Beckwith-Wiedemann Syndrome: Potassium Ascorbate with Ribose Therapy in a Syndrome with High Neoplastic Risk

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Abstract. Background: Beckwith-Wiedemann Syndrome (BWS) is a genomic imprinting disorder characterized by overgrowth and increased risk of malignancy. We studied the oxidative stress (OS) pattern of our patients with BWS and administered, for the first time, potassium ascorbate with ribose (PAR) once a day as long-term therapy in order to correct the effects induced by free radicals. Patients and Methods: We describe the clinical features of three patients examined every three months in our clinic. OS was ascertained by measuring a panel of OS biomarkers: non-protein-binding iron, total hydroperoxides, advanced oxidation protein products, isoprostanes, carbonyl groups and thiols. After the presence of OS was established, treatment with PAR was started at the dosage of 300 mg of Potassium Bicarbonate and 150 mg of Ascorbic Acid in aqueous solution and changes occurring in OS biomarkers were followed dosing every three months. Results: Our patients showed higher levels of OS biomarkers than controls at the time of diagnosis. There was a reduction in OS biomarker values for all three patients with treatment. No primary or secondary neoplastic disease was observed in 9 months of follow-up. Conclusion: This is the first report showing OS occurring in BWS. No drug until this report has been published showing efficacy against OS in any cancer. Given the limited number of patients, care must be taken to mitigate enthusiasm. We are collecting data for a large number of BWS patients to confirm these preliminary results.

Beckwith-Wiedemann syndrome (BWS) is a genomic imprinting disorder, with a frequency of 1 case per 13,700 births, characterized by overgrowth and increased risk of malignancy, first described by Beckwith in 1963 and subsequently by Wiedemann in 1964. BWS is associated with abdominal wall defects, macroGLOSSia, pre and postnatal overgrowth, neonatal hypoglycemia and visceromegaly. Patients with BWS also have an increased risk of developing embryonal tumors in childhood such as Wilm’s tumor, hepatoblastoma, neuroblastoma, adrenocortical carcinoma and rhabdomyosarcoma (1, 2). Thus the early diagnosis of this syndrome is important for the prevention of hypoglycemia-induced damage as well as for the risk of malignancy in childhood.

Recent literature showed a direct link between oxidative stress (OS) injury of human cells and cancer development. Carcinogenesis is a complex multistep process requiring an accumulation of changes before a cell becomes malignant. DNA damage is considered to be the focus in carcinogenesis and OS has a potential role in inducing mutations, such as activation of proto-oncogenes and inactivation or loss of tumor suppressor genes (3, 4). Cells show a wide range of responses under exposure to free radicals (FR), ranging from increased proliferation, prevention of cell division, senescence, necrosis and apoptosis. Thus, the role of oxidative DNA damage could be relevant in cancer etiology (5, 6). Taking into account the link between BWS and cancer development in childhood, we first assessed the characteristics of OS in BWS. Once OS was ascertained, we tested the hypothesis that the daily administration of potassium ascorbate with ribose (PAR) as antioxidant drug would reduce OS. We report here on three female patients, followed from 1999 to March 2011.

Patients and Methods

Patient 1. A female, born from twins delivered at the 35th week of pregnancy, presented overgrowth at birth, diastasis recti, facial nevus flammeus, macroGLOSSia, epicanthus, ear lobe pits, bitemporal diameter reduce, tendency for neonatal hypoglycemia, interatrial defect, and mild asymmetry of the face, chest and gluteal muscles and hepatomegaly. These is a familiar case of BWS.
mutation involved results in the hypermethylation of Imprinting Centre 1 (IC1) region, loss of insulin growth factor (IGF2/H19) imprinting and fully penetrant BWS phenotype which is maternally transmitted (7). This mutation is also associated with high neoplastic risk, specifically for Wilm’s tumor, of 25% (8). Wilm’s tumor at stage I was discovered by ultrasound when the patient was 11 months old. She had surgery and follow-up examinations at our clinic every 3 months. We evaluated the OS biomarker profile for the first time when she was 4 years and 5 months old, finding significantly higher levels than controls (twenty healthy children from 1 month to 5 years). PAR therapy (300 mg of Potassium Bicarbonate and 150 mg of Ascorbic Acid in aqueous solution every day) started immediately after.

**Patient 2.** A female, born at the 36th week of pregnancy, presented neonatal overgrowth, macroGLOSSIA, macroSTOMIA, asymmetry in the roots of the thighs, crumpled helix in the right ear, small ear lobe pits, tendency for neonatal hypoglycemia, interventricular defect, patent foramen ovale, persistent Botallo duct, voluminous umbilical cord, polyhydramnios at 6 months of pregnancy which later regressed. The patient had a normal karyotype, but molecular diagnosis showed an impairment of Imprinting Centre 2 (IC2), with an hypomethylation of KvDMR1 that is linked to a low neoplastic risk (8, 9). We evaluated an OS biomarker profile at a neonatal age (twenty children from 1 month to 5 years were used as controls) and once OS was ascertained, PAR therapy immediately started (300 mg of Potassium Bicarbonate and 150 mg of Ascorbic Acid in aqueous solution every day).

**Patient 3.** The patient was 38 years old and the mother of patient 1. Anamnesis revealed neonatal overgrowth and macroGLOSSIA. At birth, a left side congenital torticollis was diagnosed, later defined as neck hyperplasia. Molecular diagnosis showed the same mutation as that of her daughter. The mutation resulted in the hypermethylation of the IC1 region with loss of IGF2/H19 imprinting (7). High levels of OS biomarkers were ascertained at the age of 32 years. Twenty adults (aged from 25 to 40 years) were used as controls. PAR therapy (300 mg of Potassium Bicarbonate and 150 mg of Ascorbic Acid in aqueous solution twice a day) started immediately and a decrease in OS was observed. This mutation has a high neoplastic risk (>20%) (8), although the patient has not yet developed any neoplasms.

**Methods.** Heparinized blood samples were drawn and a reliable OS biomarker profile was elaborated at the time of diagnosis. Blood samples were immediately centrifuged (1000 × g for 10 min). The plasma was stored in plastic metal-free containers at −80˚C until analyses, which were performed at the Laboratory of Oxidative Stress of the University of Siena. Forty healthy individuals of the same age as our patients, i.e. 20 adults (aged from 25 to 40 years) and 20 children (aged from 1 month to 5 years), were used as controls (Table II). The biomarker profile included non-protein-binding iron (NPBI), total hydroperoxides (TH) advanced oxidation protein products (AOPP), isoprostanes (IP2), and carbonyl groups (COG). NPBI, being redox cycling active, has pro-oxidant properties because it can enter into the Fenton reaction producing a hydroxyl radical, the most oxidizing molecule in biological systems (10). TH are indices of overall FR attack, because they are indicative of intermediate oxidative products of lipids, peptides, and amino acids. Lipid and protein hydroperoxide, in the presence of traces of free iron, produce several secondary reactive radical species which can be measured collectively as organic hydroperoxide (11). AOPP are terminal products of protein exposure to FR. IP2 are recognized as the most reliable liperoxidation products. IP2 are generated by FR attack on to polyunsaturated fatty acids. Their accumulation in human tissues is a cause of cellular dysfunction. Recently there has been striking evidence implicating IP2 in cancer development (12, 13).

NPBI was measured with the method described by Paffetti (14). This method is based on preferential chelation of NPBI by a large excess of the low-affinity ligand nitrilotriacetic acid (14). TH production was measured with a d-ROMs Kit (Diacon, Rome, Italy) as described by Buonocore (11). This method makes it possible to estimate TH present in a 10 μl blood sample using a spectrophotometric procedure. Hydroperoxidic groups are attacked by iron, decompartmentalized from the transport protein in 1 ml acetate buffer at pH 4.8, to catalyze formation of reactive oxygen metabolite by Fenton’s reaction. The peroxy and alkoxy radicals produced, the quantities of which are directly proportional to peroxides present in the plasma, are trapped chemically by 10 μl chromogen in an electron transfer process leading to the formation of the radical cation of this chromogen. The purple color resulting from this reaction over time was then monitored in a UV-VIS spectrophotometer (λ, 16; Perkin-Elmer, Norwalk, CT, USA) at 505 nm. The results were expressed in conventional units, called Carr units. 1 Carr unit is equal to a concentration of 0.08 mg/l hydroperoxide. TH represents a measure of overall oxidative stress, given that they are the intermediate oxidative products of lipids, peptides, and amino acids(11). IP2 was determined using gas chromatography with mass spectrometry (GC-MS) according to the methodology of Milne and Morrow (15).

Furthermore, we measured thiols, usually considered markers of antioxidant power, in the adult patient because they are normally lower in OS-related disease. Thiols were measured with an SHp Test (Diacon) using the spectrophotometric procedure of Ellman (16). We used the method of Witko-Sarsas for AOPP, and Protein Carbonyl Assay Kit (Cayman Chemical, MI, USA) for COG.

We observed our patients quarterly over a range of times according to the different symptoms they showed and we submitted them to clinical and instrumental examinations as suggested in the literature (1). Finally, every three months OS biomarker profile of blood samples were carried out after a daily administration of PAR at the dosage of 300 mg of Potassium Bicarbonate and 150 mg of Ascorbic Acid in aqueous solution. The present study was approved by the local Ethics Committee of University Hospital of Siena.

**Results.**

Our patients showed higher OS values than the controls (Table I and II) suggesting the need to protect themselves against OS and the future risk of neoplasm (3-6), from probable DNA damage due to OS. Considering this risk, we cautiously initiated an antioxidant therapy with PAR (17, 18). PAR oral therapy started immediately after OS was ascertained and was given once a day as continuous therapy. Patients underwent clinical examinations every three months, and OS was monitored. Interestingly we observed a decrease
in OS values after treatment in all patients. For patient 3, we additionally evaluated thiol levels and observed an increase with PAR therapy, suggesting an improvement of antioxidant power after treatment. PAR therapy appeared to induce a decrease of AOPP, NPBI, CO, TH and IP2, and an increase in thiols in our patients (Table I). Biomarker values remained stable in the controls (Table II).

### Discussion

PAR is a salt resulting from ascorbic acid; it is a very powerful anti-oxidant and of particular interest for its role against degenerative diseases and especially against neoplasms (19). Ascorbate is a product without toxicity and genotoxicity (19). The first studies about this salt didn’t include ribose, which was used for the first time in 1970, so it is news in medical therapy and is added to the other compounds. The compounds are in very pure crystallized form and they are kept separate because of their instability, so we can avoid degradation processes (19). PAR therapy has been reported to allow cells to reintroduce potassium, thus restoring the physiological functions (19). PAR therapy has been used in neoplastic patients undergoing radio- and chemo- therapy, with very promising results, such as an increase of five and ten years survival (19-21). A decrease in neoplastic symptoms such as chest, abdominal and musculoskeletal pain, cough, ascites, fever and asthenia after 60 days of therapy have also been recorded (18-19). PAR therapy also showed interesting results in patients with mesothelioma and prostate cancer (23, 24). In agreement with these results, our data, the first in the literature, signal a decrease in OS suggesting the possibility of using PAR therapy as cancer prevention in BWS patients. We did not observe the appearance of primary or secondary neoplastic disease after 9 months of treatment. PAR therapy has many positive characteristics: it is not toxic, it is easy to administer, even in childhood (orally), it has no short-term side-effects and it is cheap.

In conclusion, we stress the importance to follow-up patients once the diagnosis of BWS is made, in order to monitor the antioxidant system and the risk of malignancy. Therapy with PAR stimulates the anti-oxidant system (18-24) and it may possibly be a useful instrument for the reduction of neoplastic risk in patients with BWS. Our results are preliminary and related to only three patients. Taking into account the limited number of patients, we should not be overly enthusiastic. We are collecting a larger record of data for BWS cases to confirm these preliminary results.

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References


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