Activities of Apoptotic Signal 1-Regulating Protein Kinase and Poly-(ADP-Ribose) Polymerase and Internucleosomal DNA Fragmentation in Rat Liver during Oxidative Stress Induced by Cobalt Chloride

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We revealed activation of apoptotic signal 1-regulating protein kinase, inhibition of poly-(ADP-ribose) polymerase, and intensification of internucleosomal DNA fragmentation in rat liver during oxidative stress induced by cobalt chloride.

Key Words: *apoptotic signal 1-regulating protein kinase; poly-(ADP-ribose) polymerase; internucleosomal DNA fragmentation; oxidative stress*

Apoptotic signal 1-regulating protein kinase (ASK-1) belongs to the family of protein kinases initiating phosphorylation of MAP kinases, which activate JNK and p38 MAP kinases (stress-activated protein kinases 2 and 3, SAPK-2/3) [8]. Inactive JNK binds to the major apoptotic protein p53 between the 97th and 117th amino acid residues and inhibits its activity and ubiquitin-dependent degradation [6]. JNK is involved in activation of *c-jun* expression; SAPK-2/3 phosphorylates ATF-2 protein, which binds to the *c-jun* product and forms an apoptotic complex [6].

Thioredoxin (via reactive SH groups) binds to ASK-1 and inhibits its activity [8]. Reactive oxygen species generated by xanthine oxidase [5] and NADPH+ H^+ oxidase [7] and nitric oxide interact with thioredoxin SH groups yielding sulfine anions [7] and S-nitrosothiols [9], respectively. This process leads to the release and activation of ASK-1 and, therefore, induces cell apoptosis. The effect of oxidative stress on ASK-1 activity is unknown.

Here we studied ASK-1 and poly-(ADP-ribose) polymerase (PARP) activities and internucleosomal DNA fragmentation (biochemical apoptotic markers) in rat liver during oxidative stress induced by cobalt chloride.

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MATERIALS AND METHODS

Experiments were performed on 10 female Wistar rats aging 8 months and weighing 250±10. Control animals (n=5) were intraperitoneally injected with 0.2 ml 0.9% NaCl. Experimental rats (n=5) received intraperitoneal injections of 0.2 ml $CoCl_2$ in 0.9% NaCl (30 mg/kg) [2]. All rats were decapitated 120 min postinjection, and the liver was immediately removed.

ASK-1 activity was measured as described previously [9]. The liver homogenate in 0.02 M Tris-HCl buffer (pH 7.5) containing 150 mM NaCl, 5 mM EDTA, and 1 mM NH_4VO_3 was centrifuged at 20,000g for 15 min. The supernatant (0.1 ml) was mixed with 2.7 ml incubation medium (0.02 M Tris-HCl buffer, pH 7.5; 20 mM $MgCl_2$; and 0.3 mM ATP) and 0.2 ml exogenous substrate (bovine myelin basic protein) linked to agarose through a spacer, aspartate (all components from Sigma). The samples were incubated at 37°C for 30 min, the substrate was precipitated, and the amount of phosphate groups was estimated. ASK-1 activity was expressed in units that corresponded to the amount of bound phosphate groups/min/mg protein.

PARP activity was estimated by the method [10] based on electrophoretic separation of poly-ADP-ribosilated nuclear histone proteins followed by quantitative measurements of poly-(ADP-ribose). PARP ac-

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tivity was expressed in μmol poly-(ADP-ribose) adenine/mg nuclear proteins.

Internucleosomal DNA fragmentation was studied spectrophotometrically [1].

The degree of peroxidation was evaluated by MDA content measured routinely [4].

The results were analyzed by Student's *t* test.

RESULTS

ASK-1 activity markedly increased (Table 1), PARP activity decreased, and internucleosomal DNA fragmentation was intensified in the liver of $CoCl_2$-treated rats. Thus, activated ASK-1 induced apoptosis in rat liver cells. Reactive oxygen species formed under the effect of $CoCl_2$ damage mitochondria and activate caspases 3, 6, and 7 cleaving PARP. This promotes internucleosomal DNA fragmentation.

MDA content sharply increased in the liver of $CoCl_2$-treated rats (Table 1), which indicated intensive generation of reactive oxygen species. Reactive oxygen species produce damages to DNA [3] and, therefore, activate PARP (except for conditions accompanied by initiation of apoptosis and caspase-induced disintegration of PARP). Our data on PARP activity and internucleosomal DNA fragmentation confirm induction of apoptosis in liver cells of rats treated with $CoCl_2$.

TABLE 1. Effects of $CoCl_2$ on Biochemical Markers of Apoptosis in Rat Liver ($M\pm m$, n=5)

Parameter	Control	$CoCl_2$
ASK-1 activity, U/min/mg protein	1.4±0.1	5.8±0.4*
PARP activity, μmol/mg protein	237±3	137±17*
Internucleosomal DNA fragmentation, %	3.6±0.2	4.80±0.19*
MDA content, nmol/mg protein	9.0±0.1	16.0±0.8

Note. *p<0.05 compared to the control.

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