
<td>358±41a</td>
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<tr>
<td>me-t-IMP</td>
<td>0</td>
<td>78±21a</td>
<td>161±28a</td>
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</table>

Data are given as mean±SD (n=8).
* Statistically significant (p<0.01) compared with controls.
into AMP and subsequently into ATP cannot occur optimally. These metabolic changes also might account for the observed decrease in cell proliferation indicating that an intact salvage pathway is necessary for endothelial cell growth.

UTP levels were increased within 24 hours and not found to decrease even after 48 hours incubation with AZA or 6-MP. The observed increase in UTP could be a consequence of the inhibition of HGPRT and APRT leading to the accumulation of phosphoribosylpyrophosphate (PRPP). An increase of PRPP was reported for fibroblasts [19] and MOLT4 lymphoblasts [20] in presence of 6-methyl-MPR and 6-MP. A surplus of PRPP is used for salvage and de novo synthesis of pyrimidine nucleotides probably accounting for the observed rises of intracellular UTP.

The enhanced consumption of glutathione during breakdown of AZA to 6-MP as demonstrated in the present study could account for the more pronounced cytotoxic effects of AZA. First, glutathione is one of the most important intracellular antioxidants, and its use for the conversion of AZA should lower the antioxidative potential in HUVEC leading to cellular impairment. Second, during the new formation of glutathione ATP is consumed [21,22] contributing to adenine nucleotide depletion. Bergan et al. [23] who studied the kinetics of 6-MP and thioguanine nucleotides in renal transplant recipients during azathioprine treatment found out that the mean 6-MP plasma concentration reached after administration of azathioprine was 340±290 nmol/L (range 40–2760 nmol/L). These levels are in line with the concentrations shown to be effective in our study. The mean time to the maximal 6-MP plasma concentration described by Bergan et al. was 2.0±1.8 hours, which further corresponds to our in vitro data demonstrating glutathione consumption within 4 hours.

Our findings strongly suggest that the impairment of the endothelial adenine and guanine nucleotide metabolism during treatment with AZA or 6-MP does not seem to simply reflect a general unspecific cell damage and that these changes could contribute to the development of graft vascular disease as observed in long-term transplant recipients.

We are indebted to Mrs. Anneliese Nigisch for excellent technical assistance.

References

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