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Time Course of Changes in Free Thyroid Indices, rT3, TSH, Cortisol and ACTH Following Exposure to Sulfur Mustard

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Abstract: In order to evaluate the time course of changes in serum concentration of thyroid hormones, cortisol and ACTH in patients exposed to chemical weapons containing sulfur mustard, we measured serum concentrations of hormones on the first, third and fifth week following injury in 13 soldiers and compared them to the results obtained from 34 control men. Free T4 and T3 indices were decreased and rT3, cortisol and ACTH were increased in the first week following exposure. There was a subnormal TSH response to TRH in 2 of 3 men tested. Except for an increase in FT4I and a decrease in TSH by the third week, and a steady decline in serum cortisol, serum hormone concentrations were unchanged until the fifth week after injury. The decline in serum cortisol occurred despite a constant increase in serum ACTH. By the fifth week only 1 of 13 men had serum cortisol levels $>10\,\mu g/dl$.

We conclude that exposure to chemical weapons containing sulfur mustard results in alterations in serum concentrations of thyroid and adrenal hormones and ACTH, resembling changes seen in burn trauma. Some evidence of direct effects of mustard on endocrine glands exist.

Introduction

Mustard gas (bis-2-chloroethyl-sulfide), an alkalating agent, was first manufactured for use in the First World War during which many thousands of men were poisoned by it (Beebe 1960). Two types of exposure have been investigated. Acute exposure resulting from use of the agent in the war (Dientbier 1985) or occasional exposure of fishermen to previously deposited mustard gas containers in the sea (Dahl et al., 1985), and chronic exposure in the course of its manufacture (Easton et al., 1988). Side effects of sulfur mustard on the skin, eyes, respiratory, hematological and gastrointestinal system, and its mutagenicity and carcinogenicity have been reported (Goodman and Gilman, 1955; International Agency for Research on Cancer 1975; Wada et al., 1968). However, the effect of mustard gas on the endocrine glands is unknown. Although the use of other alkylating agents has resulted in inhibition of spermatogenesis (Miller 1971) and induction of ovarian failure (Warne et al., 1973), these complications have occurred after repeated administration of a combination of chemotherapeutic agents.

According to the report of the specialists appointed by the General Secretary of the United Nations, mustard gas has been used in the Iran-Iraq conflict (Report of specialists, 1986). As a result, we had an opportunity to study serum concentrations of various hormones following exposure of Iranian soldiers to chemical weapons containing sulfur mustard (Azizi et al., 1989 A). Free thyroid indices and serum testosterone were found to be decreased and serum ACTH increased. In order to investigate the time course of changes in serum concentrations of various hormones after injury by mustard gas, we undertook further studies.

Materials and Methods

Patients

Thirteen men who were unjured by chemical warfare were studied in 1987. The suggestion that warfare contained sulfur mustard was confirmed by reported characteristics of chemical warfare in the battlefield, chemical determination, delayed appearance of symptoms by several hours after ex-

posure and pathognomonic effects on the eyes, skin and respiratory system.

All 13 men, aged 21 to 32 years, were hospitalised two to seven days after injury and were in rather serious condition upon admission. The skin and occular lesions begin to ease following the third week after exposure; however, 8 of 13 men suffered from mild to severe respiratory distress for weeks following injury. There were no symptoms or signs suggesting endocrine involvement.

This study was approved by the appropriate Human Research Review Comittee, and informed consent was obtained, in writing where possible, from each patient.

Sampling

Venous blood samples were collected between 0700 and 0800 hr. on the first, third and fifth weeks after exposure. Samples were centrifuged and serum was separated and stored at −20 °C until analysis. All samples for each patient were analyzed simultaneously in one assay.

TRH test

In three men, after a baseline sample, 400 µg TRH was injected intravenously and additional samples were obtained 15, 30, 45, and 60 minutes after TRH administration.

Hormone analysis

Serum concentrations of T4, T3, comptetive radiolimmunoassay TSH, cortisol, and T3 resin uptake tests were performed by commercial kits (Diagnostic Products Corp., Los Angeles, Ca, U.S.A.). Plasma ACTH and serum rT3 and thyroglobulin were determined by using kits from BYK-Sangtec Diagnostica, (Dietzenbach, Germany). Free T4 index (FT4I) and free T3 index (FT3I) were then calculated (Sawin et al., 1978). In all assays performed, the inter-assay and intra-assay variations were less than 10 % and 7 % respectively.

Control subjects

A group of 34 healthy men of similar age were used as normal controls.

Statistical analysis

Differences between mean values for quantitative variables were evaluated by student's t-tests; t-test to compare values between injured men and normal controls and paired t-test to evaluate values in exposed subjects on different dates.

Results

Thyroid tests and TSH

The results of free thyroid indices and serum concentrations of rT3, TSH, and thyroglobulin in injured men and normal controls are shown in Table 1. In the first week, FT4I and FT3I were decreased in men exposed to mustard as compared to normal controls (6.8 ± 1.4 vs. 8.7 ± 1.2 µg/dl and 100 ± 19 vs. 130 ± 11 ng/dl, respectively; $P < 0.001$ for both).

Mean serum concentration of rT3 in injured men was higher than mean normal values (31 ± 10 vs. 24 ± 6 ng/dl, $P < 0.01$). Serum concentrations of TSH and thyroglobulin were in the normal range. Of three men who had TRH tests, two had subnormal response of TSH to TRH administration.

Table 1 Free thyroid hormone indices, and serum concentrations of T4, T3, reverse T3, TSH and thyroglobulin in 13 young men, on the first, third and fifth weeks following injury by mustard gas

Test	Weeks after injury by mustard			Normal controls
	1	3	5	(n = 34)
T4 (µg/dl)	7.1 ± 1.5*	9.4 ± 2.2+	6.5 ± 1.3*	8.8 ± 1.2
T3 (ng/dl)	105 ± 20*	116 ± 20*	114 ± 14*	131 ± 11
FT4I	6.8 ± 1.4*	9.1 ± 2.1+	6.4 ± 1.3*	8.7 ± 1.2
FT3I	100 ± 19*	113 ± 19*	112 ± 14*	130 ± 11
Reverse T3 (ng/dl)	31 ± 10§	24 ± 6+	26 ± 6	24 ± 6
TSH (µU/ml)	2.0 ± 1.4	1.6 ± 0.9≠	2.6 ± 0.8	2.0 ± 0.8
Thyroglobulin (ng/ml)	39 ± 39	33 ± 23	31 ± 23	36 ± 19

Compared to normal controls: * $P < 0.001$, § $P < 0.01$
Compared to values in the first week: + $P < 0.025$, ≠ $P < 0.05$
All values are mean ± SD.

The increment of TSH was 1.4, 4.9 and 12.2 μU/ml after TRH injection.

In the third week following exposure, FT4I and FT3I increased significantly, as compared to the values in the first week after injury (9.1 ± 2.1 vs. 6.8 ± 1.4, $P < 0.025$ and 113 ± 19 vs. 100 ± 19, $P < 0.05$; respectively). Serum rT3 decreased from 31 ± 10 in the first week to 24 ± 6 ng/dl in the third week ($P < 0.025$), while serum TSH decreased from 2.0 ± 1.4 to 1.6 ± 0.9 μU/ml ($P < 0.05$).

Five weeks after poisoning by mustard, serum FT4I had again decreased and serum TSH increased, as compared to values in the third week. At this time FT4I and serum TSH did not significantly differ from those in the first week following injury. Serum thyroglobulin remained unchanged throughout this study.

Cortisol and ACTH

In the first week, mean serum concentrations of cortisol and ACTH were significantly increased, as compared to those in normal subjects (21 ± 9 vs. 15 ± 5 μg/dl, $P < 0.01$, and 63 ± 24 vs. 35 ± 19 pg/ml, $p < 0.001$; respectively). Eight of thirteen men had serum ACTH above 60 pg/ml. Serum concentrations of ACTH remained increased in the ensuing weeks (Table 2), while serum cortisol decreased significantly and reached lowest values by the fifth week after exposure (7 ± 5 μg/dl, $P < 0.001$, as compared to both normal controls and the values in the first week). In the fifth week following exposure, only one of 13 men had 8 AM cortisol levels above 10 μg/dl, while six of 13 men still showed ACTH concentrations above 60 and the mean serum ACTH for the group was 62 ± 42 pg/ml.

Unfortunately, this study could not be followed for a longer period; however, in five men who returned between one to three years after exposure, thyroid indices and serum cortisol and ACTH were well within normal limits.

Table 2 Serum concentrations of cortisol and ACTH in 13 young men on the first, third and fifth weeks following exposure to sulfur mustard

Test	Weeks after exposure to sulfur mustard			Normal controls (n = 34)
	1	3	5	
Cortisol (μg/dl)	21 ± 9*	15 ± 5	7 ± 5+≠	15 ± 5
ACTH (pg/ml)	63 ± 24+	55 ± 26*	62 ± 42*	35 ± 19**

Compared to normal controls: * $P < 0.01$, + $P < 0.001$
Compared to values in the first week: ≠ $P < 0.001$
** All values are mean ± SD

Dicussion

In this study we have evaluated the effect of chemical warfare containing sulfur mustard on the serum concentrations of thyroid hormones, TSH, cortisol and ACTH in young men up to 5 weeks after injury. The major findings of the present report are the significant decrease in free thyroid indices, and increase in rT3, cortisol and ACTH in the first week, followed by changes in each parameter three and five weeks following exposure.

We have previously reported that changes seen in men exposed to chemical weapons containing sulfur mustard could not be due to stress of war or environmental factors in the battlefield, since subjects injured by chemical warfare containing nerve gas in similar conditions showed no abnormalities in serum concentrations of T4, T3, TSH, cortisol and ACTH (Azizi et al., 1989 B).

Our findings of decreased FT4I and FT3I are similar to previous observations (Azizi et al., 1989 A). Increased levels of rT3 suggests increased peripheral conversion of T4 to rT3 seen in many nonthyroidal conditions including burn trauma (Azizi 1978; Vaughan et al., 1985; Becker et al., 1980; Doleček 1989). The degree of increase in rT3 is mild and disproportionate to the severe decrease in FT3I, suggesting that T3 fall is not exclusively due to decreased conversion of T4 to T3. In fact, since both FT4I and FT3I are diminished, one may assume that the secretion of hormones from the thyroid gland may have been decreased. It has been shown that even small decreases in serum concentrations of thyroid hormones are accompanied by an increase in serum TSH (Vagenakis et al., 1974). Lack of increase in TSH in the present study may have been due to inadequate sensitivity of the kit to detect small changes in serum TSH. Furthermore, the specifity of assays of TSH in sick patients has been challanged (Spencer et al., 1987). It is also conceivable that some degree of hypothalamic-pituitary dysfunction or lesion exists after mustard injury, leading to disproportionate levels of serum TSH. The results of TRH tests, showing subnormal TSH response to TRH in 2 of 3 patients, supports this hypothesis. Decreased serum levels of T4 and T3, and increased rT3 have been observed after burn trauma (Vaughan et al., 1985; Becker et al., 1980; Doleček 1989). Serum TSH and its response to TRH has been normal, but in severe cases response of TSH stimulation has been less than normal (Becker et al., 1982).

The time course of changes in thyroid hormone concentrations three weeks after exposure is puzzling. Decrease in serum rT3 and increase in FT3I indicate some normalization of peripheral T4 metabolism. However, increase in FT4I and de-

crease in serum TSH cannot be explained by re-activation of hypothalamic-pituitary axis. Primary hyperactivity of the thyroid gland may be suggested, but cannot be substantiated. By the fifth week following injury, rT3 and T3 remained unchanged, while T4 and TSH returned to values comparable to the first week. This may suggest further hypothalamic-pituitary dysfunction or direct late effect of mustard on thyroid cells.

Changes in serum cortisol and ACTH in the first week after injury are most probably due to stress and resemble results reported following sever burns (Doleček 1989; Vaughan et al., 1982). Consistent elevation of ACTH in the ensuing weeks corresponds with the clinical status of these patients who continued to suffer from systemic side effects of mustard. Decreased serum cortisol in the third and fifth weeks, despite constant increased levels of ACTH, may suggest that adrenal glands were not responsive to ACTH stimulation, probably due to the direct effect of poison. This might occur in any organ with high cellular activity, since mustard, like other alkylating agents, cross-links the twin strands of the DNA macromolecule, resulting in the prevetion of DNA replication (Lanley and Brookes, 1965).

We conclude that most of the effects of sulfur mustard on the thyroid and adrenal hormones resemble changes seen after burn trauma. However, special alterations due to direct cellular effect of mustard on hypothalamus, pituitary, thyroid and adrenal glands merits further consideration.

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