Strict cysteamine dose regimen is required to prevent nocturnal cystine accumulation in cystinosis

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Abstract

Cystinosis is an autosomal recessive disorder, caused by mutations in the lysosomal cystine carrier cystinosin, encoded by the *CTNS* gene. The disease generally manifests with Fanconi syndrome during the first year of life and progresses towards end stage renal disease before the age of ten years. Cysteamine depletes intralysosomal cystine content, postpones the deterioration of renal function and the occurrence of extra-renal organ damage. Based on the pharmacokinetic data patients with cystinosis are advised to use cysteamine every 6 hours. The aim of this study was 1) to evaluate the cysteamine dose regimen in Dutch patients with cystinosis and 2) to determine morning polymorphonuclear (PMN) leukocyte cystine content 6 versus 9 hours after the last evening cysteamine dose.

Only 5/22 of Dutch cystinosis patients ingested cysteamine every 6 hours. Morning (8 a.m.) PMN cystine content in 11 examined patients was elevated 9 hours after 12.5-15 mg/kg evening cysteamine dose compared to the value 6 hours after the ingestion of the same dose (0.73±0.81 vs 0.44±0.52 nmol cystine/mg protein, p=0.02).

In conclusion, only the minority of Dutch cystinosis patients follows the recommended strict cysteamine dose regimen. We provide evidence that cysteamine has to be administered every 6 hours, including the night, as it has much better effect for maintaining low PMN cystine levels.

Keywords: cystinosis, cysteamine, dose regimen, compliance

Introduction

Cystinosis is a rare autosomal recessive disorder caused by a defect in the lysosomal cystine carrier cystinosin, encoded by the *CTNS* gene (1,2). Cystine accumulation due to impaired cystine exit out of the lysosomes in all tissues causes a multi-organ disease with kidneys being clinically first affected. In general patients with cystinosis manifest with poor growth and generalized proximal tubular dysfunction (Fanconi syndrome) during the first year of life and develop end stage renal disease before the age of 10 years. Longer survival of these patients due to renal transplantation reveals extra-renal organ damage, such as hypothyroidism, diabetes mellitus, hypogonadism, peripheral and central neuropathy, distal myopathy and retinal blindness, usually becoming apparent after the first decade (3,4).

In 1976 Thoene et al. demonstrated that the aminothiol cysteamine lowered leukocyte cystine content in cystinosis patients (5). Cysteamine depletes intralysosomal cystine via disulfide exchange reaction with cystine resulting in a formation of cysteine and cysteine-cysteamine mixed disulfide, which exit the lysosomes via cysteine and lysine carriers respectively, therefore bypassing the defective cystine carrier (6) (Figure 1). To diagnose cystinosis and to monitor cysteamine treatment, cystine determination in the leukocytes is mandatory. Although cystine content of leukocytes might not completely reflect the accumulation in the other organs, the levels below 1 nmol ½ cystine/mg protein (= 0.5 nmol cystine/mg protein) are considered to reflect an adequate cysteamine dose (3,4,7). Cysteamine treatment slows the deterioration of renal function, improves growth and should be continued also after renal transplantation in order to postpone the occurrence of extra-renal damage (3,4,7,8). Unfortunately, renal Fanconi syndrome generally remains irreversible and some of the patients still develop renal insufficiency and extra-renal complications, despite cysteamine treatment.

Because intracellular leukocytes cystine content decreases to the minimal levels ~ 2 hours after cysteamine ingestion and than returns to pre-dose levels 6 hours after the drug intake, the adequate cysteamine dose regimen implies the administration of the drug every 6 hours (10,11). This dose regimen, however, is extremely difficult to follow, especially at night. We assumed that the nocturnal cystine accumulation might be partially responsible for the ineffectiveness of cysteamine therapy in some patients and therefore evaluated the current cysteamine dose schedule in a Dutch cohort of cystinosis patients. Additionally, we compared morning polymorphonuclear (PMN) leukocyte cystine content after 6 versus 9 hours night pause following the last evening cysteamine ingestion. PMN leukocytes were used instead of mixed leukocyte preparations as cystine preferentially accumulates in these cells and not in the lymphocytes (12,13).

Patients and methods

Questionnaire

Twenty-two patients aged 14.7±9.7 years, 14 males, filled out the questionnaire concerning the schedule of cysteamine bitartrate (Cystagon^{R}) intake. The diagnosis of cystinosis was made in all patients, presenting with renal Fanconi syndrome, by determining of elevated PMN cystine content and finding of corneal cystine crystals.

Comparison of morning polymorphonuclear cystine content 9 versus 6 hours night pause after evening cysteamine intake

In eleven compliant patients, after signing informed consent, 12.5-15 mg/kg cysteamine bitartrate was administered 4 times daily:

1) during the first week at 8 a.m. - 2 p.m. - 8 p.m. - 2 a.m.
2) during the second week at 8 a.m. - 1 p.m. - 6 p.m. - 11 p.m.

At the end of each week blood samples were taken at 8 a.m. for PMN cystine dosage prior to the ingestion of the first morning cysteamine dose.

Intracellular cystine content of PNM cells was determined by HPLC as described previously and expressed in nmol cystine/mg protein (cystine concentration x 2 = ½ cystine concentration) (13,14).

Statistical analysis

Data are presented as mean ± SD. To compare data between patients following strict (every 6 hours) Cystagon^{R} dose schedules and those not taking the drug during the night, unpaired student t-test was applied. To compare morning intracellular PMN cystine values 6 versus 9 hours night pause after the last evening Cystagon^{R} dose, paired student t-test was applied. Values were considered statistical significant at $p<0.05$.

Results

Questionnaire

Five patients (23%) followed the strict Cystagon^{R} dose regiment (group 1). Seventeen patients (group 2) received Cystagon^{R} only during the wake time. Patients of group 1 were younger compared to those of group 2 (7.5$\pm$2.7 versus 15.8$\pm$9.2 years, p=0.004). Daily Cystagon^{R} dose was not different between the two groups. The night pause between the last evening/night and the first morning Cystagon^{R} ingestion was significantly longer in patients from group 2 (8.9$\pm$2.0 versus 6.1$\pm$0.7 hrs, p=0.001). Mean PMN cystine content (the average of all determinations during 2004), determined 5-6 hours after Cystagon^{R} ingestion, was significantly higher in patients of group 2 compared to group 1 (0.6$\pm$0.3 versus 0.37$\pm$0.13 nmol cystine/mg protein, p=0.02). No relation between the age of the patients and mean PMN cystine content could be detected.

Intracellular PMN cystine content was determined 4 times yearly in all patients of group 1. In patients of group 2 it was determined 4 times yearly in eleven, 3 times in two and 2 times in four patients.

Comparison of morning polymorphonuclear cystine content 9 versus 6 hours after evening cysteamine intake

In eleven examined patients morning PMN cystine content after 9 hours night pause following the evening cysteamine dose (12.5-15 mg/kg) was significantly elevated compared to the PMN content 6 hours after the ingestion of the same dose (0.73$\pm$0.81 versus 044$\pm$0.52 nmol cystine/mg protein, p=0.02) (Figure 2).

Discussion

Cysteamine is the only available cystine-depleting drug, postponing the deterioration of the renal function, improving growth and delaying the occurrence of extra-renal complications in patients with cystinosis. To achieve the maximal effectiveness of the treatment the following recommendations have to be followed (7,15):

1) starting cysteamine as early in life as possible (target dose 60-90 mg/kg/day or 1.3 - 1.95 g/m^2/day) (3,4,7)
2) administering cysteamine every 6 hours
3) frequent monitoring of leukocyte cystine content
4) obtaining blood for cystine determination 5-6 hours after cysteamine ingestion.

The most difficult recommendation to follow is to administer cysteamine every 6 hours, which means that at least one cysteamine dose should be taken during the night.

The nocturnal accumulation of cystine in patients, do not following the strict 6 hours dose regimen might be responsible for the relative ineffectiveness of the treatment.

In this study we evaluated actual cysteamine treatment schedule in 22 Dutch patients with cystinosis. The medical advice to administer cysteamine every 6 hours was respected only by the minority of the patients (23%). In patients following the strict cysteamine dose regimen, average PMN cystine content was significantly lower compared to the other patients, despite the fact that their daily cysteamine doses were comparable.

Despite the evident pharmacokinetic data (10,11), many patients and even many physicians do not realize that 2-3 hours extra night pause may cause a significant cystine accumulation.

Our study demonstrates that the administration of the same daily cysteamine dose distributed equally during 24 hours results in significantly lower morning PMN cystine content, compared to the “only wake-time” regimen, applied by the majority of the patients.

Although it is unknown whether leukocyte cystine content reflects cystine accumulation in the other tissues, it can be suggested that in patients ingesting cysteamine only during the wake time, nocturnal accumulation of cystine may occur in the other organs.

Well preserved creatinine clearance in two siblings 15 and 8 years old with infantile cystinosis adequately treated with cysteamine, starting from the early age, supports the idea that every 6 hours dose regimen is necessary for the preservation of renal function (16).

All well-treated patients from our Dutch cohort were relatively young, meaning that their parents were responsible for the compliance with therapy. During the puberty it is often getting difficult to convince the patient to continue with strict cysteamine dose regimen, especially taking into account the annoying side affect of the drug such as bad breath odor and gastro-intestinal discomfort. The latter side effect, caused by an increased secretion of the gastric acid after cysteamine ingestion, can be successfully treated by proton pump inhibitors (17). Some breath odor improving drugs such as chlorophyll or essential oils (Breath Assure^{R}) are used by some cystinosis patients, helping to improve their compliance.

Interestingly, in all patients following strict dose regimen PMN cystine determination was performed 4 times per year compared to the less frequent measurements in 7/18 of the other patients, suggesting better awareness and responsibility of the physicians treating these patients.

In conclusion, cysteamine treatment is effective in reducing cystine accumulation in cystinosis when taken on regular basis. Hereby we provide an additional evidence that cysteamine has much better effect in maintaning low PMN cystine levels when taken every 6 hours, including the night.

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Table 1. Clinical data on Dutch patients following strict (every 6 hours) Cystagon^{R} dose regiment (Group 1) and patients receiving Cystagon^{R} only during the wake time (Group 2).

	Group 1	Group 2	P
	n=5	n=17	
Age (years)	7.5 $\pm$ 2.7	15.8 $\pm$ 9.2	0.004
Cystagon^{R} dose (mg/kg/day)	62 $\pm$ 16	53 $\pm$ 11	0.3
Night pause (hrs)	6.1 $\pm$ 0.7	8.9 $\pm$ 2.0	0.001
Average PMN cystine (nmol/mg protein)	0.37 $\pm$ 0.13	0.60 $\pm$ 0.30	0.02

Legends for figures.

Figure 1. Disulfide exchange reaction between cysteamine and cystine, resulting in formation of free cysteine and cysteine-cysteamine mixed disulfide, exiting the lysosome via cysteine and lysine carriers respectively.

Figure 2. Morning PMN cystine content (mean+SD) in 11 examined patients 6 versus 9 hours night pause following the last evening/night cysteamine ingestion.