ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. XXIII, N 1, 16 SECTIO DDD 2010

[1]Department of Neurology, [2]Department of Biochemistry and Molecular Biology,
Medical University of Lublin

KATARZYNA ŁOBEJKO[1], KRYSTYNA MITOSEK-SZEWCZYK[1],
JACEK KURZEPA[2], JERZY BERBECKI[1], ZBIGNIEW STELMASIAK[1]

Bilirubin and uric acid serum levels in patients with relapsing-remitting multiple sclerosis after the first phase of cladribine treatment

Stężenie bilirubiny i kwasu moczowego w pierwszej fazie leczenia postaci nawrotowo-remisyjnej stwardnienia rozsianego za pomocą kladrybiny

Neurodegeneration occurring in multiple sclerosis (MS) is mediated by oxidative stress and excitotoxicity, therefore the endogenous antioxidant species as uric acid (UA) and bilirubin (Bil) can play a key role in MS pathogenesis [7]. Different studies emphasize the significance of UA in the MS development; however, it is not known exactly whether and how UA is involved in MS pathogenesis. Some results suggest that UA might reflect disease activity or a treatment response in MS [8]. Previously, UA was shown to be a strong peroxynitrite scavenger – the common initiating factor of oxidative stress [4]. Unfortunately, data concerning the UA serum level during the MS course are ambiguous. Some studies indicated the decrease of UA serum level; however, other authors demonstrated that all purine compounds, including UA, are elevated in biological fluids of MS patients [1]. Bil was recognized as a potent antioxidant of physiological importance with additionally powerful immunomodulatory properties. In contrast to other similar antioxidants, Bil inhibits polyclonal T cell responses. Lastly, treatment with Bil effectively suppress experimental autoimmune encephalomyelitis in SJL/J mice [11]. Different immunomodulatory therapies can affect the oxidative status of MS patients [8]. Cladribine (2-chlorodeoxyadenosine, 2CDA) is a deaminase-resistant deoxyadenosine analogue that selectively reduces lymphocyte counts [10]. Given its toxicity towards lymphocytes and its corresponding immunosuppressive effects, it has been efficaciously studied in a variety of hematologic malignancies and recently in MS [15]. The randomized study evaluating cladribine in relapsing forms of MS showd that it was well tolerated and associated with a favorable safety profile [16]. According to our knowledge, there is no information about the influence of cladribine on Bil and UA serum level in MS; however, previously it was noticed that nucleoside analogue can modify the oxidative stress in human prostate cancer [12]. Our goal was to find the relationship of cladribine treatment with Bil or UA serum levels in MS patients.

MATERIAL AND METHODS

The study group consisted of twenty patients aged 24–45 years (12 female patients) randomly selected from the MS subjects treated with cladribine at the Department of Neurology, Medical University of Lublin, according to the modified protocol described previously [16]. The modification concerned the reduction of the dose of cladribine per course from 25 to 20 mg. Patients with active MS (confirmed by MRI of the brain) and Expanded Disability Status Scale (EDSS) scores of 1.0–7.0, who had received neither immunomodulatory nor immunosuppressive therapy, were enrolled into the cladribine treatment. The exclusion criteria were; (a) a relapse within the preceding 3 months, (b) cytopenia, (c) hepatic or renal impairment, (d) recurrent infections or required treatment for a chronic disease. Patients were scheduled to receive seven monthly courses of cladribine at a dosage of 5 mg/ day s.c., administered for four consecutive days for the first 6 months and an additional course 3 months later. Our observation concerned the first three months of treatment. Neurological examinations and venous blood samples were obtained before each treatment course and 3 months after the last dose. The progress of MS was evaluated at the same three time-points with EDSS.

Age and sex-matched control group consisting of twenty healthy donors was included to the study. Blood samples in the control group were obtained only once. The Bil and UA levels were established on the basis of patients' medical histories. Briefly: the blood samples obtained from the study group were referred to hospital laboratory. Commercially available reagents (Cormay, Lublin, Poland) were applied to automatic measurement of total Bil and UA serum levels with the usage of colorimetric method in accordance with the producer's instruction. The study protocol was approved by the Ethical Committee of the Medical University of Lublin (Poland).

The ANOVA and Linear regression were applied for statistical analysis. Kolmogorov-Smirnov test was used to check the Gaussian distributions. Statistically significant values were considered when $p < 0.05$. Statistical analysis was performed with the use of the computer assisted statistical program InStat v. 3.06. GraphPad Software (La Jolla, CA, USA).

RESULTS AND DISCUSSION

The average EDSS score of study group (5.60 ± 0.97) did not change during the study period. The means of Bil and UA serum levels decreased gradually during the first phase of cladribine treatment; however, the relatively high values of standard deviation caused that the decline of UA and Bil serum level was insignificant ($p > 0.05$ for both, ANOVA). The Bil and UA level did not differ between control and study groups (Table 1 and Fig. 1). However, the inverse correlation of mean Bil serum level with EDSS score was observed ($r = -0.53$, $p = 0.01$, linear regression; Fig. 2). An analogous relationship between UA and EDSS score was not noticed ($r = 0.05$, $p > 0.05$, linear regression).

Will I recover? MS patients very often put such a simple question. The new drugs, many researches and a large number of scientists which take up the MS issue bring the hope for patients suffering from this disease. However, the proper therapy should consider the disease causes, which are still unknown. An imbalance in oxidative status can be a candidate for initiating factor of numerous neurological disorders [5, 7]. The endogenous antioxidative compounds also seem to play a crucial role

in the MS pathogenesis. Unfortunately, there are relatively scarce data concerning the role of UA and Bil in the involvement of MS. Numerous natural compounds can change the serum levels of Bil and UA [9]. According to the literature, the cladribine administration can rise UA serum level [14]. Strong antioxidative properties of UA can play a significant role in the balance of oxidative status during MS [4]. High UA serum level might give an additional benefit of cladribine treatment in MS besides the decrease of lymphocyte count. On the other hand, some studies emphasized UA involvement in the inflammatory process [2]. UA was shown to be the endogenous inductor of interleukin-1beta (IL-1β) in the lung inflammation and fibrosis [6]. IL-1β together with tumor necrosis factor alpha (TNF-α) is the cytokine which initiates the inflammatory response also during MS (see Carrieri et al., 1992 for review). Like UA, Bil with its antioxidative and immunomodulatory properties seems to bring the benefit in MS development [11]. Previously, the abnormal values of different liver tests were observed in MS patients [17]. In our study the serum levels of Bil and UA did not differ between MS patients and healthy individuals. However, the inverse relationship between mean Bil serum level versus EDSS score confirmed the connection of antioxidative species with the neurological status of MS patients. The cladribine influence on UA and Bil serum levels was insignificant in our study.

Table 1. The profiles of bilirubin (Bil) and uric acid (UA) (both parameters expressed in μmol/L (mean, SD)) in patients' sera during the first phase of cladribine treatment (three monthly courses). A statistically insignificant decrease was observed for UA and Bil serum levels (ANOVA, $p > 0.05$)

	Control	Course I	Course II	Course III	ANOVA
Bil	9.94 (3.01)	10.78 (3.73)	9.98 (2.95)	9.12 (3.74)	p = 0.42
UA	301.05 (69.03)	293.83 (72.57)	289.67 (75.54)	239.70 (67.21)	p = 0.09

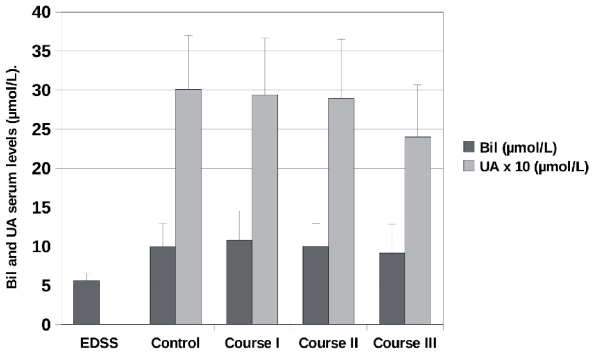

Fig. 1. UA and Bil serum levels (mean, SD) during the first phase of cladribine treatment in comparison with control values. Expressed UA values have to be multiplied by 10. UA and Bil levels did not significantly fluctuate during the study period. EDSS values did not change in any patients during the study period

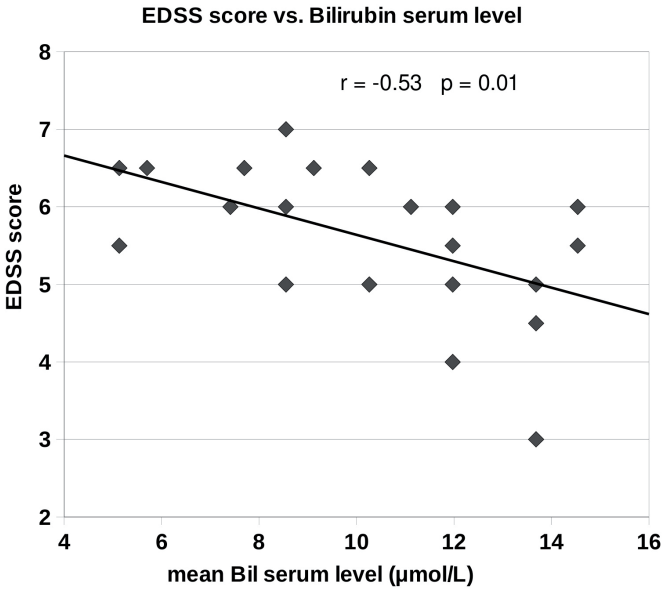

Fig. 2. The negative correlation between EDSS score *vs.* serum Bil level (linear regression)

CONCLUSIONS

The cladribine treatment does not affect UA and Bil serum levels during the first phase of cladribine treatment. The inverse correlation of mean Bil serum level with neurological status of MS patients suggests the involvement of internal antioxidant compound to the MS pathogenesis.

DECLARATION OF INTEREST. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

1. Amorini A. M., Petzold A., Tavazzi B. et al.: Increase of uric acid and purine compounds in biological fluids of multiple sclerosis patients. Clin. Bioch., 42, 1001, 2009.
2. Bandukwala F., Huang M., Zaltzman J. S. et al.: Association of uric acid with inflammation, progressive renal allograft dysfunction and post-transplant cardiovascular risk. Am. J. Cardiol., 103, 867, 2009.
3. Carrieri P.B., Maiorino A., Provitera V. et al.: Cytokines in the pathogenesis of multiple sclerosis. Acta Neurol. Napoli, 14, 333, 1992.
4. Dujmovic I., Pekmezovic T., Obrenovic R. et al.: Cerebrospinal fluid and serum uric acid levels in patients with multiple sclerosis. Clin. Chem. Lab. Med., 47, 848, 2009.
5. Ersoy M. A., Selek S., Celik H. et al.: Role of oxidative and antioxidative parameters in etiopathogenesis and prognosis of panic disorder. Int. J. Neurosci., 118, 1025, 2008.

6. Gasse P., Riteau N., Charron S. et al.: Uric acid is a danger signal activating NALP3 inflammasome in lung injury inflammation and fibrosis. Am. J. Resp. Crit. Care Med., 179, 903, 2009.
7. Gonsette R.E.: Neurodegeneration in multiple sclerosis: the role of oxidative stress and excitotoxicity. J. Neurol. Sci., 274, 48, 2008.
8. Guerrero A.L., Martín-Polo J., Laherrán E. et al.: Variation of serum uric acid levels in multiple sclerosis during relapses and immunomodulatory treatment. Eur. J. Neurol., 15, 394, 2008.
9. Karadi R. V., Palkar M. B., Gaviraj E.N. et al.: Antiurolithiatic Property of Moringa oleifera Root Bark. Pharm Biol., 46, 861, 2008.
10. Langtry H. D., Lamb H. M.: Cladribine: a review of its use in multiple sclerosis. BioDrugs, 9, 419, 1998.
11. Liu Y., Li P., Lu J., Xiong W. et al.: Bilirubin possesses powerful immunomodulatory activity and suppresses experimental autoimmune encephalomyelitis. J. Immunol., 181, 1887, 2008.
12. Minelli A., Bellezza I., Tucci A. et. al.: Differential involvement of reactive oxygen species and nucleoside transporters in cytotoxicity induced by two adenosine analogues in human prostate cancer cells. Prostate, 69, 538, 2009.
13. Peng F,. Zhang B., Zhong X. et al.: Serum uric acid levels of patients with multiple sclerosis and other neurological diseases. Mult. Scler., 14, 188, 2008.
14. Schonwald S.: Antineoplastic drugs. In C.D. Richard (ed.), Medical Toxicology (p. 502), Lippincott Williams & Wilkins, Philadelphia 2004.
15. Spurgeon S., Yu M., Phillips J. D. et al.: Cladribine: not just another purine analogue? Expert Opin. Inv. Drug, 18, 1169, 2009.
16. Stelmasiak Z., Solski J., Nowicki J. et al.: Effect of parenteral cladribine on relapse rates in patients with relapsing forms of multiple sclerosis: results of a 2-year, double-blind, placebo-controlled, crossover study. Mult. Scler., 15, 767, 2009.
17. Tremlett H., Seemüller S., Zhao Y., et al.: Liver test abnormalities in multiple sclerosis: findings from placebo-treated patients. Neurology, 67, 1291, 2006.

SUMMARY

Bilirubin (Bil) and uric acid (UA), endogenous antioxidant compounds, can be involved in the pathogenesis of multiple sclerosis (MS). Our goal was to find the influence of 2-chlorodeoxyadenosine (cladribine), applied for MS treatment, on Bil and UA serum levels. Twenty patients aged 24–45 years (12 female patients) with relapsing-remitting (RR) MS were enrolled. The observation period covered the first phase (three months) of cladribine infusion. The drug was administrated monthly at a dosage of 5 mg/ day s.c. for four consecutive days. Neurological examination (Expanded Disability Status Scale, EDSS) and venous blood samples were obtained before each treatment. The cladribine influence on UA and Bil serum levels was statistically insignificant. The average EDSS score of the study group (5.60 ± 0.97) did not change during the study period. The inverse correlation of mean Bil serum level with EDSS score was observed (r = -0.53, p = 0.01, linear regression). An analogous relationship between UA and EDSS score was not noticed (r = 0.05, $p > 0.05$, linear regression). The inverse relationship between mean Bil serum level versus EDSS score confirmed the relationship of antioxidative species with the neurological status of MS patients.

STRESZCZENIE

Bilirubina (Bil) i kwas moczowy (KM), wewnętrzne związki przeciwutleniające, biorą udział w patogenezie stwardnienia rozsianego (SR). Celem pracy było znalezienie wpływu 2-chlorodeoxyadenosy (kladrybiny) na stężenie Bil i KM surowicy krwi pacjentów z postacią nawrotowo-remisyjną SR. Do badania zostało włączonych dwudziestu pacjentów w wieku 24–45 lat (12 kobiet, 8 mężczyzn). Okres obserwacji obejmował pierwszy etap (trzy miesiące) leczenia kladrybiną. Lek podawano raz na miesiąc w dawce 5 mg / dobę przez cztery kolejne dni. Badanie neurologiczne (Expanded Disability Status Scale, EDSS) i próbki krwi żylnej były uzyskiwane przed każdym cyklem leczenia. Wpływ kladrybiny na stężenie KM i Bil był statystycznie nieistotny ($p>0.05$). Średni EDSS wynik grupy badanej ($5,60 \pm 0,97$) nie zmienił się w okresie badania. Stwierdzono ujemną korelację średniego stężenia Bil z wartościami EDSS ($r = -0,53$; $p = 0,01$; regresja liniowa). Analogiczne relacje między KM i EDSS nie zostały zauważone. Odwrotna zależność pomiędzy stężeniem Bil oraz stanem neurologicznym pacjentów wskazuje na pozytywny wpływ związków antyoksydacyjnych na przebieg SR.