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METHEMOGLOBINEMIA ASSOCIATED WITH LOXOSCELISM

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SUMMARY

In twenty five patients who presented the cutaneous form of loxoscelism, serum haptoglobin and lactic dehydrogenase, erythrocyte glucose-6-phosphate dehydrogenase, glutathione reductase, glutathione peroxidase, methemoglobin, bilirubin and reticulocytes were investigated after bite. No hemolysis was detected but an increase in methemoglobin was found in 54% of the cases; in 7% it was between 1.1% and 2%, in 27% it ranged from 2.1% to 4%, and in 20% from 4.1% to 8%.

Blood samples of a normal, blood group 0 individual and of a patient who exhibited methemoglobinemia after Loxosceles bite were incubated separately with antisera against **Loxosceles gaucho, Crotalus terrificus, Bothrops jararaca,** with **Loxosceles gaucho** venom and 0.3% phenol. No methemoglobin was found after 1, 4, 8 and 15 days in both sets of samples. At the 25th day all the samples, including the controls, exhibited similar methemoglobin reductase decrease. The data suggest that the methemoglobinemia which occurs in 50% of the patients probably arises from *in vivo* venom metabolism, inasmuch as the crude venom does not induce methemoglobinemia.

KEY WORDS: Loxoscelism; Methemoglobinemia.

INTRODUCTION

There are two clinical forms derived from spider **Loxosceles sp** bite: the cutaneous form, with painful and necrotic lesion and the rare viscerocutaneous form, in which a severe hemolysis occurs in addition to the skin lesion. In order to detect hemolysis and/or metabolic changes in the erythrocyte of patients with the cutaneous form, the pertinent tests for detecting hemolysis and erythrocytes enzymes and methemoglobin were performed, disclosing an increased methemoglobin in most cases.

MATERIAL AND METHODS

In a period of two years 25 patients referred to a special hospital (Vital Brazil Hospital, Butantan Institute) were studied. All presented the cutaneous form of loxoscelism and were assayed for serum haptoglobin (BARRETTO et al.[1]), and lactic dehydrogenase (E.C.1.1.1.27), erythrocyte

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glucose-6-phosphate dehydrogenase (E.C.1.1.1.49), glutathione reductase (E.C.1.6.4.2), glutathione peroxidase (E.C.1.11.1.9) (BEUTLER[3]) and methemoglobin (EVELYN & MALLOY[5]) besides bilirubin and reticulocytes. Antisera employed in *in vitro* tests were prepared by the Butantan Institute and all the chemicals used were from Sigma Co.

The enzymes were assayed in a Gilford 2451 recording spectrophotometer at 37°C. The methemoglobin determination, as percentage of total hemoglobin, was performed at room temperature.

RESULTS

Reticulocytes counting, conjugated bilirubin, serum lactic dehidrogenase and haptoglobin concentrations were found to be within the normal range, as well as the red cell enzymes. Methemoglobin levels, however were increased in 54% of the patients.

Results of methemoglobin assays performed in the patients after **Loxosceles sp.** bite are depicted in table 1. The patients who presented methemoglobin below 1% of total hemoglobin were grouped. The time elapsed between the bite and the blood examination was 53 ±29 hours. The patients who exibited more than 1.1% methemoglobin were tested 97.5 ± 42 hours after bite ($p < 0.01$). Data from **in vitro** studies with a normal individual and with a patient who presented increased methemoglobinemia after bite (patient no. 23) are depicted in tables 2 and 3, which show a gradual and equal decrease of methemoglobin reductase in both patient and control.

DISCUSSION

In this series of patients, we intended to search for slight hemolysis and red cell metabolic changes in patients with loxoscelism. Hemolysis was not found but in 54% of the cases increased methemoglobinemia was present. To the best of our knowledge methemoglobinemia in loxoscelism has not been reported to date. This alteration may worsen the clinical condition of the patients but so far it has not been taken into account. In 12 of the 25 studied patients, methemoglobinemia did not increase, in two it ranged from 2.1% to 4%, and in 5 it was between 4.1% and 8.8%, indicating that methemoglobinemia may be severe in loxoscelism.

This discrepancy could be related to the different species of **Loxosceles** involved, as there are some differences in venom composition of **L. laeta** and **L. reclusa** (SCHENONE et al.[12]). Usually the patient who suffers **Loxosceles** bite does not bring the spider to be identified, inasmuch as it is not even seen most of the times. Although the spider only seldom can be identified, it is known that in São Paulo area the **Loxosceles gaucho** is the most frequent species (CARDOSO et al.[4]). Another possible explanation is the presence of genetic factors which could either protect against or predipose the patient for the increased methemoglobinemia. It has also been a matter of great dispute that only a few patients following the **Loxosceles sp** bite exhibit the severe viscerocutaneous form. Among other factors involved in this selective hemolysis, complement (KNIKER & MORGAN[8]; KNIKER et al.[9]; MORGAN et al.[10]) and a glucose-6-phosphate dehydrogenase deficiency (NANCE[11]; BARRETTO et al.[2]) have been mentioned. Oxidative drugs employed in loxoscelism treatment could well explain the methemoglobinemia, but only promethazine, prednisone and **Loxosceles** antiserum are routinely prescribed. No other drugs which could account for the increased methemoglobin generation have been used to treat our patients. Anyhow, to assess if the antiserum itself could account for the methemoglobinemia, we incubated 3 ml of normal group 0 whole blood with 30 μl of anti-**Loxosceles**, anti-**Crotalus** and anti-**Bothrops** antisera, in addition to incubation with 30 μl phenol 0.3% in saline, as phenol is the conservating agent used for the antiserum produced in the Butantan Institute; 10 μl of 1mg vacuum dried/ml **Loxosceles gaucho** venom was also added to 3 ml of whole blood. The samples were kept at room temperature (around 25°C) and aliquots were taken for methemoglobin and NADH-methemoglobin reductase assays under aseptic conditions after 1, 4, 8, 15 and 25 days, thus ruling out the antisera as the cause of methemoglobinemia seen in most patients. Neither 0.3% phenol in saline nor the **L.gaucho** venom did induce methemoglo-binemia. However, by the 25th day methemoglobin increased in all samples, including the controls, probably due to the slight NADH-methemoglobin

TABLE 1

Methemoglobin (% of total hemoglobin) in patients victims of Loxosceles sp. bite.

Patient no.	Methemoglobin %	Time after bite (hours)
1	0.4	50
2	0.4	120
3	0.4	48
4	0.4	?
5	0.4	51
6	0.4	48
7	0.5	57
8	0.6	?
9	0.8	72
10	0.5	50
11	0.8	30
12	0.5	04
13	1.8	?
14	1.7	58
15	2.5	82
16	3.7	79
17	2.5	216
18	3.1	96
19	3.2	120
20	2.6	120
21	5.7	192
22	6.4	96
23	4.1	87
24	6.6	96
25	8.8	128

Student "t" test employed for time elapsed between bite and assay between normal group (patients 1 to 12 : X = 53 hours, S = 29 hours) and higher than 1.1% methemoglobin group (patients 13 to 25 : X = 97.5 hours, S = 42.5 hours) showed "t" = 2.88 ($p<0.01$)

reductase activity decrease, as shown in tables 2 and 3.

The same experiment was performed with red blood cells from a patient who suffered **Loxosceles sp.** (patient no. 23) bite and exhibited methemoglobinemia 10 months ago (tables 2 and 3). Again, no increase in methemoglobin was observed up to the 15th day. The samples at the 25th day also exhibited high levels of methemoglobin, and the slight methemoglobin reductase activity decrease may have similarly been responsible for this finding. It is noteworthy that in this patient the **L.gaucho** venom did not induce methemoglobinemia **in vitro**, although it occured **in vivo** after the bite, what suggested that the venom **in natura** is not oxidative (methemoglobin inducer). Possibly, a product of its metabolism would be the oxidizing agent.

There are no studies on the **L.gaucho** venom composition, but for **L.laeta** and **L.reclusa**, proteases, phospholipases and hyaluronidase have been reported (SCHENONE & SUAREZ[13]). These proteins could well exist also in **L.gaucho**, what would help in cleaving the red cells membrane and allow macromolecules to enter the cells and thus lead to the methemoglobin formation.

The elapsed time for the methemoglobin assay after the bite (table 1) shows that the average level of methemoglobin is significantly higher in those patients who were investigated later, what suggests that the development of some venom metabolite is necessary for methemoglobin formation. It should be recommended that methemoglobin assay be performed in all patients who suffer **Loxosceles** bite and according to the observed levels, riboflavin (KAPLAN & CHIROUZE[7]) and ascorbic acid (JAFFÉ[6]), known as good reducing agents for methemoglobinemia, could be prescribed, what could avoid increasing the methemoglobin levels and ameliorate the general condition of the patients.

RESUMO

Meta-hemoglobinemia associada ao loxoscelismo

Vinte e cinco pacientes que apresentaram a forma cutânea do loxoscelismo foram estudados após a picada, determinando-se a glicose-6-fosfato desidrogenase, glutationa redutase e glutationa peroxidase eritrocitárias, haptoglobina e latico desidrogenase séricas, bilirrubina, reticulócitos e meta-hemoglobina. Não foi observada hemólise aumentada, mas foi detectado aumento da meta-hemoglobina em 54% dos casos: em 7% entre 1,1% e 2%, em 27% variou de 2,1% a 4%, e em 20% de 4,1 a 8%.

Amostras de sangue de um indivíduo normal do grupo 0 de uma paciente que exibiu meta-hemoglobina após picada por **Loxosceles** foram incubadas separadamente com anti-soros contra **Loxosceles gaucho, Crotalus terrificus e Bothrops jararaca**, com veneno de **Loxosceles**

TABLE 2

Serial methemoglobin assay (g% of total hemoglobin) in blood samples incubated with anti-sera, **L. gaucho** venom and 0.35% phenol

	O hour		1st. day		4th. day		8th. day		15th. day		25th. day	
	C*%	P**%	C%	P%	C%	P%	C%	P%	C%	P%	C%	P%
1. Non sterile blood	0.3	0.3	0.3	0.3	0.3	0.4	0.3	0.4	0.4	0.4	0.2	2.58
2. Sterile blood	0.3	0.3	0.3	0.2	0.3	0.4	0.4	0.4	0.4	0.4	1.2	2.52
3. Sterile blood + anti-**Loxosceles** anti-serum	0.4	0.3	0.4	0.3	0.3	0.3	0.4	0.3	0.3	0.4	1.3	3.14
4. Sterile blood + anti-**Bothrops** anti-serum	0.4	0.3	0.4	0.4	0,3	0.4	0.3	0.4	0.4	0.4	0.58	0.61
5. Sterile blood + anti-**Crotalus** anti-serum	0.3	0.3	0.4	0.4	0.3	0.3	0.4	0.3	0.4	0.4	0.58	0.61
6. Sterile blood + 0.35% Phenol	0.4	0.3	0.3	0.4	0.4	0.4	0.4	0.4	0.4	0.4	1.2	2.85
7. Sterile blood + **Loxosceles gaucho** venom (1mg/ml)	0.4	0.3	0.3	0.3	0.3	0.4	0.3	0.4	0.4	0.4	0.62	0.68

* C = Control: group 0 whole blood from a normal donor
** P = Blood from patient no. 23, who presented methemoglobinemia following **Loxosceles** bite

TABLE 3

Serial determinations of red cell methemoglobin reductase activity (U.I./GHb/MIN/37°C) in blood samples incubated with anti-sera, **L. gaucho** venom and 0.3% phenol

	O hour		8th. day		15th. day		25th. day	
	C*	P**	C	P	C	P	C	P
1. Non sterile blood	15.5	19.0	15.4	19.2	14.5	18.8	11.1	18.5
2. Sterile blood	16.7	18.9	16.2	19.1	16.3	19.0	16.0	18.9
3. Sterile blood + anti-**Loxosceles** anti-serum	16.0	19.2	15.9	18.9	15.0	18.9	14.5	14.5
4. Sterile blood + anti-**Bothrops** anti-serum	16.0	19.0	16.0	19.0	15.4	19.2	13.5	18.6
5. Sterile blood anti-**Crotalus** anti-serum	16.1	19.1	16.3	19.3	15.8	19.0	14.4	18.5
6. Sterile blood + 0.35% phenol	16.5	19.2	16.5	19.1	16.4	19.2	14.8	18.2
7. Sterile blood + **Loxosceles gaucho** venom (1mg/ml)	16.5	18.9	16.5	19.0	16.2	19.1	14.1	16.8

* C = Erythrocyte methemoglobin reductase form a control: group 0 normal donor
** P = Erythrocyte methemoglobin reductase from patient no. 23 who presented methemoglobinemia following **Loxosceles** bite

gaucho e fenol a 3%, e não se detectou aumento de meta-hemoglobina depois de 1, 4, 8 e 15 dias em todas as amostras. Por ocasião do 25º dia, todas as amostras, inclusive os controles, exibiram discreta diminuição da atividade da meta-hemoglobina redutase. Os dados sugerem que a meta-hemoglobina que ocorreu em 54% dos pacientes provavelmente decorreu do metabolismo do veneno, uma vez que o veneno **in natura** não induziu meta-hemoglobinemia.

REFERENCES

1. BARRETTO, O.C. DE O.; NONOYAMA, K.; MIZUKAMI, S. & MASPES, V. – Método modificado de dosagem de haptoglobina por eletroforese. **Rev. bras. Pat. clín., 20**:104-106, 1984.
2. BARRETTO, O.C. DE O.; CARDOSO, J.L. & CILLO, D.M. de – Viscerocutaneous form of loxoscelism and erythrocyte glucose-6-phosphate dehydrogenase deficiency. **Rev. Inst. Med. trop. S.Paulo, 27**:264-267, 1985.
3. BEUTLER, E. – A manual of biological methods. 2nd. ed. New York, Grune & Stratton, 1975
4. CARDOSO, J.L.; EICKSTEDT, V.R.D.; BORGES, L.; NOGUEIRA, M.T. & FRANÇA, F.O.S. – Loxoscelismo: estudo de 242 casos (1980/1984). In: **Congresso Brasileiro de Toxicologia,** 5., Salvador, 1987. **Anais**. p.13.
5. EVELYN, K.A. & MALLOY, N.T. – Micro determination of oxyhemoglobin, methemoglobin and sulfhemoglobin in a single sample of blood. **J. biol. Chem., 126**:655-658, 1938.
6. JAFFE, E.R. – Methaemoglobinemia. **Clin. Haemat., 10**:99-122, 1981.
7. KAPLAN, J.C. & CHIROUSE, M. – Therapy of recessive congenital methemoglobinemia by oral riboflavine. **Lancet, 2**:1043-1044, 1978.
8. KNIKER, W.T. & MORGAN, P.N. – An inactivator in spider of the fifth component. **Fed. Proc., 26**:362, 1967.
9. KNIKER, W.T.; MORGAN, P.M.; FLANIGAN, W.J.; REAGEN, P.W. & DILLAHA, C.J. – An inhibitor of complement in the venom of the brown recluse spider, Loxosceles reclusa. **Proc. Soc. exp. Biol. (N.Y.), 131**:1432-1434, 1969.
10. MORGAN, B.B.; MORGAN, P.N. & BOWLING, R.R. – Lysis of human erythrocyte by venom from the brown recluse spider Loxosceles reclusa. **Toxicon, 16**:85-88, 1978.
11. NANCE, W.E. – Hemolytic anemia of necrotic arachnidism. **Amer. J. Med., 31**:801-807, 1961.
12. SCHENONE, H.; RUBIO, S.; VILLARROEL, F. & ROJAS, A. – Epidemiologia y curso clinico del loxoscelismo. Estudio de 133 casos por la araña de los rincones (Loxosceles laeta). **Bol. chil. Parasit., 30**:6-17, 1975.
13. SCHENONE, H. & SUAREZ, G. – Venoms of Schystodidae. Genus Loxosceles. In: BETTINI, S., ed. – **Handbook of experimental pharmacology**. New York, Springer-Verlag, 1978. v.48, p.247-275.

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