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Case Report: Possible Links between Sickle Cell Crisis and Pentavalent Antimony

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Abstract. For over 60 years, pentavalent antimony (Sb^v) has been the first-line treatment of leishmaniasis. Sickle cell anemia is a disease caused by a defect in red blood cells, which among other things can cause vasooclusive crisis. We report the case of a 6-year-old child with leishmaniasis who during treatment with meglumine antimoniate developed a sickle cell crisis (SCC). No previous reports describing the relationship between antimonial drugs and sickle cell disease were found. Reviews of both the pathophysiology of SCC and the mechanism of action of Sb^v revealed that a common pathway (glutathione) may have resulted in the SCC. ChemoText, a novel database created to predict chemical-protein-disease interactions, was used to perform a more expansive and systematic review that was able to support the association between glutathione, Sb^v, and SCC. Although suggestive evidence to support the hypothesis, additional research at the bench would be needed to prove Sb^v caused the SCC.

INTRODUCTION

Leishmaniasis is a disease caused by the parasite *Leishmania* and transmitted by sand flies from the genus *Lutzomyia*. It may affect the skin, mucosal surfaces, or the viscera. *Viannia* is the predominant subgenus in Colombia with the species *Leishmania panamensis*, *Leishmania braziliensis*, and *Leishmania guyanensis* being most prevalent.[1] Cutaneous leishmaniasis has a wide distribution throughout Colombia with the southern Pacific Colombian coast being historically endemic. The population is mainly of Afro-Colombian heritage and access to health services is difficult. The Sb^v continues to be the first line of treatment. Second line options include miltefosine, amphotericin B, and pentamidine among others. Side effects of meglumine antimoniate (MA) have included constitutional symptoms, renal failure, hepatotoxicity, cardiotoxicity, and pancreatitis.[2]

Sickle cell anemia (SCA) is a hereditary disease caused by the mutation of a nucleotide at the sixth position of the β-globin chain provoking the replacement of the amino acid glutamate by valine. This change facilitates a hemoglobin polymerization setting off the characteristic sickle deformity of the red blood cells (RBC). This structural alteration is accompanied, among other consequences, by cellular rigidity and an increased adherence to vascular endothelium. Ultimately, vasooclusion occurs generating a painful crisis and damage to target organs. The half-life of RBCs is also diminished without the bone marrow being able to supply the RBC demand leading to anemia.[3] The SCA has a higher prevalence in the Afro-descendent population and in the Colombian southern pacific region, prevalence is estimated between 10% and 18%.[4]

No prior reports of interactions between these two diseases or their treatments could be found in the medical literature. ChemoText is a recently described database and data mining approach that can be used to investigate hypothetical chemical-protein-disease relationships based on MeSH term co-annotations in the literature.[5] We describe a patient with both SCA and leishmaniasis. We then use both manual literature search methods and ChemoText analysis to explore hypothetical mechanisms underlying the potential pathophysiological cause of a sickle cell crisis (SCC) coincident temporally with treatment of his leishmaniasis.

Background clinical case observation. A 6-year-old Afro-Colombian male, from the rural area of Tumaco (Nariño – Colombia) presented with two ulcerated lesions on his right ear of 6 months duration (Figure 1). *Leishmania* amastigotes where identified in the direct microscopic examination of tissue smears.

His past medical history included anemia (hemoglobin level of 8.3 g/dL) and a hospitalization at 4 years of age with fever and knee pain.

With the diagnosis of cutaneous leishmaniasis, MA was started at a dose of 20 mg/kg/d intramuscular for 20 days as established by the protocol of the Colombian Ministry of Social Protection. Before administration of the first dose a hemoglobin level of 8.8 g/dL was documented. Three days after the treatment was started the patient presented to the emergency service complaining of abdominal pain associated with diarrhea without fever. He was hospitalized and MA was suspended. Gastroenteritis was suspected therefore a coprologic exam and urinalysis was performed. Ceftriaxone treatment was started. During hospitalization, pain in the lower limbs was reported and splenomegaly was confirmed by abdominal ultrasound. The results of the urinalysis and coprologic exam ruled out an infectious process. His hemoglobin level had decreased to 6.8 g/dL and a peripheral blood smear showed moderate hypochromia, anisocytosis, microcytosis, poikylocytosis, koilocytes, and acanthocytes. The sodium metabisulfate test (for sickle cell trait) was positive. With this result, and the absence of an infectious process to explain the symptoms, the diagnosis of SCC was made. Folic acid was started and the patient was discharged eventually in good condition and without pain.

Hemoglobin electrophoresis revealed hemoglobin F = 20.7%, hemoglobin S = 74.3%, and a band compatible with hemoglobin A2 = 5.1%. No blood transfusion had been received in the previous 3 months. This confirmed the patient as homozygous for sickle cell disease.

Once the SCC was overcome, the patient's lesions were noted to have significant improvement (Figure 2). Therefore,

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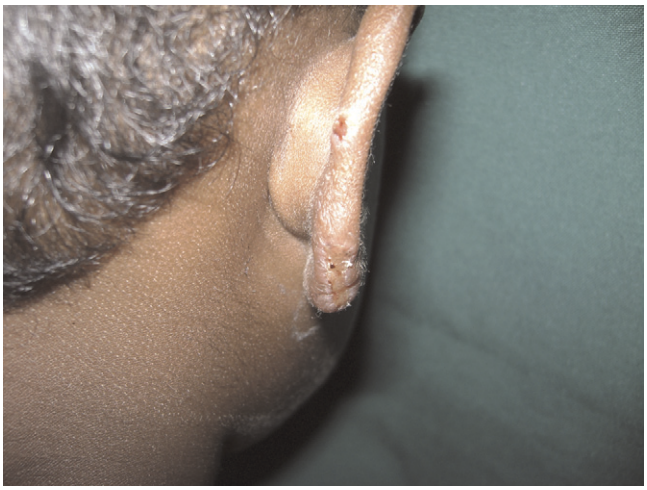

FIGURE 1. Lesions previous to treatment with meglumine antimoniate.

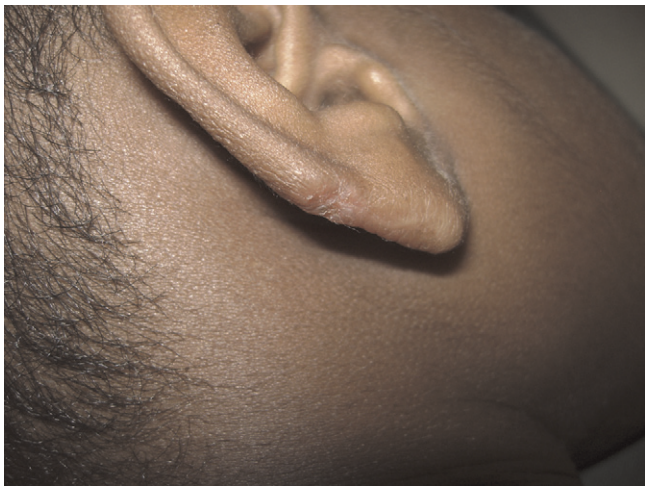

FIGURE 3. Lesions after 1 year of follow-up, previous to treatment with miltefosine.

no additional treatment was administered. The lesions, however, never fully recovered after 12 months of follow-up and miltefosine was administered at a dose of 2.4 mg/kg/day for 28 days (Figure 3). At the end of the treatment, lesions had resolved and no recurrence was observed after 3 months of follow-up. During the same period no other SCC occurred (Figure 4).

Results of manual search and automated literature mining. The patient's clinical presentation and the hemoglobin electrophoresis established the diagnosis of SCA. The coincidence of the treatment administration with the episode of SCC raised the question of a possible causal relationship. A manual literature search was conducted through PubMed and LILACS (last search on July 2011). Using "AND" combinations of all search terms, no prior reports were found. We also consulted with experts in hematology and in the treatment and clinical management of leishmaniasis. Expert consultants also had no obvious explanation. The second step was a thorough review of the pathophysiology of SCC together with the mechanism of action of Sb^{v} in search of a possible interaction. As a result of these discussions and research, the glutathione pathway captured our interest.

The role of redox imbalance has been described in the pathophysiology of SCC. Physiologically RBCs are exposed to an elevated oxidative stress. In the case of sickle cell patients, RBCs undergo even higher levels of oxidative stress[6]; to counteract this situation, RBCs possess diverse antioxidant molecules that allow them to achieve homeostasis. Glutathione is part of the antioxidant system and has been considered the principal buffer thiol of redox reactions inside the RBC.[7] This system has been studied in erythrocytes of individuals with SCA and its role in the pathophysiology has been characterized. Glutathione levels in the RBCs of patients with SCA are diminished when compared with healthy controls. This decrease occurs despite a higher

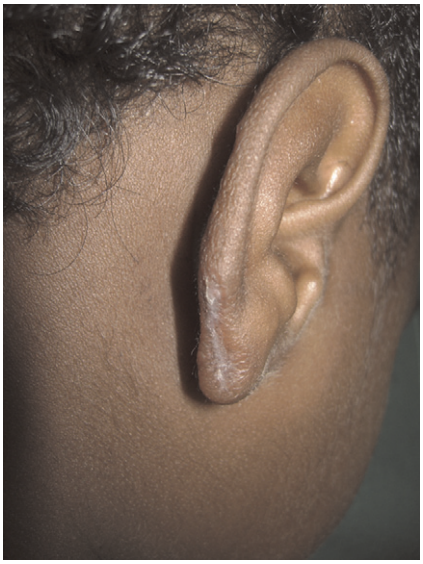

FIGURE 2. Lesions after treatment with meglumine antimoniate.

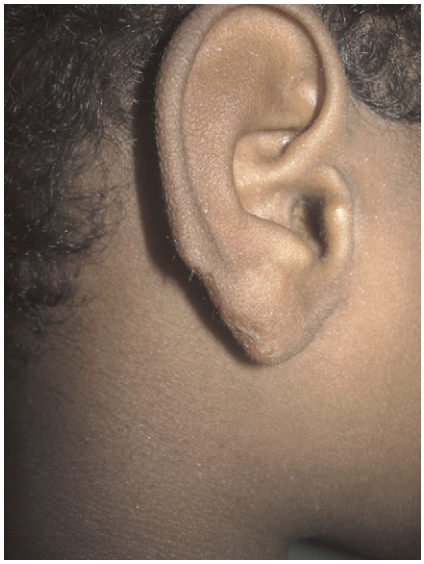

FIGURE 4. Lesions after treatment with miltefosine.

TABLE 1

Proteins that have been co-annotated in PubMed articles with MeSH terms *Anemia*, *Sickle Cell*, AND (*Meglumine Antimoniate* OR *Antimony Sodium Gluconate* OR *Antimony*) and the number of articles in which each co-annotation occurs*

Protein	Article counts			
	Sickle cell	Meglumine antimoniate	Antimony sodium gluconate	Antimony
1-Phosphatidylinositol 3-kinase	1	0	1	0
5-Aminolevulinate synthetase	2	0	0	1
Acetylcholinesterase	2	0	0	1
Acetylcysteine	3	0	0	1
Acid phosphatase	6	0	0	1
Adenosine triphosphatases	14	0	0	7
Adrenocorticotropic hormone	4	0	0	1
Alkaline phosphatase	25	1	2	3
Aminobutyric acids	1	0	0	1
Amylases	1	2	3	0
Antilymphocyte serum	2	0	0	1
Antiporters	2	0	0	1
Buthionine sulfoximine	1	1	1	1
Ca(2+) Mg(2+)-ATPase	10	0	0	1
Calcium channels	1	0	0	1
Calcium-transporting ATPases	19	0	0	1
Catalase	8	0	0	2
Cholinesterases	3	0	0	1
Collagen	14	0	0	1
C-reactive protein	14	1	0	1
Creatine kinase	1	0	1	1
Cysteine	14	0	1	8
DNA-binding proteins	8	0	2	2
DNA-directed DNA polymerase	4	0	0	5
DNA-directed RNA polymerases	1	0	0	2
Endonucleases	2	0	0	1
Erythropoietin	74	0	1	0
Ether-A-Go-Go potassium channels	1	0	0	1
Glucuronidase	6	0	0	2
Glutamates	9	0	0	2
Glutathione	31	1	2	7
Glutathione peroxidase	8	0	0	2
Glutathione reductase	6	0	1	1
Glutathione transferase	2	0	2	1
Glycoside hydrolases	2	0	0	1
Granulocyte-macrophage colony-stimulating factor	12	0	3	0
Heme oxygenase (decyclizing)	1	0	0	3
Heme oxygenase-1	4	0	1	1
Hemoglobins	639	1	4	4
Hexokinase	8	0	1	0
Histones	2	0	0	1
HMOX1 protein, human	2	0	1	1
HSP70 heat-shock proteins	2	0	0	1
Hydrolases	1	0	0	1
Immunoglobulin A	24	1	1	0
Immunoglobulin G	61	3	5	3
Immunoglobulin M	36	2	1	0
Interferon-gamma	6	5	8	1
Interferons	1	0	1	2
Interleukin-1	6	0	1	0
Interleukin-10	3	4	2	0
Interleukin-2	3	1	1	2
Interleukin-3	8	0	1	0
Interleukin-4	2	1	4	1
Interleukin-6	11	0	1	0
Lactoferrin	2	0	0	1
Lectins	3	1	0	0
Lectins, C-type	1	0	0	1
L-lactate dehydrogenase	22	0	1	1
Methionine	11	0	0	2
Mitogen-activated protein kinases	2	0	1	0
NAD	10	0	0	3
NADH, NADPH oxidoreductases	1	0	0	1
Neuraminidase	3	0	0	1
Nitric oxide synthase	22	0	1	1
Nitric oxide synthase type II	10	0	2	0
Nos2 protein, mouse	4	0	1	0
Oxidoreductases	3	0	1	4
Penicillamine	1	0	0	2
Peroxidase	7	0	0	1
Peroxidases	5	0	0	1
Phosphofructokinase-1	3	0	1	4
Phosphoprotein phosphatases	3	0	1	0
Phosphotransferases	5	0	0	1
Phytohemagglutinins	6	0	1	0
Porphobilinogen synthase	3	0	0	1
Proline	1	0	0	1
Protein tyrosine phosphatases	2	0	4	1
Protein-tyrosine kinases	4	0	1	0
Proteoglycans	2	0	1	0
Purine-nucleoside phosphorylase	1	0	0	1
Pyruvate kinase	14	0	1	0
Receptors, IgG	3	0	1	0
Receptors, immunologic	6	0	0	1
Receptors, tumor necrosis factor	1	0	1	0
Repressor proteins	1	0	0	1
Ribonucleo proteins	1	0	0	1
RNA-directed DNA polymerase	1	0	0	3
Serine	6	0	0	1
Serine endopeptidases	1	0	1	0
Superoxide dismutase	12	0	0	3
Transferrin	35	0	0	1
Transforming growth factor-β	2	0	1	0
Trypsin	17	0	0	1
Tuftsin	2	0	1	0
Tumor necrosis factor-α	22	3	0	2
Tyrosine	13	0	1	0
Urease	2	0	0	1

*The proteins were defined as any annotations mapping to the MeSH Tree level D12 (http://www.nlm.nih.gov/mesh). The version of ChemoText used was built from baseline 2010 MEDLINE.

rate of production, indicating that the defect does not reside in the antioxidant production but in an elevated consumption caused by the great oxidative stress to which the cell is submitted.[7–10] Low glutathione levels have also been associated with hemolysis probably explaining why antioxidant therapies developed to target the glutathione pathway have had good results.[11,12] By the same logic, the supplementation with precursors such as L-glutamine successfully diminish the phenomenon of endothelial adhesion.[12,13]

Although the mechanism of action of Sb^v is not yet fully understood, one pathway that has been described involves antioxidants of the thiol group. It has been shown that Sb^v in its trivalent form (active molecule) depletes glutathione and tripanothione levels of parasites of *Leishmania donovani* probably through an efflux system. Additionally, Sb^v has the capacity to inhibit both the tripanothione reductase enzyme of the parasite and also human RBC glutathione reductase.[14,15] Finally, Sb^v exerts the same action on human macrophages suggesting Sb^v could be a chemotherapeutic agent for neoplasias, such as acute promyelocytic leukemia.[16]

The published literature thus supports the hypothesis that Sb^v could potentially trigger an SCC. The hypothetical mechanism would be an alteration in the redox balance in RBCs of patients that are facing an even greater oxidative stress caused by their disease. After uncovering this possible explanatory pathway we sought to expand our review of potential interactions using ChemoText, a multivariate search tool designed to investigate possible chemical-protein-disease relationships. We attempted to compare and contrast our results with those generated by the ChemoText automated literature mining tool. At the outset, we hypothesized that the exercise might validate our ideas about glutathione and indicate other potential pathways linking sickle cell and Sb^v.

The goal of the ChemoText analysis was to find proteins implicated in both the mechanism of action behind antimony drugs *and* the physiology of SCA. We first queried ChemoText for all protein annotations occurring in articles where the chemical annotations *Meglumine Antimoniate*, *Antimony Sodium Gluconate*, or *Antimony* were present and the drugs were the subject of the article. Next, we queried ChemoText for all protein annotations co-occurring in articles with the disease annotation *Anemia, Sickle Cell*. These queries resulted in four sets of proteins that were then evaluated to find the overlapping entries. After refining the list to include only proteins with meaningful specificity (i.e., removal of non-specific protein terms like *Blood Proteins*), 98 proteins remained. These proteins are listed in Table 1 along with the number of published articles in which they are annotated both with the antimonial drugs and with SCA. In support of our earlier findings by manual search, glutathione and its associated enzymes were also detected by ChemoText. Glutathione itself has been annotated in 31 articles about SCA, and in articles about each of the antimonial drugs.

DISCUSSION

The results presented here describe a potential relationship between SCC and MA and highlight the need for thoughtful consideration of unknown relationships between chemicals, proteins, and diseases. This one interaction will require additional research at the bench to determine if there is a true causative link between the drug and the crisis. However, the comparison of a manual search with automated literature mining for underlying mechanistic connections, illustrates the potential for high throughput data mining to identify potentially important pathways. ChemoText independently confirmed our findings about the glutathione pathway. Even more interesting was discovering the number of biochemical and molecular pathways relating to antimonial drugs and sickle cell pathophysiology. Although the mechanistic connections identified by manual search and ChemoText are purely speculative at this stage, these results open the door for additional theoretical evidence to be discovered and investigated.

Literature mining or text mining has been used by a number of researchers to extract and organize known relationships between biomedical entities, and to use those known relationships to predict or infer new relationships. Using such text-based inference, researchers have predicted new therapies for disease, novel applications of existing drugs, and connections between diseases.[5,17–20]

ChemoText was constructed by extracting the MeSH annotations from each article in MEDLINE, the database behind the National Library of Medicine's PubMed. The annotations were processed and organized into a database that allows known relationships between chemicals, proteins, and disease to be explored and new relationships to be inferred. (A publicly accessible version of ChemoText is under development and available to the public at http://chembench-dev.mml.unc.edu:8082). There are other data repositories containing combinations of chemical, disease, and protein information, including STITCH, CBioC, KEGG, DrugBank, and the Comparative Toxicogenomics Database.[21–25] Whereas many of these sources are curated, the data in ChemoText is extracted automatically from MEDLINE and receives no curation. Although the quality of the data in ChemoText may not rival the curated sources, ChemoText has greater coverage of the literature, reflecting the broad reach of PubMed.

In earlier work using ChemoText in drug research, explicit relationships between diseases and proteins were used to infer new, possibly therapeutic, relationships between chemicals and diseases.[5] In the current study, we took a slightly different approach and found the overlap between the set of proteins related to sickle cell in the literature and the proteins related to antimony drugs in the literature. The list of overlapping proteins has the potential to suggest possible mechanisms in common between the activity of MA and the pathology of SCC.

Despite these search results, a causal link between MA and SCC is clearly not known. The effect of the drug has been demonstrated *in vitro* and it is not known if the same action occurs *in vivo*. Furthermore, ChemoText was able to find potentially important disease-protein-drug relationships; however, because a correlation cannot be established by a single case, one must consider that this outcome could have been a random event. It would therefore be important, on the basis of theoretical findings and advanced data mining technologies, to perform a more thorough case-controlled investigation or more mechanistic studies *in vitro*. Addressing these hypothetical interactions would allow physicians to offer better therapeutic options in certain populations where both diseases coexist.

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