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Fort Detrick/SMUFD ltr dtd 17 Feb 1972

TRANSLATION NO. 2382

DATE: Jan 1969

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DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

EXPERIMENTAL ADRENAL GLAND NECROSIS BY MASSIVE INTRAVASCULAR COAGULATION AND THE EFFECT OF ACTH

Translation No. T-680-1

JANUARY 1969

U. S. ARMY
BIOLOGICAL LABORATORIES
FORT DETRICK, FREDERICK, MARYLAND

EXPERIMENTAL ADRENAL GLAND NECROSIS BY MASSIVE INTRAVASCULAR COAGULATION AND THE EFFECT OF ACTH

(Following is the translation of an article by A. Pataki, Pathology Institute if the Kanton Hospital, St. Gallen, published in the German language periodical J. Ges. Exptl. Med. 142, 1967, pages 75-86. Translation performed by Constance L. Lust.)

It is now extablished that the general Schwartzman-phenomenon or Schwartzman-Sanarelli-phenomenon (SSP) consists of a massive intravascular thrombin formation from fibrin or a fibrinoid substance. In rabbits this is illicited by two doses of endotoxin from gram negative bacteria given at a specific time and amount. Anatomically one regularly sees fibrinthrombins in the glomerula and eventual bilateral adrenal necrosis (Krecke, 1964, McKay 1965). Among the human pathological equivalants of this is the Waterhouse-Frederichsen-Syndrome (WFS). A sepsis with hemorrhagic adrenal gland necrosis (Margaretten and McAdams 1958, Stuber and Hitzig 1960, Krecke et al 1963, Siebenmann 1966). In the WFS ill human, the same disturbance of coagulation can be demonstrated as in experimental SSP. (Krecke et al 1963). For the morphologist two obvious discrepancies appear between the findings in SSP in the experimental animal and WFS of humans. Clasically the SSP is caused in rabbits by a two time IV injection of a gram (-) endotoxin. This leads to a high percentage of bilateral cortical necrosis, but to few adrenal lesions. This is mentioned briefly in many reports (Gerber, 1936, Black et al, 1947, Thomas and Good, 1952, Krecke, 1964, McKay, 1965). However, in WFS in humans even in cases with massive hemorrhagic necrosis of the adrenal only seldom is bileteral necrosis of the cortex found (Siebenmann, 1966). We agree with (Krecke et al, 1963) that not all factors of human and animal disease are identical. Nevertheless, the problem as to why so many severe adrenal lesions appear in humans remains.

Pathologically one must consider the following factors for adrenal infarction:

a.) the closing of capsule arterioles by means of fibrin thrombi followed by ishemic or hemorrhagic necrosis, whereby massive bleeding like a hemorrhagic diathesis may occur.

b.) the functional state of the adrenals, especially a stimulation as a stress reaction during sepsis.

c.) a direct breaking of the walls of the cappillaries, followed by bleeding.

It should be possible to elucidate the importance of these factors in animal experiments, but only if the intravascular coagulation is not

illicited by SSP, but rather in some other way. We tried to produce adrenal lesions in rabbits via massive intravascular coagulations without a toxic effect on cappillaries. Then we tested the effect of ACTH in stimulating the adrenal during this process.

Methods

Animals: 42 male and female rabbits, weight 1750-4530 g. fed "Nafag", Gossau SG and water ad libitum:

Intravascular coagulation: We ellicited this response by a single injection of Sodium polyanetholsulfonate (liquoid, Roche) as based on the work of Rodriguez-Erdman, Krecke et al (1960). The coagulation and morphology caused in this way was studied extensively by these authors and Krecke (1964). This resulted in an immediate (hours) general hemmorrhagic diethisis. Since this was undiserable for the present work we modified the method. We noticed that not only the dose, but also the duration of injection was important in order to get a general intravascular formation of coagulation and can cause a longer time to death of animals. We infused for several hours (Lee 1962, Simon) epsilon-amino-capróic acid. In this way fibrin thrombin formation is enhanced and longer. This mechanism is not understood as yet; perhaps inhibition of fibrinolysis as well as blockade of RES.

The following method was optimal for us. Liquoid (12 mg/kg) as a 3.4% solution is injected over 3-5 min. into a marginal ear vein followed by an infusion for 2.5-3 hours of 90 ml saline containing 1 g/kg epsilon amino caproic acid. Lungs, kidney and adrenals were studied histoloigically in sacrificed animals and in those that died. Stain: HE, PAS, phosphotungstic and hematoxilin to demonstrate fibrin. In the tissues the number of fibrin clots were gradual from (+) - (+++++).

ACTH stimulation: In 1934 Anselmino et al showed that the adrenals of rabbits responded somewhat to the corticoid hormone, contrary to rat and mouse. Histological changes are less general in rabbits than in mice or guinea pigs (Bachman 1954). Now it can be shown biochemically that the rabbit adrenal is stimulated by administration of ACTH (Kass et al 1954, Krum and Glenn 1965).

We used "Cortrophin-Z-" Organon in a dose of 15 IU/kg IM. According to the manufacturer (Organon, Holland) a maximal response occurs in 12-24 hours. We found a response in 12-14 hours after injection. No increase in weight of adrenals was noted; no lipid storage. In no control animal did necrosis, thrombosis or hyperemnia occur. This was tested via staining with Sudan III.

Group I. 19 rabbits, "liquoid" and epsilon amino caproic acid; 2 animals sacrificed in first 8 hours; 8 animals died spontaneously in first 3 hours, 5 more at 12-35 hours; 4 were sacrificed at 69 hours.

Group II. 18 rabbits, ACTH injection and 12-14 hours later liquoid and epsilon amino caproic acid. 4 died in first 8 hours after liquoid injection. 4 died 28-57 hours, the rest were sacrificed between 17-72 hours.

Results

Results of Group I confirmed the work of Rodriguez-Erdman et al (1960); a single IV injection of sodium polyanetholsolfunate causes a generalized intravascular coagulation and precipitation of fibrin clots in terminal vessels especially in kidney and lung. Simultaneously a severe generalized hemorrhagic diathisis results. Only in 3 of 19 rabbits, sacrificed at 70 hours was no fibrin thrombin or bleeding demonstrated. The results of the other 16 animals (3 organs) are summarized in table 1. The animals used by Rodriguez et al (1960) usually died in 2-5.5 hours; 6 of 16 in one trial survived 125 hours and some even lived 22 hours. It is unknown whether this was due to a low dose of liquoid (12 mg instead of 15 mg/kg) or infusion of caproic acid. Morphologically the results were similar as before. Lungs, kidney and adrenals contained numerous fibrin clots which stained with PAS and PWSH. They were visible in two animals that died right after injection and were missing in only some organs in animals sacrificed 22-69 hours. The mechanism of fibrin formation is still unknown, but it is definite that most clotting factors and platelets are diminished (Krecke 1964). In lungs fewer clots appeared. This may be due to solution of the clots once formed, but we have no definitive results on this. We do not definitely know whether fibrinolysis occurred.

The flow is altered due to fibrin clots and this may have caused bilateral adrenal necrosis in the animals that survived for longer times. These are fully visible from 22 hours on. The discussion of these anemic, occasionally hemorrhagic zones remains to be done.

The adrenals show numerous fibrin coagulations in the sinusoide capillaries. This was not seen in one animal sacrificed at 69 hours. These clots are less numerous than in the kidney, in agreement with earlier reports (Krecke 1964, figure 13, page 52). Nevertheless some thrombi appear in the adrenal. The "anemic adrenal necrosis" was seen as was also described previously (Rodriguez et al 1960). Only rarely did we observe bleeding at the edge of the necroses. At times the adrenal necrosis appeared simultaneously with bilateral kidney necrosis, but then only 12 hours after liquoid injection. Of 6 rabbits with bilateral kidney necrosis, 5 also showed adrenal necrosis. Only in one animal, which had few fibrin thrombi, were they missing.

Bleeding was noted in the lungs in the early phase and in the kidney and adrenals in the late phase. It may be assumed that those animals that died early did so because of extensive bleeding in the lungs. This hemorrhagic diathisis is characteristic of liquoid and based on coagulation-physiological studies is due to a direct action of the sodium polyanetholsulfanate on the clotting mechanism (Krecke 1964). The hemorrhagic diathisis caused by liquoid is distinctly different from that caused by endotoxin is SSP. Earlier (Krecke 1964) liquoid was reported to cause severe bleeding in the adrenal. In our case this was not so extensive; they were lacking completely in 50% of all animals. We assumed this was because of infusion of epsilon amino caproic acid following liquoid injection.

The effect of an "early" ACTH stimulation under otherwise identical experimental conditions (group II) is presented in table 2. As in group I, 3 of 18 animals showed no pathological alterations when sacrificed at 72 hours. Those percentages with "no response" is therefore the same for both groups. Only 4 of 15 rabbits died in the first 5 hours, contrary to 8 in group I.

The intravascular fibrin formation is qualitatively not modified by pretreatment with ACTH. In lungs and kidneys no quantitative difference was seen. In the adrenals it was, however, very clear that ACTH treatment resulted in less adrenal clot formation than in untreated animals. The difference, which is presented in table 3, is statistically very significant (p. 5%).

The semiquantitative measure of fibrin clot formation in kidney was used as a control figure. When the kidneys were (+++) in fibrin clots then the adrenals were compared. The adrenals were then treated separately.

Bilateral adrenal necroses appeared in greater numbers in those animals that survived longer. Eleven animals that survived 17-22 hours, or were sacrificed at this time, all showed typical necroses which could not be distinguished from those of rabbits treated with ACTH. In the adrenal less necroses were found after ACTH. When necroses were found they were indistinguishable from those of group I in number, size and histologically.

As for bleeding, the difference between the two groups was only that treatment with ACTH cause more generalized bleeding in animals surviving for 5 hours or more. This was most noticeable in lungs. In animals not treated, only in those that survived 22 hours showed bleeding in the lung. Treating with ACTH resulted in longer survival time in those animals which had more generalized lung bleeding. The longer survival time, over those lung bleedings which caused early death, may be because of increased resistance in general due to the elevated secretions of adrenal hormones as ellicited by ACTH.

It was found that treatment of ACTH does not result in a general increase in hemorrhagic diethesis as caused by liquoid. However, the appearance of adrenal bleeding appeared to be enhanced by ACTH.

In our experiments stimulation with ACTH is expected only in the first hours after liquoid injection. During this time 6 rabbits of group I showed lung bleeding of (+ to +++) and only 2 adrenal bleeding of (+ to ++). No animal without lung bleeding had adrenal bleeding. Only 4 animals can be studied in the "safe adrenal stimulation" period. Two animals with lung bleeding had extensive adrenal damage. A third had no lung bleeding.

In summary; our results show that IV injection of Na-polyanethol-sulfonate followed by epsilon-amino-caproic acid causes in rabbits a generalized intravascular fibrin clot formation in lungs, kidney and

adrenal. It causes anemic necroses as well as classical bilateral adrenal necrosis. Pretreatment with ACTH leads to a significant decrease of clot formation and ishaemic necrosis in the adrenal. The common hemorrhagic diathesis caused by liquoid is not affected, but ACTH does cause increased adrenal bleeding. We showed anemic necrosis of adrenals. The basis for this is still not known. The effect of an ACTH stimulation of fibrin clot formation and necrosis could be studied.

Discussion

The ishemic adrenal necroses of our work were not enhanced by previous ACTH stimulation, but rather were lessened. Presently we can not give an adequate explanation for this corticotropic stimulation. This at first appears to contradict results of other workers who found ACTH stimulation increases the occurance of adrenal lesions caused by intravascular coagulation.

Margeretten et al (1964) produced in rats adrenal bleeding by giving thrombin and simultaneously giving epsilon amino caproic acid to inhibit fibrinolysis. These lesions appeared much more frequently after first giving ACTH. This was explained as follows: in rats ACTH causes a dilation of the sinus membrane which allows greater thrombin formation with secondary bleeding. The extent of thrombin formation was not ascertained in the work. Further work by the same group (Margeretten et al 1965) confirmed these results. In animals with extensive hemorrhagic adrenals they found intravascular thrombin in only 3 of 10 animals. Ishemic infarction did not occur. Gabbiani et al (1965) produced "thrombin-hemorrhagic" lesions in numerous organs by thorium dioxide. After pretreatment with ACTH bleedings occurred in adrenals and in other organs. These experiments were only done in rats and occurrance of thrombin was not measured.

All these trials point to a favoring of hemorrhagic adrenal infarction via a corticotropic stimulation of the organ. Therefore, they cannot be compared with our results. Our findings are not in opposition to these. Besides the different method used to illicit intravascular coagulation, species differences may also account in part for certain discrepancies.

ACTH causes a hyperemia in the cortex of rats; in rabbits this did not occur. This was also found by Harrison and Hoey (1960). In any case, we found that ACTH stimulation results in more frequent adrenal bleeding as caused by liquoid than in non-stimulated rabbits. Further work must be done to elucidate whether ACTH simply elevates the capability for bleeding in adrenals; furthermore, whether the bleeding in cortex of rats also are caused this way and not via intravascular coagulation.

With the methods we used we could not reproduce the hemorrhagic adrenal infarction of the human WFS. Pathogenically it was concluded that intravascular fibrin clot formation is the adrenal is not the only and maybe not the most important pathogenic factor. Our data clearly show that intravascular coagulation in the cortex leads to anemic

infarction. For the adrenal lesions of humans the bleeding of the cortex tissue is important, whether it is caused by ishemic or toxic vessel lesions, or simply by a general propensity to bleed. Hemorrhagic lesions are very probably enhanced by the stress-required endogenous cortex stimulation.

Summary

Waterhouse-Friderichsen-syndrome is considered today as one of the human manifestations of the generalized Shwartzman-phenomenon with disseminated intravascular coagulation. There is however a discrepancy between the findings in the phenomenon in the rabbit and the human disease, in that hemorrhagic infarction of the adrenals is inconspicious in the first and frequent in the latter. The pathogenesis of the adrenal changes were studied by examining the effect of massive intravascular coagulation induced by intravenous heparinoid (Liquoid)-followed by an infusion of epsilon-amino-capronic acid-on the adrenal cortex. With our method ishemic infarction of the adrenal cortex could be produced in a high percentage of animals. Simultaneous administration of ACTH reduces significantly the number of fibrin thrombi and the extent of ishemic necrosis in the adrenal cortex. On the other hand it probably enhances the well known cortical hemorrhages in the early phase of the experiment.

Tabelle 1. *Intravasale Gerinnung durch Liquoid und E-ACS bei 16 Kaninchen*

Nr.	t (h)	Thromben L	Thromben Ni	Thromben NN re/li	Nekrose Ni	Nekrose NN re/li	Blutung L	Blutung Ni	Blutung NN re/li
847	sofort	+++	(+)	(+)	0	0	++	0	0
846	sofort	++	+	(+)	0	0	0	0	0
57	1 sp.*	+	+	(+)	0	0	+	+	0
50	1½ sp.	++	++	(+)	0	0	+++	0	+
860	1½ sp.	+++	++	(+)	0	0	+++	0	++/
856	1½ sp.	+++	++	(+)	0	0	++	0	0
848	2 sp.	++	+++	+	0	0	++	0	0
2a	2	+	++	+	0	0	0	0	0
845	3 sp.	++	+++	++	0	0	(+)	+	(+)
4a	8	++	+++	+++/++	0	0	0	0	0
21a	12-14 sp.	++	+++	(+)	+	+	0	0	++
863	22 sp.	+	+++	(+)	+++	0	++	+++	0
29	33 sp.	0	+++	+++	+++	+++	0	++	++
3a	66 sp.	++	+++	+++	+++	+++	0	+	+
62	35 sp.	+	+++	+++	++	+++	0	+	+
60	69	0	+++	(+)/0	++	+/0	0	+	0

* sp. = spontan verstorben.

Table 1 - Intravascular coagulation by liquoid and epislon-amino-capronic-acid.
Blutung = bleeding; sofort = right away; sp = sponteaneous death;
L = lung; Ni = kidney; NN = adrenals

Tabelle 2. *Intravasale Gerinnung durch Liquoid bei 15 ACTH-vorbehandelten Kanin*

Nr.	t (h)	Thromben L	Thromben NI	Thromben NN re/li	Nekrose NI	Nekrose NN re/li	Blutung L	Blutung NI	Blutung NN re/li
65	2 sp.*	++	(+)	(+)	0	0	+++	0	+-
62	2 sp.	+++	++	+	0	0	0	(+)	0
64	3 sp.	+++	++	+	0	0	+++	0	+-
69	5-8 sp.	+	++	++	0	0	0	+	+-
58	17-20	+	+++	(+)	+++	0	+	++	0
61	20	+	+++	+	++	0	+	+	0
857	28 sp.	++	+++	(+)/+	+++	0	+++	++	0
861	32 sp.	+	+++	+++	+++	+++	+	+++	+
853	45 sp.	+	+++	(+)/0	+++	0	++	++	0
841	57 sp.	+	+++	+++/++	+++	+++/++	+	++	0/-
59	69	+++	+++	(+)/+	+++	0/++	0	++	0
68	69	0	(+)	0	+	0	0	0	0
856	71	0	++	++	++	+++	+	++	+-
850	71	0	+	0	++	0	+	++	0
864	72 sp.	+	+++	(+)	+++	0	+	++	0

* sp. = spontan verstorben.

Table 2.- Intravascular coagulation by liquoid after pretreatment of rabbits with ACTH
Legend same as Table 1

Tabelle 3. *Ausmaß der Nebennierenthrombose der Kaninchen mit maximaler Nierenthrombose*

Thrombosenausmaß	0 bis +	++ bis +++	Total
9 Tiere mit +++ Nierenthrombose ohne ACTH	8	10	18 NN
8 Tiere mit +++ Nierenthrombose mit ACTH	12	4	16 NN

Statistische Signifikanz der ACTH-Wirkung: n = 1, χ^2 = 3,26, P nahezu 5%.

Table 3 - Extent of adrenal clots of rabbits with maximal kidney coagulation -
9 animals with +++ kidney thrombin (no ACTH)
8 animals with +++ kidney thrombin (+ACTH)

Tabelle 4. *Kaninchen mit Nierennekrosen nach intravasaler Gerinnung*

	NN Nebennierennekrosen	Ohne Nebennierennekrosen
6 Tiere ohne ACTH	5	1
11 Tiere mit ACTH	4	7

Statistische Signifikanz der ACTH-Wirkung: n = 1, χ^2 = 3,42, P nahezu 5%.

Table 4 - Rabbits with kidney necroses after IV coagulation.

	+necrosis	−necrosis
6 animals (no ACTH)	5	1
11 animals (+ACTH)	4	7

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