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Army Research Office memo dtd 15 Feb 1968
THE EFFECT OF EDTA ON THE EXTENT OF TISSUE DAMAGE CAUSED BY THE VENOMS OF BOTHROPS ATRIX (FER-DE-LANCE) AND AGKISTRODON PICTIVORUS (COTTONMOUTH MOCASSIN)

Capt. Herschel H. Flowers, VC
Charles R. Goucher, Ph.D.

3 October 1963
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Report Submitted 23 September 1963

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The animals used in this study were handled in accordance with the "Principles of Laboratory Animal Care" established by the National Society for Medical Research.

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REPORT NO. 596

THE EFFECT OF EDTA ON THE EXTENT OF TISSUE DAMAGE CAUSED BY THE VENOMS OF BOTHROPS ATROX (FER-DE-LANCE) AND AGKISTRODON PSEIVORUS (COTTONMOUTH MOCASIN)

Capt Herschel H. Flowers, VC
Charles R. Goucher, Ph.D.

Pathology Division
US ARMY MEDICAL RESEARCH LABORATORY
Fort Knox, Kentucky

3 October 1963

In-House Laboratory Initiated Research and Development
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ABSTRACT

THE EFFECT OF EDTA ON THE EXTENT OF TISSUE DAMAGE CAUSED BY THE VENOMS OF BOTHROPS ATROX (FER-DE-LANCE) AND AGKISTRODON PISCIVORUS (COTTONMOUTH MOCCASIN)

OBJECT

To determine the effect of EDTA on the extent of tissue damage caused by the venoms of Bothrops atrox (fer-de-lance) and Agkistrodon piscivorus (cottonmouth moccasin).

RESULTS

The chelating agent EDTA injected immediately following and in the area of envenomation greatly minimizes hemorrhage and necrosis which are characteristics of these particular venoms.

CONCLUSIONS

The highest therapeutic value of EDTA is derived by immediate subcutaneous infiltration of this chemical directly into the area of envenomation. The dosage administered depends upon the tissue structure to be infiltrated. EDTA affects the local activity of venom and appears to be a useful adjunct to specific antivenin therapy.
THE EFFECT OF EDTA ON THE EXTENT OF TISSUE DAMAGE
CAUSED BY THE VENOMS OF BOTHROPS ATROX
(FER-DE-LANCE) AND AGKISTRODON PISCIVORUS
(COTTONMOUTH MOCCASIN)

I. INTRODUCTION

Envenomation by the Crotalidae of Central and North America
results in fulminating necrotising wounds, the vast majority of which
are inflicted subcutaneously. Extensive hemorrhage and tissue loss
are suffered in regions joined by lymphatics to the point of envenoma-
tion as well as in areas surrounding that point.

The initially confined and superficial character of the snakebite
wound has suggested the use of "incision and suction" for the removal
of venom from tissue or the inactivation of venom by the use of rea-
gents deleterious to tissue as well as to venom.

The present paper reports a study of the effect of ethylenediaminetetraacetic acid (EDTA) on the course of tissue damage which
followed the subcutaneous injection of Bothrops atrox and Agkistrodon
piscivorus venom in the rabbit and in the chimpanzee. Evidence is
presented here that infusion of an innocuous quantity of EDTA in the
vicinity of envenomation dramatically diminished the hemorrhage and
necrosis caused by these venoms.

II. MATERIALS AND METHODS

The Agkistrodon piscivorus venom used was part of a pooled
sample obtained from reptiles in the serpentarium of this laboratory.
The venom was extracted into a container at ice bath temperatures.
It was then frozen with a dry ice-acetone mixture and lyopholized.
The lyopholized material was pooled with other venom collected over
the course of two years in the same manner. Bothrops atrox venom
was obtained commercially and it was collected and treated in es-
sentially the same way as the cottonmouth moccasin venom. The
venoms were dissolved in physiological saline and immediately in-
jected.

For injection, the disodium salt of EDTA was used. These so-
lutions were adjusted to pH 7.4 with concentrated NaOH.

In this study, the rabbits employed were 3 to 4 kg New Zealand
males. The chimpanzee used was a 45-pound male, approximately
5 years old. Physical inspection of the chimpanzee revealed no abnormalities except an apparently congenital absence of the second toe of the right side.

Preliminary to inoculation with venom, the chimpanzee was captured in a net, covered with a sheet, and open drop ether was administered. After induction, which was accompanied by the usual violent struggle, the animal was placed in a supine position on a table and open drop ether was continued with the use of a mask.

The animal went into deep 4th plane anesthesia, and into a period of apnea. Immediate intratracheal catheterization, suction, and positive pressure artificial respiration were instituted. Normal respiration resumed after about 5 minutes. Cardiac rate and rhythm and the quality of the heart sounds and pulse were unaffected during the apneic interval. The animal did not become cyanotic. Recovery of consciousness was rapid. Fifteen hours after the administration of venom, the chimpanzee became apneic, and efforts at resuscitation failed.

III. RESULTS AND DISCUSSION

The ability of the Crotalidae venoms to produce tissue necrosis has been attributed in part to their content of proteolytic enzymes (1, 2). Deutsch and Diniz (3) reported that the digestion of hemoglobin by cottonmouth moccasin venom was inhibited completely by EDTA, and Philpot and Deutsch (4) stated that EDTA inhibition of venom proteases was partially reversed by phosphate and serum kochsoft. Philpot (5) reported that sodium bicarbonate reversed EDTA inhibition of venom proteases and that the treatment of venom with EDTA prior to intraperitoneal injection in the mouse did not alter its toxicity.

These reports appeared to have discouraged research on the usefulness of EDTA in snakebite therapy. However, in this laboratory, Goucher and Flowers (6) were not able to demonstrate a complete inhibition of protease activity by EDTA nor a diminution of EDTA inhibition by sodium bicarbonate. Furthermore, in the course of these studies (6) strong suggestive evidence was obtained that the venom constituent which caused visible and rapid tissue erosion could not be identified, as Ohsaka suggested (7), solely with the proteases investigated by Deutsch, Diniz, Philpot, and others. Therefore, a reappraisal was attempted of the value of EDTA as a possible therapeutic agent in the treatment of bites of Agkistrodon piscivorus, the cottonmouth moccasin, and Bothrops atrox, the fer-de-lance.
In contrast to the rabbit, the skin of the hands and feet of the chimpanzee is similar to the human in that it is firmly bound to muscle and to underlying tissue. Thus, it was felt that the effectiveness of EDTA in diminishing injury resulting from envenomation in the chimpanzee would parallel to a large extent the effectiveness of this chelate compound in the treatment of snakebite in man.

A chimpanzee was chosen for the experimentation and 5 mg (0.1 ml) of venom was injected into the pulp of the distal phalanx of both index fingers. The pulps of the distal, middle, and proximal phalanges were infused with approximately 1.0, 2.0, and 3.0 ml, respectively, of EDTA. The quantity of EDTA used was the maximum permitted by the anatomy of the structure and the underlying skin and tissue. An additional 4 - 5 ml of EDTA was infused in and around the first web space. The administration of EDTA was started immediately after envenomation and was completed within 5 minutes of that time. The left index finger was untreated and maintained as a control.

The feet of the chimpanzee were injected in essentially the same manner. Ten mg of venom (0.2 ml) was injected into the dorsal region of the first web space of each foot. The right foot was treated by infiltrating 35 ml of 0.1 M EDTA into the onvenomated area.

About 15 hours after the administration of venom, the chimpanzee died. Physical examination at that time revealed little apparent difference between the two hands. The pulp of the index fingers of both hands was distended and firm to the touch and quite dark in color.

The lower extremities were markedly different in external appearance. The left leg was edematous as far as the knee. The treated right leg showed no signs of swelling. The first web space of both feet showed moderate swelling which extended to the plantar surface of the web in each case.

After physical examination, the skin of the extremities was reflected back and the EDTA treated and control areas were examined and photographed. Even though few external differences distinguished the fingers and hand treated with EDTA from the non-treated, striking differences were evident after reflection of the skin (Figs. 3a and 3b). The untreated finger showed necrosis of the pulp of each phalanx. Hemorrhages were present along the tendon sheaths extending as far up as the carpus. Severe general hemorrhage and necrosis were present in the subcutaneous tissue in the dorsal metacarpal region extending upward to the carpus and distally into the first web space.
Hemorrhage extended up the arm along the path of the lymphatics to the axillary nodes. These glands were swollen and hemorrhagic. The treated finger demonstrated much less necrosis and hemorrhage. The pulp of the distal phalanx of the treated finger was dark red and hemorrhagic. However, the pulp of the middle phalanx showed little discoloration and the pulp of the proximal phalanx appeared normal. Slight hemorrhage appeared in the first web space and the subcutaneous tissue of the dorsal metacarpal area; this was minor compared with the control. A strip of hemorrhage extended up the arm following the path of the lymphatics (Fig. 3b). There was swelling and congestion of the axillary lymph glands.

Upon reflection of the skin, the difference between the EDTA treated and non-treated lower limbs was revealed to be greater than the difference between the upper extremities (Figs. 4a and 4b). In physical examination, as noted above, the control foot and leg were considerably swollen. Reflection of the skin showed that this swelling resulted from massive subcutaneous hemorrhage and edema. Necrosis extended above the knee and along the medial surface of the thigh. The inguinal lymph nodes were entirely necrotized and little normal tissue structure appeared to remain. Hemorrhage was present in the abdominal cavity adjacent to the inguinal area. The mesenteric lymph glands were involved but to a lesser extent than the inguinal lymph glands.

The EDTA treated limb was affected by the venom only slightly in comparison with the control. A hemorrhagic area was apparent at the site of venom injection. Hemorrhage was present along the lymphatics of the lower leg. Several hemorrhagic areas were present in the lymphatics of the thigh. The inguinal lymph nodes and adjacent tissues in the abdominal cavity were normal.

In evaluating the use of EDTA it must be recognized that, in addition to locally toxic components, these crotalid venoms also contain systemically toxic components which are not inactivated by EDTA and for which the indications for antivenins remain unaltered whether or not EDTA is used to minimize local effects.

It is apparent that EDTA will be most effective when used against those crotalid venoms which have locally necrotising components (enzymes) which are inactivated by direct contact with EDTA. The results of this study suggest that the prompt local use of EDTA is helpful in diminishing local edema, hemorrhage, and necrosis following cotton-mouth and fer-de-lance envenomation.
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