Sodium Stibogluconate (Pentostam) induced Nephrotoxicity in Mice.

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Abstract:

Pentavalent antimonials (Pentostam) (SSG) are one of the first-line drugs recommended by the Word Health Organization (WHO) for treating leishmaniasis. The reported serious adverse effects after SSG handling was cardiotoxicity, clinical pancreatitis and very few trials have reported renal side effects with these compounds. The present study is aimed at evaluating the nephrotoxicity effect of sodium stibogluconate (Pentostam) in mice. In addition to a control group, adult male albino mice were divided into three groups, 7 mice each, and i.p. injected with 20mg/kg of pentostam for 14 days. After 14 therapeutic days. Group II, III and VI were sacrificed after 1, 3 and 6 weeks later. The mice' serum and kidney tissues were collected, and biochemical and histo-pathological studies were carried out. Biochemical analysis of the serum obtained showed a significant increase in the levels of creatinine and blood urea in group II and III when compared with control group. In parallel, the histo-pathological assessments of the kidney tissue proved tubular necrosis. From this study, it can be concluded that the antimonial pentostam has nephrotoxicity effect on treated mice.

Keywords: Pentostam,histo-pathological studies, Nephrotoxicity, Leishmaniasis

الملخص

يعتبر عقار البنتوستام أول دواء أعلنت عليه منظمة الصحه العالميه لعلاج مرض اللشمانيا وقد نشرت العديد من الدراسات إن عقار البنتواستام يسبب أعراض جانبيه كثيره منها فشل عضلة القلب والتهاب البنكرياس والتسمم الكبدي ودراسات قليله فقط سجلت اعراض تسمم كلوي بهذا العقار. لذلك الهدف من هذه الدراسة هو تقييم التسمم كلوي بعقار البنتوستام في الفئران. واعتمدت التجربة على استخدام ذكور فئران الالبينو البالغة حيث تم تقسيمها إلى ثلاثة مجموعات,7 فئران في كل مجموعه بالإضافة الي المجموعة الضابطة وحقنت الفئران بالبنتوستام بجرعة 20 ميكروجرام لكل كيلوجرام من وزن الجسم في التجويف البروتوني لمدة 14 يوم ثم شرحت المجموعات الثلاثة حسب الترتيب بعد اسبوع وبعد ثلاثة اسابيع وبعد سته اسابيع من اخر جرعه. جمعت عينات الدم لكل مجموعه بالإضافة إلى الأنسجة الكلوية لإجراء التحاليل البيوكيميائية والاختبارات النسيجية. وقد أظهرت الدراسة ارتفاع في أنزيم اليوريا والكرياتينين في المجموعة الثاني والثالثة بالمقارنة مع المجموعة الضابطة كما بينت الاختبارات النسيجية حدوث تنخر ونزيف في الأنابيب الكلوية في هذه المجموعات. وبذألك فان هذه الدراسة تشير إن عقار البنتوستام سبب حدوث تسمم كلوي في الفئران.

الكلمات المفتاحية: السمية الكلوية، داء الليشمانيات.

1. Introduction

Leishmaniasis is an infection caused by protozoan parasites of the genus Leishmania, (family trypanosomatidae). which are transmitted to a susceptible host by phlebotomine sand flies (Diptera: Psychodidae) in the old and new worlds, In humans, leishmaniasis is divided into three general clinical patterns according to the form of the disease including: cutaneous, visceral and mucocutaneous (Svobodová et al., 2009). Most leishmania species cause cutaneous leishmaniasis in people. Cutaneous leishmaniasis consists of skin ulcers, which vary in number and are often self-healing. It is usually caused by L. major and L. tropica and by members of the L. Mexicana complex and the L.viannia subgenus. Depending on the species of leishmania, ulcers, smooth nodules, flat plaques or hyperkeratotic wart like lesions may be seen (Salman et al., 1999).

Treatment of leishmaniasis is medical therapy, cryotherapy, heat therapy, surgery and cauterization (Alvajhi, 2003; Berman, 1997; Talari, 1996). The customary drug used in medical therapy over the past several years for treatment of leishmaniasis is antimonial sodium stibogluconate (Pentostam) (SSG). In 1982, WHO (unpublished data) supported treatment with 20 mg of SSG / kg of body weight, with a maximum dose of 850 mg / day (Herwald and Berman, 1992). However, pentavalent antimonials can be used where the parasites are sensitive to these drugs, but resistance is a major problem in some areas (Maltezou, 2010). Similar to any drug, pentostam has multiple acute and chronic adverse effects which can be decreased by using the lowest effective dose. This toxic antimonial compound has a narrow therapeutic window (Mitropoulos, 2010; WHO, 2017). Moreover, the toxic effects of SSG have seldom been reported in previous studies (Ballesteros et al., 1991; Delgado et al., 1997). Franke et al., 1990 reported that these medicines could cause not only changes in blood indices also renal, hepatic, cardiac, gastrointestinal and pancreatic side effects. On the other hand, Veiga et al., 1983 has observed renal dysfunction in patient treated with pentostam which prompted them to conduct experiments on the rats. They observed nephrotoxicity in the rats after treatment with both meglumine antimoniate and SSG described by disturbances in urine concentrating capacity (Veiga et al., 1990). The present study was carried out in order to evaluate the effect of pentavalent antimonials drugs (Pentostam) on the renal function of normal mice.

2. Materials and methods

2.1. Experimental design

Adult male albino mice weighting 25-35 g at the age of 8-10 weeks were housed in standard laboratory conditions. The animals were separated into four groups of 7 animals each. Group I (control group), was treated with distilled water as a vehicle for 14 days. All the animals of groups II, III &IV

were intra-peritoneal (IP) injected with pentostam at a dose of 20 mg/kg b.w. once a day for 14 days. 7 days after receiving the final dose. The mice of group II were sacrificed while group III were sacrificed after 3 weeks and group IV after 6 weeks from final dose were sacrificed.

2.2. Biochemical analysis

Samples of blood were placed into glass tubes and centrifuged at 4000 rpm at 4°C for 15 min to get serum, which was kept at -80 °C . Creatinine and blood urea nitrogen (BUN) were assayed spectro-photo-metrically using commercially available kits (Sigma Ltd, U.K.) according to standard laboratory techniques (Horder et al., 1981).

2.3. Histo-pathological Studies

The kidney of animals for each group was dissected and preserved in the formalin solution for histo-pathological study. The specimens were fixed in paraffin to prepare for sectioning (4-5 µm) thereafter subjected to hematoxylin and eosin (H & E) for photo-microscopic observations of the kidney histo-pathological architecture (Galigher and Koyloff, 1971).

3. Results:

3.1. Effect of IP injection of Sodium Stibogluconate (Pentostam) on Blood urea nitrogen (BUN), and Creatinine levels (U/L) in albino mice compared to normal control group.

Biochemical results of blood urea nitrogen (BUN) and creatinine levels

are summarized in Table 1. In the serum of mice treated with pentostam; BUN and creatinine levels were found significant higher in group II and group III than control group. After 6 weeks; BUN and creatinine levels were near to normal which was clear in group IV in comparison to control group.

Table 1: Effect of IP injection of Sodium Stibogluconate (Pentostam) on Blood urea nitrogen (BUN), and Creatinine levels (U/L) in albino mice compared to normal control group

Enzyme	Control group	Group II	Group III	Group IV
BUN (U/ L)	16.14± 0.63	37.85 ± 0.55**	30.28 ± 1.5**	18.28 ± 0.42
Creatinine (U/L)	14.42 ± 0.42	39.85 ± 0.50*	30.85 ± 0.63*	19.28 ± 0.60

All vaues are presented as Mean ± SEM, n =7, *P ≤ 0.001,** P ≤ 0.0001.

3.2. Histo-pathological study

The results of kidney histopathologic examination are shown in figour 1. Kidneys of the control group animals showed a

normal histologic morphology. kidney tissue of the group II showed; hyperemia with hyperemia in tubular glomerular capillary and inter-tubular range, dilatation in the tubule lumen, degeneration in the tubular epithelium. Stained sections of the pentostam treatment mice after 3 weeks; group III maintained a better morphology with clear reduction in the degree of tubular necrosis and very few degeneration in tubular epithelium. However, the kidney sections of the fourth group did not show any significant histo-pathological changes.

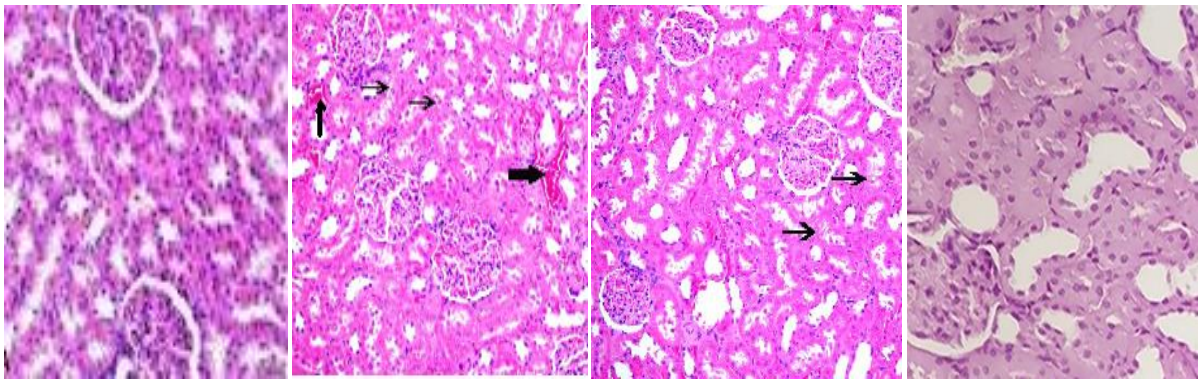

Fig 1 (H&E, ×400):
A: Photomicrograph of kidney tissue of control group showing a normal histologic morphology
B: Photomicrograph of kidney tissue of mice treated with pentostam and were sacrificed after 7 days (Gr II) showing hyperemia with hyperemia in tubular glomerular capillary and intertubuler range.
C: Photomicrograph of kidney tissue of mice treated with pentostam and were sacrificed after 21 days (Gr III) showing reduction in the extent of tubular necrosis. and very fewdegenerationin tubular epithelium
D: Photomicrograph of kidney tissue of mice treated with pentostam and were sacrificed after 6 weeks (Gr VI) showing normal kidney structure.

4. Discussion

The present results showed that intraperitoneal injection of pentavalent antimonial pentostam in normal mice in a daily dose of 20 mg /kg b.w during a14 days period caused renal tubular dysfunction. This was characterized by increased of urea and creatinine in serum. Dwinnell and Anderson, 2012 have reported that retrogradation of renal function results in elevations of blood urea nitrogen and serum creatinine concentrations. These substances are very sensitive markers employed in the diagnosis of kidney diseases. Although several factors such as excessive protein intake, shock, gastrointestinal hemorrhage etc. could also contribute to this (Anderson, 1996). Serum urea and creatinine levels may be indicators of acute tubular necrosis caused by chemicals toxicity (Furhan et al., 2004; Hole, 1992). Veiga et al., 1990 was one of the earlier studies aimed at evaluating nephrotoxicity by pentavalent antimonial compounds. They observed turmoil in urine concentrating capacity in rats treated with both meglumine anti-moniate and pentostam. Moreover, our results of histological examination of kidney tissue are

in agreement with Furhan et al., 2004. Present study results showed dilatation in the tubuler lumen, degeneration in the tubuler epithelium and necrosis in group II and III as he described that the dilation of glomerular capillaries, necrosis of hematopoietic tissue, vaculoation of tubuler cells and degeneration of epithelial cell lining are some of the pathological changes observed in kidney of various toxins. As noted in group VI, these histological changes needed abaut 6 weeks to return nearly to normal. On the other hand, Functional and histopathological alterations, suggestive of tubular necrosis were found at higher doses of the drugs (Rodrigues et al., 1999). Further, the chances of renal toxicity are more when antimonials are administered at higher doses for a longer period. When administered at low doses and for a short period, antimonials have low renal toxicity (Oliveira et al., 2011). In this study, after 14 pentostam therapeutic days, the mice were sacrificed in different periods (after 7, 21 and 42 days), the clear histopathological changes were observed in the kidney tissue of treatment groups compared to the control group. these histological changes needed to about 6 weeks to return to normal.

Conclusion: The treatment of mice with therapeutic doses of pentostam induced renal tubular dysfunction characterized by clear histological changes in the kidneys, these histological changes were needed to about 6 weeks to returned to normal. Consequently, pentavalent antimony pentostam may be included in the large list of offending agents that can cause drug induced nephrotoxicity that requires more researches.

5. References

- Alvajhi A. A. 2003: Cutaneous leishmaniasis of the Old World. Skin Therapy; 8(2):1-4.
- Anderson W. A. 1996: Kidneys in Anderson's Pathology, 5th edition (CV Mosby Company), St Louise BatlomorePhiladephia Toronto 610-621.
- Ballesteros A, Buades J, Salva F, Villalonga C, 1991: Visceralleishmaniasis: another HIV-associated opportunistic infection?Report of eight cases and review of literature. AIDS 5:201–207
- Berman J. D.1997: Human Leishmaniasis ; clinical, diagnostic, and chemotherapeutic developments in the last 10 years. Clin Infect Dis; 24: 684-703.
- Delgado M, Garcı´a M. A, Martos F, Regura J. M, Jime´nez F, ColmeneroJ.D. 1997: Caracterı´sticasclı´nicas y evolutivas de la leishmaniasis visceral en pacientes con infeccio´n por VIH. An Med Interna 14: 506–510
- Dwinnell B.G and Anderson R.J. 2012: Diagnostic Evaluation of the Patient with Acute Renal Failure, Atlas of Diseases of the Kidney. ISN Informatics Commission and NKF cyber Nephrology.
- Franke E.D, Wignall F.S, Cruz M. E, Rosales E, Tovar A.A, Lucas C.M. 1990 : Efficacy and toxicity of sodium stibogluconate for mucosal leishmaniasis. Ann Intern Med; 113(12):934-40.
- Furhan I , Irfan Zia Q and Muhammad A. 2004: Histopathological Changes in the kidney of common carp, Cyprinuscarpio, following nitrate exposure. J. res. Sci.,15(4), 411-418
- Galigher, A.E. and Koyloff, E.N. 1971: Essential of Practical Microtechnique, 2nd Ed, Lea and Febiger, Philadelphia, pp.77

- Herwald B.L, Berman J.D. 1992: Recommendations for treating leishmaniasis with sodium stibogluconate (Pentostam) and review of the pertinent clinical studies. Am J Trop Med Hyg 46:296–306.
- Hole J. W. 1992: Essentials of human anatomy and physiology. 4th ed. Wm. C. Brown Publishers, Dubuque, 745-749
- Horder H, Gerhardt W, Harkonen M, Magid E, Potkanen E, Stromme J.H, Theodorsenl and Waldenstrom J.1981: Experiences with the Scandinavian recommended methods for determinations of enzymes in blood. A report by the Scandinavian Committee on Enzyme. Scan. J. Clin. Lab. Invest., 41, 107 ± 116.
- Maltezou H.C. 2010: Drug resistance invisceralleishmaniasis. Journal of Biomedicine and Biotechnology, 61, 7521-30.
- Mitropoulos P, Konidas P, Durkin-Konidas M. 2010: New World Cutaneous leishmaniasis: updated review of current and future diagnosis and treatment. J Am AcadDermatol ; 63(2):309–22.
- Oliveira R.A, Diniz L.F, Teotônio L.O.2011: Renal tubular dysfunction in patients with American cutaneous leishmaniasis. Kidney Int ;80:1099-106
- Rodrigues M.L, Costa R.S, Souza C.S, Foss N.T, Roselino A.M.1999: Nephrotoxicity attributed to meglumineantimoniate (Glucantime) in the treatment of generalized cutaneous leishmaniasis. Rev Inst Med Trop Sao Paulo ;41:33-7.
- Salman S.M, Rubeiz N.G, Kibbi A.G. 1999: Cutaneous leishmaniasis: Clinical features and diagnosis. Journal of Clinical Dermatology, 17, 291-296
- Svobodová M, Alten B, Zídková L, Dvořák V, Hlavačková J, Myšková J.2009:Cutaneous leishmaniasis caused by Leishmania infantum transmitted byPhlebotomustobbi. Int J Parasitol ;39:251–6.
- Talari S.1996: Toxicity of glucantime in blood element, in cutaneous leishmaniasis, Faze – Kashan university; 10:18-21
- Veiga J.P, Khanam R, Rosa T.T. 1990: Pentavalent antimonial nephrotoxicity in the rat. Rev Inst Med Trop Sao Paulo ;32:304-9.
- Veiga J. P,Wolff E. R, Sampaio R. N and Marsden P. D. 1983: Renaltubular dysfunction in patients with mucocutaneous leishmaniasis treated with pentavalentantimonials . Lancet, 2: 569.
- http://apps.who.int/iris/handle/10665/255104. Accessed on 10 May 2017.