CITATION NOTE: DETECTING CASPASE ACTIVITIES IN MOUSE LIVER

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Liu, D. *et al.* (2004) Nuclear import of proinflammatory transcription factors is required for massive liver apoptosis induced by bacterial lipopolysaccharide. *J. Biol. Chem.* **279**, 48434–42.

This study investigated the role of cSN50 peptide on mouse liver apoptosis. cSN50 is an inhibitor of the nuclear import of stress-responsive transcription factors (SRTFs). Mouse animal models of acute liver injury treated with the cSN50 peptide before and after challenge with lipopolysaccharide (LPS) and 2-amino-2-deoxy-D-galactosamine (D-Gal) displayed a decrease in liver apoptosis compared to animals that were treated with vehicle only before and after challenge with LPS and D-Gal. This work included experiments demonstrating initiator caspase-8 and -9 and effector caspase-3/7 activities in liver tissue homogenates of mice undergoing LPS/D-Gal treatment.

Measuring Caspase-3/7, -8 and -9 Activities in Mouse Liver Homogenates with the Caspase-Glo® 3/7, 8 and 9 Assays

Female C57BL/6 mice were injected intraperitoneally with LPS and D-Gal. The mice were then divided into two groups. The experimental group received the cSN50 peptide through seven intraperitoneal injections before and after being challenged with LPS and D-Gal. The control mice received injections of 5% DMSO (vehicle only).

To detect caspase-3/7, -8 and -9 activities in mouse livers, the authors used the Caspase-Glo® 3/7, 8 and 9 Assays[a,b] (Cat.# G8090, G8200 and G8210, respectively) with a slightly modified procedure. This modified procedure employed a hypotonic extraction buffer (25mM HEPES [pH 7.5], 5mM $MgCl_2$, 1mM EGTA, 1mM Pefabloc®, and 1µg/ml each pepstatin, leupeptin and aprotinin) during Dounce homogenization of livers. After homogenization the extracts were cleared by centrifugation at 13,000rpm for 15 minutes at 4°C. Protein concentrations of the cleared extracts were adjusted to 1mg/ml prior to storage at –80°C. To perform the caspase assays, the authors mixed an equal volume of diluted (10µg/ml) extract with the appropriate Caspase-Glo® Reagent in 96-well, white-walled plates. The assays were incubated for one hour at room temperature before reading on a plate-reading luminometer.

Data from the caspase-3/7 assays indicate that C57BL/6 mice treated with vehicle alone (5% DMSO; no peptide inhibitor) and then challenged with LPS and D-Gal showed increased levels of activated caspase-3 and -7 in liver tissue as assessed by the Caspase-Glo® 3/7 Assay. The largest increase in caspase-3/7 activity occurred 6 hours after the

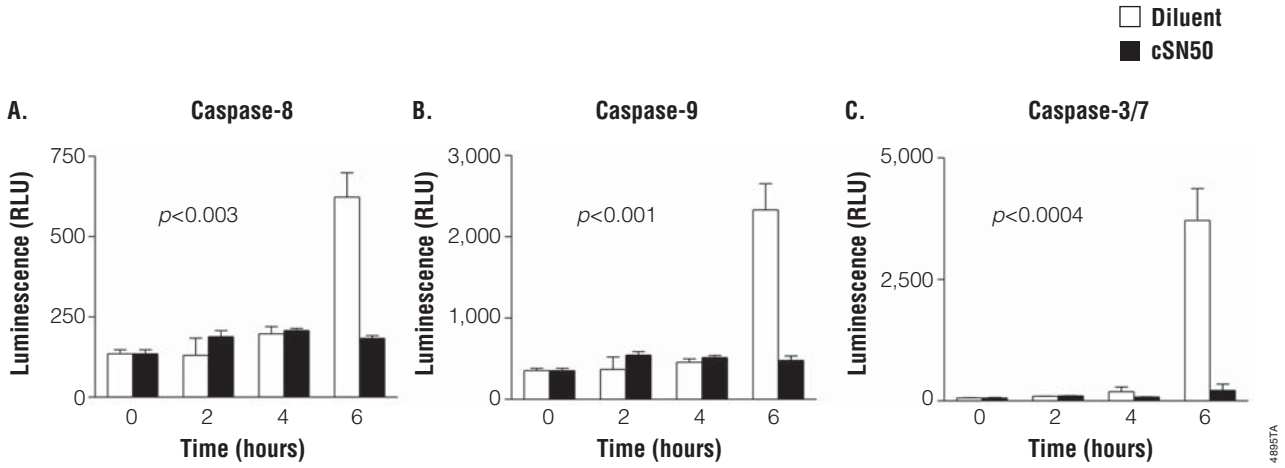

Figure 1. Time-dependent activation of initiator and effector caspases in control and cSN50 peptide-treated mice. Wildtype C57BL/6 mice were treated with cSN50 peptide (0.7mg in 200µl of 5% DMSO) or diluent before and after intraperitoneal administration of LPS with D-Gal according to the protocol described in Liu, D. *et al.* 2004. Caspase activities in liver were measured after LPS and D-Gal challenge in diluent controls (open bar) and cSN50 peptide-treated animals (solid bar). Error bars indicate the ± standard error of the mean value in four mice that are represented by each data point. *p* values represent the significance of the difference between the control and the cSN50 peptide-treated groups (two-way ANOVA) RLU, relative light units. Reprinted with the kind permission of Dr. Jacek Hawiger, Vanderbilt University, and *The Journal of Biological Chemistry.*

challenge with LPS and D-Gal (Figure 1). Most interestingly, mice that had been treated with the cSN50 peptide did not display this same increase in liver cell apoptosis.

The authors also describe their analysis of intrinsic and extrinsic apoptosis pathway induction by studying the activities of the initiator caspases, caspase-9 (intrinsic pathway) and caspase-8 (extrinsic pathway). The activities of both caspases were monitored in liver extracts at various time points (0–6 hours) after the LPS and D-Gal challenge. Both caspase-8 and -9 activities dramatically increased at 6 hours after treatment with LPS and D-Gal in the control mice that did not receive cSN50 peptide. Like caspase-3/7, caspase-8 and -9 activities decreased when mice were treated with the cSN50 peptide. The authors noted that, although inflammatory cytokine TNF-α levels increased within an hour of LPS/D-Gal treatment, caspase-8 activity in the liver did not increase until 6 hours into the treatment.

Based on liver caspase activity data and other supporting data the authors conclude that nuclear import of SRTFs is important for the initiation of apoptosis and liver injury caused by the release of cytokines by macrophages (Figure 2). This finding is ultimately important for understanding the biology and pathogenesis of massive liver apoptosis mediated through macrophage expression of inflammatory cytokines. ■

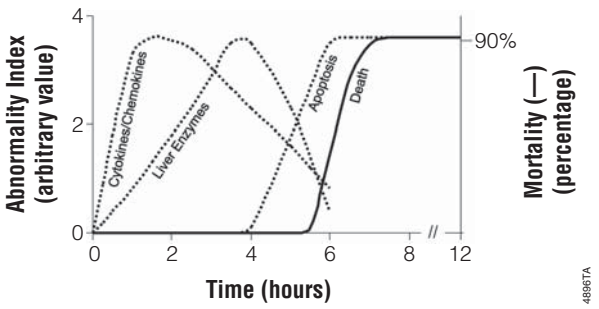

Figure 2. Schematic depiction of time course of cytokine/chemokine activation, hepatocyte injury as indicated by release of liver enzymes, apoptosis, and death in this model of LPS-induced liver injury. Abnormality index represents the -fold increase in the parameter studied. Reprinted with the kind permission of Dr. Jacek Hawiger, Vanderbilt University, and *The Journal of Biological Chemistry.*

Protocols

Caspase-Glo® 3/7 *Assay Technical Bulletin* #TB323, Promega Corporation (www.promega.com/tbs/tb323/tb323.html)

Caspase-Glo® 8 *Assay Technical Bulletin* #TB332, Promega Corporation (www.promega.com/tbs/tb332/tb332.html)

Caspase-Glo® 9 *Assay Technical Bulletin* #TB333, Promega Corporation (www.promega.com/tbs/tb333/tb333.html)

Ordering Information

Product	Size	Cat.#
Caspase-Glo® 3/7 Assay	2.5ml	G8090
	10ml	G8091
	100ml	G8092
Caspase-Glo® 8 Assay	2.5ml	G8200
	10ml	G8201
	100ml	G8202
Caspase-Glo® 9 Assay	2.5ml	G8210
	10ml	G8211
	100ml	G8212

For Laboratory Use.

[a]U.S. Pat. No. 6,602,677, Australian Pat. No. 754312 and other patents pending.

[b]The method of recombinant expression of *Coleoptera* luciferase is covered by U.S. Pat. Nos. 5,583,024, 5,674,713 and 5,700,673.