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RESEARCH HIGHLIGHT

Down regulation of acrolein on corticosterone secretion in male rats

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Acrolein is a small unsaturated aldehyde and can be found in a wide range of resources including all types of smoke and exhaust gases from gasoline engines. Although the toxicity and damage of acrolein have been recognized, the action mechanisms of acrolein, especially that of acrolein on the response of stresshormones are still unclear. The present study hypothesized that administration of acrolein altered the secretion of both adrenocorticotropin (ACTH) and corticosterone *via* the regulation of steroid biosynthetic pathway in rat zona fasciculata-reticularis (ZFR) cells. Both *in vivo* and *in vitro* approaches were uased. In the *in vivo* study, intraperitonal injection of acrolein (2 mg/ml/kg) once daily for 1 or 3 days resulted in a reduction of plasma levels of ACTH and corticosterone as well as the intracellular cAMP and ACTH-induced secretion of corticosterone. The protein expression of ACTH receptor (ACTHR) in rat ZFR cells was also reduced by 40-60% after treatment of acrolein for 1 day and 3 days, respectively. In the *in vitro* study, rat ZFR cells were prepared and chanllenged with ACTH (10^{-9} M), forskolin (an adenylyl cyclase activitior, 10^{-5} M), 8-Br-cAMP (a permeable synthetic cAMP, $5x10^{-5}$ M), 25-OH-cholesterol (10^{-5} M) ± trilostane (an inhibitor of 3β-hydroxysteroid dehydrogenase, 3β-HSD, 10^{-5} M). The evoked release of corticosterone by ACTH, forskolin, 8-Br-cAMP and the induced release of pregnenolone in response to 25-OH-cholesterol plus triolostane were decreased. Since the accumulation of pregnenolone after blocking 3β-HSD by trilostane represents the activity of $P450_{scc}$, the rate-limiting step of steroid biosynthesis, we suggest that not only the cAMP pathway was inhibited, but also the enzyme activity of $P450_{scc}$ was attenuated following administration of acrolein. Although insignificant, the protein expression of steroidogenic acute regulatory protein (StAR) was decreased by 40% in ZFR cells after treatment of acrolein *in vivo*. Incubation of ZFR cells with acrolein (10^{-9}~10^{-7} M) also decreased the *in vitro* release of corticosterone. These results suggest that administration of acrolein inhibited corticosterone production *via* the attenuation of cAMP pathway, StAR protein expression, and the enzyme activity of $P450_{scc}$. The attenuation of protein expression of ACTHR (also named melanocortin 2 receptor, MC2R) and reduced secrection of ACTH indicated that the hypothalamus-pituitary-adrenal (H-P-A) axis was also down- regulated by the administration of acrolein.

Keywords: acrolein; corticosterone; rats; ZFR cells; ACTH; forskolin; 8-Br-cAMP; StAR; P_{450scc}, MC2R; H-P-A axis

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It is well known that acrolein is a toxic pollutant in air pollution [1]. The sources of air pollution include smoke, wastes of industry, drugs, or fried food [2, 3, 4]. Some toxic effects of acrolein have been mentioned, e.g. rapidly bind to and deplete cellular nucleophiles, inhibit the production of proinflammatory cytokines [5], and suppress the activation of NFκB [6]. Although the levels of gonadotropin are not changed, the levels of plasma testosterone are altered under long-term exposure to cigarette smoke [7]. The histology of testes has revealed that portion of Leydig cells is decreased by the long-term exposure of cigarette smoke [7]. Since glucocorticoid is also an important steroid hormone associated with metabolic and immunologic responses [8], we suspect that the biosynthesis and release of glucocorticoids might be altered by the exposure of acrolein. In the present study, we hypothesized that acrolein may alter the secretion of corticosterone *via* a direct action on the adrenocortical cells.

In order to confirm the effect of acrolein on the pituitary-adrenal axis, both *in vivo* and *in vitro* studies were performed. The concentrations of adrenocorticotropin (ACTH) in plasma and that of corticosterone in plasma and media following cell culture were measured by radioimmunoassay. The protein expressions of ACTH receptor (ACTHR) and steroidogenic acute regulatory protein (StAR) were analyzed by the western blots. The levels of pregnenolone in media and that of intracellular cAMP were detected by enzyme immunoassays. In the *in vivo* study, male Sprague-Dawley rats were intraperitoneal injected with or without acrolein (2 mg/ml/kg) once daily for 1 or 3 days before catheterization *via* right jugular vein and challenged with a single injection of ACTH (5 μg/ml). The control rats received normal saline. Blood samples were collected through catheters continuing the following 2 h. The result showed that the plasma concentrations of corticosterone were rapidly stimulated by ACTH within 10 min. Administration of acrolein dose-dependently attenuated the increase of corticosterone release in response to ACTH challenge. But pre-treatment of acrolein for 3 days resulted in higher basal level of corticosterone. During ACTH challenge the total secretion of corticosterone was decreased and the corticosterone response to ACTH was delayed.

In the *in vitro* study, rats were decapitation after exposure to acrolein for 0, 1 and 3 days. Rat blood samples were collected and the concentrations of both corticosterone and ACTH were measured by RIA. The zona fasciculata-reticularis cells (ZFR) were prepared and cultured *in vitro* with or without ACTH, 8-Br-cAMP, or forskolin. Administration of acrolein resulted in a significant decrease of plasma levels of corticosterone and ACTH as well as *in vitro* release of corticosterone in response to ACTH, 8-Br-cAMP, or forskolin, and the protein expression of ACTHR in ZFR cells. The protein expressions of StAR were reduced by 40% and the levels of intracellular cAMP in response to forskolin were also reduced by 18-32% following administration of acrolein. Pre-treatment of acrolein 3 days decreased the enzyme activity of $P450_{scc}$ *via* the release of pregnenolone following incubation of ZFR cells with trilostane (a blocker of 3β-hydroxysteroid dehydrogenase, 3β HSD). Incubation of rat ZFR cells with acrolein (10^{-9}~10^{-7} M) *in vitro* decreased the basal and ACTH-induced release of corticosterone.

In the present study, we have demonstrated that administration of acrolein either *in vivo* or *in vitro* decreased the secretion of corticosterone. Our *in vivo* data indicated that administration of acrolein increased the basal level of plasma corticosterone, but attenuated the corticosterone secretion in response to ACTH including the maxium response and total amount of corticosterone release during 2 hours. The *in vitro* data indicated that acrolein inhibited the biosynthesis of corticosterone *via* the mechanisms including the down regulation of the generation and function of cAMP, $P450_{scc}$ activity, and the protein expressions of ACTH and StAR in rat ZFR cells. Although more pathways might be involved, the evidence of our study revealed that acrolein is toxic for our endocrine and immune systems. The down regulation of glucocorticoid production may inhibit the anti-inflammatory effects and induce a hyperfunction of immune system. Since acrolein is one of major pollutant during cigarette smoking, we expect to get more damage for endocrine and immune systems after long-term exposure of smoke from any sources. Many studies reported that the concentration of acrolein in mean ambient concentration is 14.3 $\mu g/m^3$ (6.2 ppb) ranging from 8.2 to 24.6 $\mu g/m^3$ (3.6 to 10.7 ppb) and the exhaust gases from gasoline engines and diesel engines are 0.05-27.7

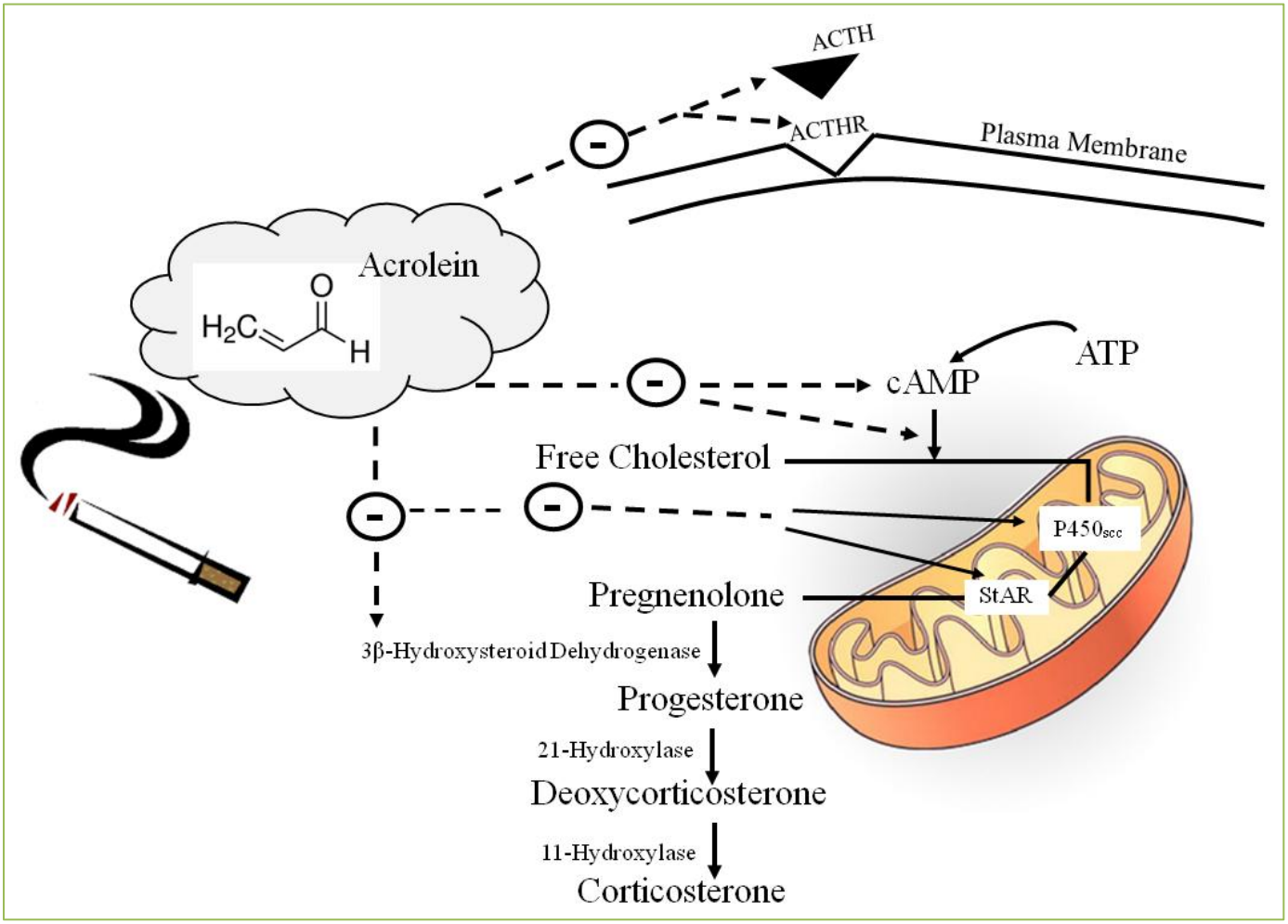

Figure 1. Propose mechanism of acrolein-induced inhibition on the biosynthesis and release of corticosterone in rat zona fasciculate-reticularis (ZFR) cells. Acrolein, a toxic pollutant exists in the smoke, downregulats the activity of H-P-A axis *via* a reduction of （1） ACTH secretion, （2） protein expression of ACTHR (MC2R), （3） the generation and action of cAMP, （4） the enzyme activity of $P450_{scc,}$ and （5） protein expression of StAR.

mg/m^3 and 0.12-0.21 mg/m^3, respectively [4]. The indoor level of acrolein is generally ranged from 2.3 to 275μg/m^3. Mainstream smoke contains the level of acrolein between 10 and 140 μg per cigarette, and side-stream smoke may reach a higher level of 100-1700 μg per cigarette [9]. We did not detect the real level of acrolein in rat circulation after intraperitoneal injection of acrolein (2 mg/ml/kg), whereas this dosage for 3 days was enough to induce a serious negative effects on glucocorticoid biosynthesis. More investigations in the immune system and other physiological systems in response to acrolein might be helpful or useful to prevent the damage caused by smoke, from either cigarette or gasoline engines.

Conflicting interests

The authors have declared that no conflict of interests exist.

Author contributions

J.-C.C. wrote, prepared and finalized the manuscript, Y.-H.Y. and T.-C.W. performed the experiments, C.S. and S.H. performed data and statistical analyses, G.I. edited the manuscript and data interpretation, F,-K.L., P.S.W. and S.-W.W conceived, designed and supervised the experiments.

Abbreviations

ACTH: adrenocorticotropin; 3β-HSD: 3β hydroxysteroid dehydrogenase; MC2R: melano 2 corpin receptor; StAR: steroidogenic acute regulatory protein; ZFR: zona faciculate-reticularis; H-P-A axis: hypothalamus-pituitary-adrenal axis.

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