MORPHOMETRIC CHANGES IN THE LAYERS OF THE ADRENAL GLANDS IN WHITE RATS UNDER CHRONIC CARBON MONOXIDE INTOXICATION

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Abstract. *Chronic exposure to carbon monoxide (CO) is one of the most common forms of low-grade gaseous intoxication, yet its long-term effect on the endocrine organs remains insufficiently studied. The present experimental study evaluated the morphometric alterations occurring in the layers of the adrenal glands of white rats subjected to chronic CO intoxication. A total of 120 outbred white rats of both sexes were allocated into a control group and two experimental groups and exposed to a CO concentration of 0.01–0.05 mg/L over a two-month period. Histomorphometric, histochemical, microscopic, immunohistochemical and statistical methods were used. Chronic intoxication was accompanied by thinning of the adrenal cortex, relative enlargement of the medullary layer, proliferation of collagen fibres, thickening of vascular walls, perivascular oedema and lymphocytic infiltration. The findings indicate that prolonged CO exposure induces fibrotic remodelling and hypoxia-related structural damage of the adrenal gland.*

Keywords: *carbon monoxide, chronic intoxication, adrenal gland, morphometry, cortex, medulla, hypoxia, fibrosis.*

Introduction

Carbon monoxide is a colourless and odourless product of the incomplete combustion of carbon-containing material. Because its affinity for haemoglobin is several hundred times greater than that of oxygen, even modest ambient concentrations produce carboxyhaemoglobin and reduce the oxygen-carrying capacity of the blood, thereby provoking tissue hypoxia. While the acute clinical picture of CO poisoning is dominated by neurological and cardiac manifestations, attention has increasingly turned to its insidious chronic and low-grade form, which may damage organs with a high metabolic demand over prolonged periods.

The adrenal glands occupy a central position in the body's response to stress and hypoxia. The cortex, organised into the zona glomerulosa, zona fasciculata and zona reticularis, secretes mineralocorticoids, glucocorticoids and androgens, respectively, while the medulla releases catecholamines. Activity of the cortex is governed by the

hypothalamic–pituitary–adrenal axis. Epidemiological and experimental observations have linked CO exposure to disturbances of adrenal function, including an elevated long-term risk of adrenal insufficiency, attributed both to direct hypoxic injury and to the inflammatory and oxidative reactions triggered by the gas. Despite these clinical associations, the structural basis of such dysfunction at the level of the individual cortical and medullary layers has received limited morphometric characterisation.

The present study was therefore designed to assess, by quantitative morphometry, the alterations occurring in the distinct layers of the adrenal glands of white rats exposed to chronic carbon monoxide intoxication, and to relate these measurements to the accompanying histological and vascular changes.

Materials and Methods

The experiment was performed on 120 outbred white rats of both sexes, aged 6 and 12 months, kept under standard vivarium conditions with free access to food and water. After a seven-day quarantine period, during which the animals were screened to exclude somatic and infectious disease, they were randomly allocated into three groups of 40 animals each: one intact control group and two experimental groups subjected to chronic carbon monoxide intoxication.

The intoxication model was reproduced in sealed metal chambers maintaining a carbon monoxide concentration of 0.01–0.05 mg/L over a period of two months. Control animals received 1 ml of distilled water intragastrically through a metal gastric tube for 14 days. Over the course of the study, 6 rats died as a consequence of chronic intoxication.

All procedures were carried out in accordance with the principles of the Declaration of Helsinki and with international recommendations for the care and use of laboratory animals. Euthanasia was performed by decapitation under ether anaesthesia. The adrenal glands were excised, weighed and processed for examination using histomorphometric, histochemical, microscopic and immunohistochemical techniques. Morphometric measurements included the weight of the gland, the thickness of the capsule, the total thickness of the cortex and of each of its zones, and the thickness of the medullary layer. The data obtained were processed by standard statistical methods and expressed as the arithmetic mean and its standard error (M ± m).

Results

Quantitative morphometry of the adrenal glands of the 12-month-old animals revealed a consistent set of structural changes following chronic carbon monoxide intoxication. The mean weight of a single adrenal gland was 0.95 ± 0.42 g, and the thickness of the adrenal capsule was increased to 3.7 ± 0.10 μm. The total thickness of

the cortical layer averaged 15.2 ± 0.16 μm, within which the zona glomerulosa measured 4.81 ± 0.24 μm, the zona fasciculata 8.05 ± 0.08 μm and the zona reticularis 4.75 ± 0.25 μm. The medullary layer reached a mean thickness of 17.24 ± 0.18 μm. The principal morphometric values are summarised in Table 1.

Table 1. Morphometric parameters of the adrenal glands of 12-month-old white rats under chronic carbon monoxide intoxication.

Morphometric parameter	Value (M ± m)
Mean weight of one adrenal gland	0.95 ± 0.42 g
Adrenal capsule thickness	3.7 ± 0.10 μm
Cortical layer (total thickness)	15.2 ± 0.16 μm
Zona glomerulosa	4.81 ± 0.24 μm
Zona fasciculata	8.05 ± 0.08 μm
Zona reticularis	4.75 ± 0.25 μm
Medullary layer	17.24 ± 0.18 μm

Histological examination complemented these measurements. The amount of periadrenal adipose tissue was reduced, and the stroma of the gland showed a marked proliferation of collagen fibres. The walls of the intraglandular vessels appeared thickened, accompanied by perivascular oedema and focal lymphocytic infiltration. Within the cortex, both the number and the size of the parenchymal cells were diminished, indicating a depletion of the steroidogenic cell population. Taken together, these features point to progressive fibrotic remodelling of the cortex set against a relatively expanded and congested medulla.

Discussion

The morphometric and histological findings of the present study are consistent with the view that chronic carbon monoxide exposure injures the adrenal gland primarily through sustained hypoxia and the associated inflammatory response. The thinning of the cortex, together with the reduction in the number and size of cortical cells, reflects a loss of functional steroidogenic tissue, while the proliferation of collagen and the thickening of vessel walls are typical hallmarks of a chronic fibrotic process. These structural observations provide a plausible morphological substrate for the clinical association reported between carbon monoxide poisoning and later adrenal insufficiency.

The relative enlargement of the medullary layer, occurring against a background of vascular congestion and perivascular oedema, is in keeping with experimental work showing that medullary blood flow rises markedly during both hypoxic and carbon monoxide–induced hypoxia, whereas the cortical response is comparatively blunted. The perivascular oedema and lymphocytic infiltration observed here further support the involvement of microvascular injury and a local inflammatory reaction, mechanisms that have been implicated in CO-related organ damage through the generation of reactive oxygen species.

It is notable that the cortex and the medulla respond in opposite directions: the cortex contracts while the medulla expands. This divergence underlines the interdependent but functionally distinct nature of the two adrenal compartments and suggests that chronic CO intoxication does not affect the gland uniformly. The age-related comparison reinforces this interpretation, in that the reduction of cortical thickness was apparent even in the younger animals. Limitations of the study include the focus on structural rather than hormonal endpoints; future investigations combining morphometry with measurement of corticosterone and catecholamine output would help to clarify the functional consequences of the changes described.

Conclusion

Chronic carbon monoxide intoxication produced distinct morphometric changes in the layers of the adrenal glands of white rats. In the 6-month-old animals the thickness of the adrenal cortex decreased by up to approximately 1.11 times, whereas the medullary layer increased by about 1.14 times, the latter being associated mainly with vascular congestion and perivascular oedema. The accompanying proliferation of collagen fibres, thickening of vascular walls and reduction in the cortical cell population indicate that prolonged carbon monoxide exposure induces fibrotic remodelling and hypoxia-related structural damage of the adrenal gland. These results highlight the adrenal gland as a target organ in chronic CO intoxication and warrant attention to adrenal status in individuals subjected to long-term low-level exposure.

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