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ORIGINAL ARTICLE

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The response to cyclophosphamide in steroid-sensitive nephrotic syndrome is influenced by polymorphic expression of glutathion-S-transferases-M1 and -P1

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Abstract Glutathione-S-transferases (GST) play a central role in the inactivation of toxic drugs like cyclophosphamide (CP). These enzymes depict several polymorphisms with altered activity, and it has been shown that different polymorphisms influence the risk of malignancies and the outcome after chemotherapy. To prove the hypothesis that CP efficacy in children with nephrotic syndrome is influenced by polymorphic expression of GSTs, the genotype of 26 patients was analyzed and correlated with the outcome after CP treatment. All 26 children with steroid-sensitive nephrotic syndrome and frequent relapses or steroid dependency were treated with CP at a mean age of 6.7±4.0 years. CP was given in a dose of 2 mg/kg/day for 12±1 week. GST-M1, GST-P1 and GST-T1 polymorphisms were detected by PCR. In patients with GST-M1 null polymorphism, a significantly better rate of sustained remission was seen than in patients with the heterozygous or homozygous GST-M1 wildtype (0 versus 29%, P <0.01). In contrast, children with GST-P heterozygous or homozygous polymorphism had a significantly lower rate of sustained remission compared to homozygous wildtype (7 versus 38%, P <0.02). The GST-T1 genotype did not influence the outcome after CP treatment (P =0.32). Patients with the combination of GST-M1 null and GST-P1 wildtype did not relapse in 50%, compared to 6% in other children (P <0.01). We conclude that the polymorphic expression of GST-M1 and -P1 did significantly influence the long-term remission rate after CP treatment of steroid-sensitive nephrotic syndrome in children. Whereas GST-M1 null will increase cyclophosphamide efficacy, GST-P1 polymorphism seems to be related to enhanced susceptibility to further relapses.

Keywords Nephrotic syndrome · Cyclophosphamide · Children · Sustained remission · Glutathion S-transferase · Polymorphism

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Introduction

Children with steroid-sensitive nephrotic syndrome and imminent steroid toxicity because of frequent relapses or steroid dependency require an alternative treatment such as cyclophosphamide (CP) to achieve long-term remission [1]. However, the success of CP is difficult to predict, and a recent meta-analysis estimated that only one third of all children treated with CP went into sustained remission [2]. Evidence exists that the total dose per BSA [3], HLA-genotype [4] or status as a frequent relapser or being steroid dependent [3, 5] may influence the effect of CP.

The pharmacokinetic pathway of CP is complex and involves several enzymes, including cytochrom P 450 systems, aldehyde dehydrogenase and glutathione-S-transferases (GST) [6]. The GSTs are a group of cytosolic enzymes and play a central role in the detoxification of various substances and toxic drugs such as CP. These enzymes depict several polymorphisms with reduced enzyme activity: GST-M1 and GST-T1 exhibit a deletion polymorphism, which in the homozygous state (GST-M1 null and GST-T1 null) leads to an absence of enzyme

activity [7, 8]. GST-P1 polymorphism with an exchange of isoleucin to valine in position 105 leads to reduced enzyme activity in the heterozygous and homozygous condition [9].

It has been shown that the GST genotype predicts susceptibility to certain malignancies [10, 11, 12, 13] and the outcome after therapy in breast and ovarian cancer or childhood leukemia [14, 15, 16, 17]. To prove our hypothesis that CP efficacy in children with nephrotic syndrome is influenced by the polymorphic expression of GSTs, we analyzed the genotype of 26 patients and compared it to the outcome after CP treatment.

Patients and methods

Twenty-six children were included in the study (15 boys and 11 girls). These children were part of a larger group whose cases have been published recently [3]. Patients were included if they fulfilled the following criteria: (1) typical steroid-sensitive nephrotic syndrome with frequent relapses or steroid dependency, (2) minimal changes in the biopsy result, (3) no other treatment beside prednisone before CP, (4) CP treatment at a dose of 2 mg/kg/day for 12±1 weeks and (5) informed consent for GST polymorphism testing from the caregivers. The definition of relapse was heavy proteinuria (dipstick >+++) on 3 consecutive days requiring steroid medication [18]. Genotyping of GST polymorphisms was performed by multiplex PCR followed by restriction fragment length polymorphism (RFLP) analysis for GST-M1, GST-T1 [19] and GSTP1 [13]. PCR primers used were: GST-M1:5'-GAACTCCCT-GAAAAGCTAAAGC-3', 5'-GTTGGGCTCAAATATACGGTGG-3', GST-P1:5'-ACCCCAGGGCTCTATGGGAA-3', 5'-TGAGG-GCACAAGAAGCCCCT-3', GST-T1:5'-TTCCTTACTGGTCCT-CACATCTC-3' and 5'-TCACCGGATCATGGCCAGCA-3'.

PCR was carried out in a total volume of 100 μl containing 100 ng genomic DNA, 1 μM of each of the six primers, PCR buffer (50 mM KCl, 10 mM tris-HCl, pH 8.3, Perkin Elmer), 0.2 μM each of dNTPs, 2.5 mM $MgCl_2$ (Perkin Elmer), 5 units Amplitaq-Gold DNA polymerase (Perkin Elmer) and aqua ad 100 μl. Enzyme activation was done at 95°C for 10 min. Cycling conditions (35 cycles) were: denaturation at 95°C for 60 s, primer annealing at 62°C for 90 s and elongation at 72°C for 6 min. This method was used to distinguish between individuals with a homozygous deletion of GST-M1 or GST-T1 (GST-M1-null and GST-T-null) from homozygous or heterozygous for wildtype. In order to identify polymorphisms in the GST-P1 gene (homozygous Val/Val, heterozygots Ile/Val), the PCR product (10 μl) was digested with 20 units BsmA1 (New England BioLabs Inc.) at 55°C for 1 h. All products were separated on a 2% agarose gel and subsequently stained with ethidium bromide to visualize the bands.

Statistical analysis was done with SPSS 11.0 for Windows. The Mann-Whitney U-test was used to analyze differences between means, and log-rank to compare survival. Kaplan-Meyer analysis was performed to calculate the percentage of patients in complete remission. A P value of less than 0.05 was defined to indicate a significant difference.

Results

The mean age at the first presentation of nephrotic syndrome was 5.3±3.5 years (range 1.6–14.9), and the age at CP treatment was 6.7±4.0 years (range 2.4–20.4). Follow-up after CP treatment was 5.6±4.7 years. Sustained remission was seen in six children (23%); all others relapsed after a median interval of 0.6 years. Results of

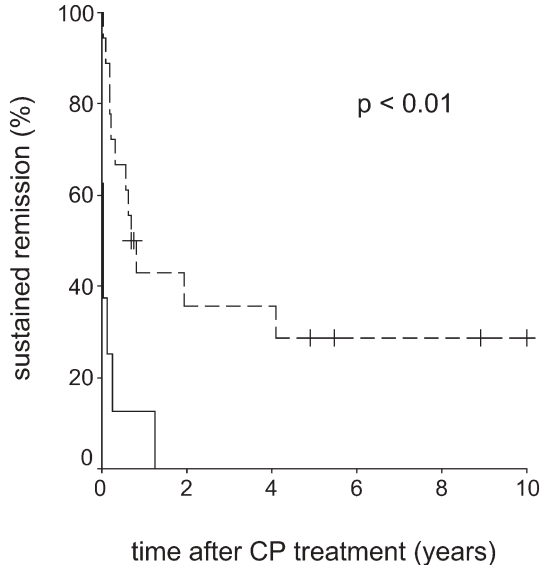

Fig. 1 Sustained remission in patients with GST-M1 null polymorphism (*dotted line*, n =18) and heterozygous or homozygous GST-M1 wildtype (*solid line*, n =8)

GST polymorphisms were: GST-M1 heterozygous or homozygous wildtype was detected in 18 (69%) and GST-M1 null in 8 children (31%). Eleven children had GST-P1 wildtype (42%); 15 (58%) were heterozygous or homozygous for the codon 105 polymorphism. GST-T1 heterozygous or homozygous wildtype was seen in 19 children (73%), and null-polymorphism in 7 (27%). These distributions of polymorphisms did not differ from the published incidence in the Caucasian population [7, 20, 21], albeit the number of patients is too small to be able to draw firm conclusions.

Cumulative sustained remission in children with GST-M1 null-polymorphism was significantly better (29 versus 0%, P <0.001) and median time to first relapse longer (0.7 versus 0.03 years, P <0.01) than in children with the homozygous or heterozygous wild type GST-M1 (Fig. 1). In contrast, children with heterozygous or homozygous GST-P1 polymorphism (Fig. 2) had a significantly worse remission rate than those with the wildtype GST-P1 (7 versus 38%, P <0.02) and a shorter time to first relapse (0.2 versus 0.8 years, P =0.06). The GST-T1 genotype did not show any obvious difference (Fig. 3). Patients who had the combination of GST-M1 null and GST-P1 wildtype (n =9) had the best sustained remission rate (50 versus 6%, P <0.01, Fig. 4). With respect to gender, age or total CP dosage per kilogram of bodyweight, no significant difference between groups with different GST genotypes could be detected.

Discussion

The efficacy of CP treatment in children with steroid-sensitive nephrotic syndrome and steroid dependency or toxicity is difficult to predict. Recent data suggest that the

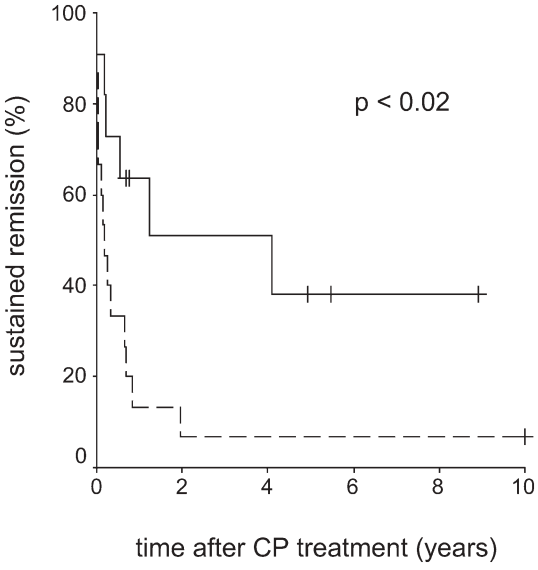

Fig. 2 Sustained remission in patients with GST-P1 wildtype (*solid line*, *n* =11) and heterozygous or homozygous GST-P1 codon 105 polymorphism (*dotted line*, *n* =15)

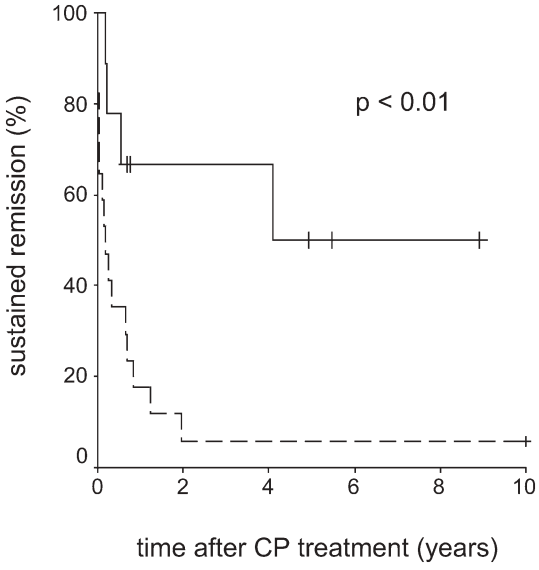

Fig. 4 Sustained remission in patients with the combination of GST-M1 null polymorphism and GST-P1 homozygous wildtype (*solid line*, *n* =9) and all other patients (*dotted line*, *n* =17)

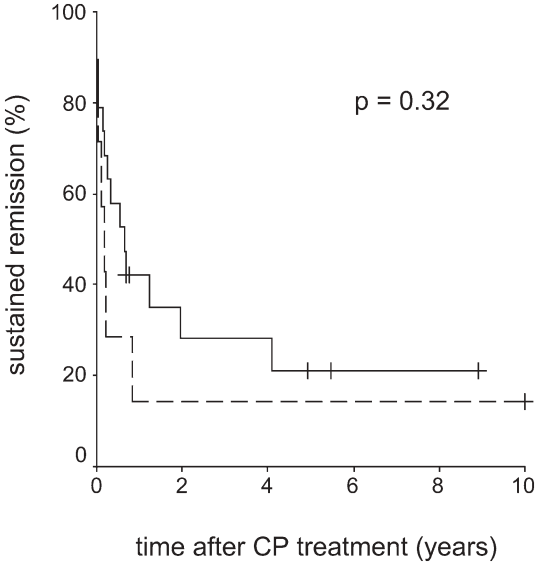

Fig. 3 Sustained remission in patients with GST-T1 null polymorphism (*dotted line*, *n* =7) and heterozygous or homozygous GST-T1 wildtype (*solid line*, *n* =19)

total dose per body surface area, but not per kilogram bodyweight [3], HLA [4] or status as a frequent relapser or steroid dependency [3, 5] may influence the outcome. GSTs are known to participate in detoxifying carcinogenic substances. Patients with reduced activity due to a polymorphism are known to have an increased risk of malignancies, and it has been speculated that this is due to a decreased clearance of carcinogenic substances [10, 11, 12, 13]. Besides naturally occurring toxins, GSTs are also involved in the complex pathways of drug metabolism including CP [6, 22, 23].

From studies with patients with CP treatment of malignancies, an influence of GST polymorphisms on survival in breast cancer [14, 15], ovarian cancer [16] or risk of relapse after childhood leukemia [17, 19] has been described.

Therefore, we speculated that GST polymorphisms might alter the course after CP in children with nephrotic syndrome as well. Albeit our study was retrospective and had a small cohort, we assume that the genetically determined ability to metabolize CP may influence the rate of sustained remission. However, results for different GST enzymes did show divergent results: GST-M null-allele (with reduced activity) patients did indeed have a significantly better rate of sustained remission after CP and a longer period until relapse. This can be explained by an enhanced CP exposure and thus better results. On the other hand, we found a reduced remission rate in children with polymorphic GST-P1. As GST-P1 is dominantly expressed in the kidney and not in the liver [24], it is tempting to speculate that GST-P1 is not primarily involved in CP metabolism, but is more responsible for susceptibility for further relapses. Patients with the combination of GST-M1 null and GST-P1 wildtype did have the most profit from CP therapy.

In conclusion we found evidence of an influence of the GST enzyme system on sustained remission after CP in children with steroid-sensitive nephrotic syndrome. As our study was retrospective and consisted of a small cohort, we propose further prospective studies to verify our findings. These studies need to incorporate pharmacokinetic and pharmacogenetic data and monitor the toxicity of CP to optimize cytotoxic treatment in childhood nephrotic syndrome.

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