Desensitization of the Permeability Transition Pore by Cyclosporin A Prevents Activation of the Mitochondrial Apoptotic Pathway and Liver Damage by Tumor Necrosis Factor-α*

Maria Eugenia Soriano‡§, Luca Nicolosi‡§, and Paolo Bernardi‡§**

From the ‡Consiglio Nazionale delle Ricerche Institute of Neuroscience at the Department of Biomedical Sciences, University of Padova, Viale Giuseppe Colombo 3, I-35121 Padova, Italy and the §Venetian Institute of Molecular Medicine, Via Orus 2, I-35129 Padova, Italy

Received for publication, May 12, 2004
Published, JBC Papers in Press, June 16, 2004, DOI 10.1074/jbc.M405297200

We studied the effects of cyclosporin A (CsA) administration 1) on the properties of the permeability transition pore (PTP) in mitochondria isolated from the liver and 2) on the susceptibility to hepatotoxicity induced by lipopolysaccharide of Escherichia coli (LPS) plus NGalN in rats. CsA exerted a marked PTP inhibition ex vivo, with an effect that peaked between 2 and 9 h of drug treatment and decayed with an apparent half-time of about 13 h. Administration of LPS plus NGalN to naive rats caused the expected increased serum levels of tumor necrosis factor (TNF)-α, liver inflammation with BID cleavage, activation of caspase 3, appearance of terminal deoxynucleotidyltransferase-mediated dUTP nick-end labeling-positive nuclei, and release of alamine aminotransferase and aspartate aminotransferase into the bloodstream. Treatment with CsA before or within 5 h of the administration of LPS plus NGalN protected rats from hepatotoxicity despite the normal increase of serum TNF-α and BID cleavage. These results indicate that CsA prevents the hepatotoxic effects of TNF-α by blocking the mitochondrial proapoptotic pathway through inhibition of the PTP and provides a viable strategy for the treatment of liver diseases that depend on increased production and/or liver sensitization to TNF-α.

Key advances are being made in understanding the role of mitochondria in pathophysiology, in particular as mediators of the amplification and execution phases of apoptosis (1). These advances may in turn yield novel drugs and treatments for high-prevalence conditions such as cancer and degenerative diseases (2). Mitochondria release proteins that cause cell death through both caspase-dependent and -independent mechanisms. The release of these apoptogenic factors may be triggered by the engagement of surface receptors such as the TNF-α receptor (3–5). The ensuing caspase 8 activation causes cleavage of BID, the truncated form of which, tBID, targets mitochondria and causes the release of cytochrome c (6–8) in a process modulated by proteins of the BCL-2 family. Indeed, antiapoptotic members inhibit (9, 10), whereas proapoptotic members favor the release process (11–15). Release of cytochrome c and other apoptogenic factors can also be caused by opening of the PTP, an inner membrane channel that is modulated by a variety of proapoptotic signals (15–21). The two mechanisms are not mutually exclusive, because the PTP participates in the release of proapoptotic proteins through at least two mechanisms: swelling-dependent rupture of the outer mitochondrial membrane and remodeling of cristae with increased availability of cytochrome c for release through the specific pathways activated by outer membrane tBID insertion (19, 20).

The importance of PTP regulation in pathophysiology in vivo has been recently highlighted by two studies from our laboratory. We were able to cure dystrophic Col6a1−/− mice, a model of Bethlem myopathy (22), with the PTP inhibitor cyclosporin A (CsA) through a demonstrably mitochondrial effect (23), and we found that up-regulation of mitochondrial BCL-2 caused resistance to apoptosis through desensitization of the PTP during tumor promotion by the hepatocarcinogen 2-acetylamino-fluorene (24). The latter observation is of specific relevance to hepatotoxic diseases that depend on liver sensitization to TNF-α (25, 26) because feeding with 2-acetylaminofluorene confers resistance to the hepatotoxicity otherwise caused by treatment with LPS plus NGalN (24).

TNF-α triggers other well-characterized proapoptotic pathways whose effector signals may converge on mitochondria. Activation of sphingomyelinas (27) generates ceramide (28) and GD3 ganglioside (29, 30), which directly affect mitochondrial function by increasing the PTP open time (31–35). Generation of ceramide-1-phosphate by ceramide kinase activates cytosolic phospholipase A2, resulting in release of arachidonic acid (36), which in turn provides a key amplification loop through further stimulation of sphingomyelin breakdown (37) and PTP opening (18, 21). An additional mechanism specifically linked to activation of acidic sphingomyelinase is down-regulation of liver methionine adenosyltransferase 1A, which leads to depletion of glutathione and sensitizes hepatocytes to TNF-α (38).

The present study was undertaken to test whether the mitochondrial PTP is the effector system of TNF-α-triggered hepatotoxicity by evaluating the protective effects of CsA on 1) PTP 

* This work was supported in part by Grants from the Associazione Italiana per la Ricerca sul Cancro and Telethon-Italy (to P. B.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† These authors contributed equally to this work.

‡ Presented in partial fulfillment of the requirements for the Ph.D. degree in the School of Biosciences, Program in Cell Biology of the University of Padova.

§ To whom correspondence should be addressed. Fax: 39-049-827-6361; E-mail: bernardi@bio.unipd.it.

¶ Abbreviations used are: TNF, tumor necrosis factor; tBID, truncated BID; CsA, cyclosporin A; LPS, E. coli lipopolysaccharide; NGalN, N-galactosamine; PTP, permeability transition pore; CRC, calcium re-

tention capacity; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TUNEL, terminal deoxynucleotidyltransferase-mediated dUTP nick-end labeling; MOPS, 3-(N-morpholino)propanesulfonic acid; CyP, cyclophilin.
were anesthetized by inhalation of 1.5–3% isofluorane/O2 when reaccess to a standard diet and were kept under controlled conditions of

Nonidet P-40 in 0.3 mannitol, 5 mM Tris-MOPS, pH 7.4, 4 mM KH2PO4, analyzed with the use of a Leica DMR optical microscope.

5 anti-fluorescein antibody conjugated with horseradish peroxidase detection kit, which includes a fluorescein-DNA labeling system and an

diene difluoride membranes (for tBID and actin) (Millipore), and sequentially immunooblotted with antibodies against BID, cleaved caspase 3, and actin. Signals were visualized with ECL reagents.

RESULTS

Properties of the PTP in Isolated Liver Mitochondria after Treatment of Rats with CsA—To assess whether the PTP was actually inhibited after administration of CsA to living animals, we treated rats with vehicle or with CsA and isolated liver mitochondria by differential centrifugation 2 h later. The occurrence of PTP inhibition was then estimated by comparing the CRC of mitochondria isolated from vehicle-treated animals with that of CsA-treated ones. The CRC is a sensitive and quantitative measure of the propensity of mitochondria to open the PTP after Ca2+ uptake (40). The experiments reported in Fig. 1, trace a, show that mitochondria isolated from the liver of a vehicle-treated rat accumulated a train of about 10 pulses of 10 μM Ca2+ before the precipitous Ca2+ release that marks onset of PTP opening. As expected, the addition of 1 μM CsA to the incubation medium caused an increase of >3-fold in the CRC (trace a’), which is consistent with desensitization of the PTP to Ca2+ (40). Mitochondria prepared from the liver of rats treated with 5 mg of CsA/kg of body weight for 2 h displayed a striking increase of the CRC, which was more than 2-fold higher than that of vehicle-treated mitochondria (compare trace b with trace a). Addition of 1 μM CsA to the assay cuvette increased the CRC further (trace b’), yet the maximum CRC obtained in the presence of 1 μM CsA was the same for mitochondria prepared from CsA- and vehicle-treated rats (compare trace b’ with trace a’). We next determined the dependence of PTP inhibition on the dose of CsA in protocols identical to those described in Fig. 1. Fig. 2A shows that measurable PTP inhibition could be detected already at 2.5 mg of CsA/kg, that maximum inhibition was achieved at 5 mg/kg, and that inhibition decreased as the CsA dose was raised further to 10 mg/kg. At 5 mg of CsA/kg, PTP inhibition was maximal between 2 and 9 h of treatment, with a decay curve indicating a half-life of about 13 h for the inhibitory effect (Fig. 2, B). PTP inhibition could be maintained at the peak level for 24 h by a second administration of 5 mg of CsA/kg 10 h after the first dose (results not shown).

![Fig. 1. Effect of treatment with CsA in vivo on the CRC of isolated liver mitochondria.](http://www.jbc.org/content/368/10/6041/F1)

Liver mitochondria were isolated 2 h after treatment of rats with vehicle (traces a and a’) or with 5 mg/kg of CsA (traces b and b’). The incubation medium contained 0.2 mM sucrose, 10 mM Tris-MOPS, 5 mM succinate-Tris, 1 mM Pi-Tris, 10 μM EGTA-Tris, 2 μM rotenone, and 1 μM calcium green-5N. Final volume was 2 ml, pH 7.4, 25 °C. All the experiments were started with the addition of 0.6 mg/ml of mitochondria (not shown). Where indicated (arrows), pulses of 10 μM Ca2+ were added. In traces a’ and b’, the medium was supplemented with 1 μM CsA. The experiments are representative of four replicates for each condition.
Inhibition of Liver Apoptotic Signaling by Cyclosporin A

Protection from LPS Plus d-GalN Hepatotoxicity by CsA—Having established the kinetics of PTP inhibition with CsA ex vivo, we tested whether rats could be protected with the same time course from the short-term hepatotoxic effects of the combination of 20 μg of LPS (which stimulates TNF-α production by the macrophages) plus 700 mg of d-GalN/kg of body weight (which greatly sensitizes the liver to the cytotoxic effects of TNF-α through a transcriptional block) (25, 26). Treatment of rats with LPS plus d-GalN caused the expected liver damage, which could be easily appreciated from the hematoxylin-eosin stained slides. Most hepatocytes displayed nuclear alterations ranging between chromatolysis (the pale nuclei with a rim of residual chromatin at the periphery) and pyknosis to karyolysis and cytoplasmic vacuolization; inflammatory cells could be detected within the liver parenchyma (Fig. 3a, a). The occurrence of apoptosis was confirmed by the TUNEL reaction (Fig. 3a, a′), and by cleavage of caspase 3 (Fig. 3B, lanes 2–4); the occurrence of necrosis was documented by release of AST and ALT into the bloodstream (Fig. 3C, open bars). Treatment of rats with 5 mg/kg CsA 1 h before LPS + d-GalN fully protected rats from liver damage as assessed from hematoxylin-eosin liver staining (Fig. 3a, b), from the absence of TUNEL-positive nuclei (Fig. 3a, b′), from lack of caspase 3 cleavage (Fig. 3B, lanes 5–7), and from the marginal increase in serum AST and ALT levels (Fig. 3C, closed bars). It must be mentioned that the levels of TNF-α increased to comparable levels after treatment with LPS plus d-GalN, peaking after 1–2 h at values of 11.8 ± 3.3-fold and 12.7 ± 3.6-fold over the basal level in vehicle- and CsA-treated animals, respectively (n = 4) and that BID underwent cleavage (not shown, but see Fig. 4), consistent with activation of apical caspases.

CsA Hepatoprotection before and after the TNF-α Increase—A question of great relevance is whether hepatoprotection by CsA requires pretreatment of the animals or can also be obtained after the hepatotoxic insult. We therefore studied the effect of the time of administration of CsA relative to that of LPS plus d-GalN on liver BID cleavage and caspase 3 activation, and on release of AST and ALT into the circulation. Fig. 4A demonstrates that treatment with CsA (lanes 2–10) did not prevent cleavage of BID. On the other hand, Fig. 4B shows that pretreatment with CsA (lane 2), treatment at the same time as LPS plus d-GalN (lanes 3 and 4), or treatment 1.5 h (lanes 5 and 6) or 3 h (lanes 7 and 8) after the addition of LPS plus d-GalN prevented caspase 3 activation, whereas treatment after 8 h was ineffective (lane 9). These results are fully consistent with inhibition of transaminase release when CsA was added up to 5 h after LPS plus d-GalN (Fig. 4C).
Inhibition of Liver Apoptotic Signaling by Cyclosporin A

**DISCUSSION**

In this study, we have shown that administration of CsA to living animals leads to in situ inhibition of the PTP, which can still be detected in mitochondria subsequently isolated from the liver of treated animals. By studying the CRC of isolated mitochondria, we have established the dose-dependence and the kinetics of PTP inhibition by CsA in vivo, and we have shown that CsA suppresses the TNF-α-dependent mitochondrial liver proapoptotic pathway with the same time course. Indeed, treatment with CsA conferred full resistance to the short-term effects of LPS plus d-GalN at doses of the latter that would otherwise cause extensive liver damage. These results integrate previous studies on death receptor signaling to mitochondria in vivo (38, 41–43), document the role of the PTP as the final common pathway for TNF-α liver toxicity in vivo, and provide a strong rationale for the use of CsA in conditions under which hepatic damage is caused by increased production and/or sensitization to the proapoptotic effects of TNF-α.

**Cell Death Pathways in TNFα-dependent Hepatotoxicity**—Fig. 5 summarizes the pathways involved in liver apoptotic signaling triggered by engagement of the TNF-α CD120a receptor. The involvement of all these pathways in the onset of liver damage after treatment with LPS plus d-GalN or with Fas is clearly documented by the protective effects observed after genetic ablation of BID (41, 43), of the adapter protein FAN (factor associated with neutral sphingomyelinase activation), which is essential for activation of neutral sphingomyelinase (42, 44, 45), and of acidic sphingomyelinase (46). Taken together, these findings indicate that there is no redundancy between the effector mechanisms triggered by activation of caspase 8, of neutral sphingomyelinase, and of acidic sphingomyelinase and suggest that specific, permissive signals arising from each pathway must be present for the death program to proceed. This pattern of activation is remarkably similar to...
that established for the PTP, which responds to a wide variety of signals that act in a combinatorial fashion at discrete sites to determine the overall open probability (47).

Fig. 5 also illustrates how the signaling molecules generated by BID cleavage and by activation of sphingomyelinas may converge on the PTP, which, based on the protective effects of CsA, must be downstream of these pathways. It should be noted that the prevailing mode of cell death caused by PTP opening (apoptosis with caspase 9 and 3 activation versus other forms of cell death, necrosis being the extreme end of a continuum (48)) probably depends on the PTP open time (49). Indeed, openings of short duration could cause intramitochondrial cytochrome c redistribution, which stimulates cytochrome c release through the tBid/BAX-dependent pathway without outer membrane rupture and loss of pyridine nucleotides (19, 20). The maintained ATP levels would allow formation of an active apoptosome leading to cleavage of caspase 9 and then caspase 3, eventually resulting in apoptosis. On the other hand, openings of long duration would cause swelling of matrix with outer membrane rupture, depletion of pyridine nucleotides with respiratory inhibition, irreversible depolarization, and mitochondrial hydrolysis of ATP (49). The resulting ATP depletion would prevent activation of caspase 9 and switch cell death to a different subroutine (50, 51), where caspase-independent events may predominate (48). Thus, a continuum of modes of cell death is possible that is consistent with the spectrum of morphological changes recorded by histology.

Inhibition of the PTP after CsA Administration in Vivo—CsA exerts all of its known biological effects after binding to CyPs, a family of proteins possessing peptidyl-prolyl-cis-trans-isomerase activity that is inhibited by CsA (52, 53). The mitochondrial effects of CsA (54, 55) are mediated by its binding to CyP-D, a unique CyP located in the mitochondrial matrix (56–58). Because of the high-affinity CsA-CyP-D interaction (the concentration of CsA required for 50% inhibition is about 100 nM), the propensity of the PTP to open is decreased through an effect that does not require calcineurin inhibition (57). It must be stressed that PTP opening in the presence of CsA is still possible (e.g. by increasing the load of matrix Ca2+ (Fig. 1) or by the effect of additional inducing factors such as oxidants and thiol reagents (see Ref. 47 for a review). The effect of CsA on the PTP is thus best described as “desensitization”, an effect that can be quantitated with the CRC test (Fig. 1). Remarkable findings of our work are 1) that the mitochondrial effects of CsA display an optimum at a dose of 5 mg of CsA/kg of body weight, whereas higher concentrations are less effective; and 2) that the maximum effect is observed between 2 and 9 h of administration, with return to the basal level within 24 h of treatment. The first observation is probably caused by the overlapping effect of CsA toxicity, which is mediated by overproduction of reactive oxygen species (59, 60) that may counterbalance the desensitization effects of CsA on the PTP (47); the second observation rules out the possibility that immunosuppression, which requires several days to develop, may be involved in the short-term hepatoprotective effects of CsA. Both observations help clarify outstanding questions about the effector mechanisms of hepatotoxicity by TNF-α and of hepatoprotection by CsA in various experimental models.

Protection against Liver Damage by CsA—The hepatoprotective effects of CsA have been tested in three in vivo animal models of toxicity (i.e. treatment of rats with LPS after liver sensitization with heat-inactivated Propionibacterium acnes (61) or L-GalN (62), treatment of cats with LPS alone (63), and treatment of mice with an anti-Fas antibody (64)) and in one cohort of cases of fulminant viral hepatitis in combination with interferon-β (65). In the animal models, the dose of CsA ranged between 6 (63), 10 (61, 62) and 100 mg/kg of body weight (64), which conferred variable degrees of protection; the time of administration relative to the hepatotoxic treatment ranged from pretreatment with a single dose (61, 63, 64) to repeated administrations of the same dose at different time intervals (62). This latter study revealed that the time of CsA administration was a critical factor, in that maximum protection was achieved with treatment by CsA at the same time as the hepatotoxic treatment with LPS plus L-GalN, followed by repeated CsA treatments. It is remarkable that pretreatment with CsA for 24 h and then 2 h before LPS plus L-GalN made the damage worse (62), suggesting that toxicity may become a problem as the overall dose of CsA is increased above an optimum (see Fig. 2A). Our results offer unique clues on the basis for these variable effects of CsA and provide a rationale for its use in a clinical setting. Indeed, the protective effects of CsA in vivo were strictly correlated with inhibition of the PTP, and determination of the time course of PTP inhibition by CsA provided a strong rationale to optimize the CsA dose and time of treatment to achieve maximum protection from hepatic damage. If it is permissible to extrapolate our results to other experimental settings, it should be possible to maximize liver protection by treatment with 5 mg of CsA/kg of body weight within 5 h of the hepatotoxic insult, followed by the same maintenance dose every 12 h during the acute phase. Very encouraging results were obtained with an initial dose of 3 mg of CsA/kg by continuous drip infusion for 2 days, followed by a maintenance dose of 1 mg/kg in a clinical trial on 13 patients with fulminant viral hepatitis. The simultaneous use of interferon-β prevents an assessment of whether the protective effects depended on CsA alone (65). Based on the present results, however, we believe that CsA should be considered a potential hepatoprotective drug in diseases that depend on liver sensitization to apoptosis triggered by TNF-α.

REFERENCES

1. Orenesovs, S., Zhivotovsky, B., and Neotera, P. (2003) Nat. Rev. Mol. Cell. Biol. 4, 552–565
2. Reed, J. C. (2002) Nat. Rev. Drug Discov. 1, 111–121
3. Pfeffer, K., Matsuyama, T., Kundig, T. M., Wakeham, A., Kishihara, K., Shabahinian, A., Wiegmann, K., Ohashi, P. S., Kronke, M., and Mak, T. W. (1993) Cell 73, 457–467
4. Rothe, J., Lesslauer, W., Lhotscher, H., Lang, Y., Koebel, P., Kempten, F., Althage, A., Zinkernagel, R., Steinmetz, M., and Blumthelm, H. (1993) Nature 364, 798–802
5. Wallach, D., Varfolomeev, E. E., Malinin, N. L., Golsteyn, Y. E., Kovaleenko, A. V., and Boldin, M. P. (1999) Annu. Rev. Immunol. 17, 331–367
6. Li, H., Zhu, H., Xu, C. J., and Yuan, J. (1998) Cell 94, 491–501
7. Luo, X., Budhijardo, I., Zou, H., Slaughter, C., and Wang, X. (1998) Cell 94, 481–490
8. Gross, A., Yin, X. M., Wang, K., Wei, M. C., Jockel, J., Milliman, C., Erdjument-Bromage, H., Tempst, P., and Korsmeyer, S. J. (1999) J. Biol. Chem. 274, 1156–1163
9. Yang, J., Liu, X., Bhalla, K., Kim, C. N., Ibrado, A. M., Cai, J., Peng, T. I., Jones, D. P., and Wang, X. (1997) Science 275, 1129–1136
10. Klicic, R. M., Bossy-Wetzel, E., Green, D. R., and Newmeyer, D. D. (1997) Science 275, 1122–1126
11. Cosulich, S. C., Worrall, V., Hedge, P. J., Green, S., and Clarke, P. R. (1997) Curr. Biol. 7, 915–920
12. Masson, S., Chauhari, B., and Guerin, M. (1997) FEBS Lett. 415, 29–32
13. Jungersen, M. J. M., Xie, Z., Deveraux, Q., Ellery, L., Bredesen, D., and Reed, J. C. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 4997–5002
14. Ekes, R., Antonsson, B., Osen-Sand, A., Montessuit, S., Richter, C., Sadowski, R., Mazzu, G., Nichols, A., and Martinou, J.-C. (1998) J. Cell Biol. 143, 217–224
15. Pastorino, J. G., Chen, S. T., Tufani, M., Snyder, J. W., and Farber, J. L. (1998) J. Biol. Chem. 273, 1770–1775
16. Susin, S. A., Zamzami, N., Casamento, M., Hirsch, T., Marchetti, P., Maicho, A., Daugas, E., Geuskens, M., and Kroemer, G. (1996) J. Exp. Med. 184, 1331–1341
17. Bradham, C. A., Qian, T., Streit, K., Trautwein, C., Brenner, D. A., and Lemsasters, J. J. (1998) Mol. Cell. Biol. 18, 6353–6364
18. Susin, S. A., Penzo, D., Petronilli, V., Fagano, F., and Bernardi, P. (2001) J. Biol. Chem. 276, 12035–12040
19. Scorrano, L., Ashiya, M., Buttle, K., Weiler, S., Oakes, S. A., Mannella, C. A., and Korsmeyer, S. J. (2002) Dev. Cell 2, 55–66
20. Bernardi, P., Petronilli, V., Di Lisa, F., and Forte, M. (2001) Trends Biochem. Sci. 26, 112–117
21. Penzo, D., Petronilli, V., Angelin, A., Cusan, C., Colonna, R., Scorrano, L.,
Inhibition of Liver Apoptotic Signaling by Cyclosporin A

Pagan, F., Prato, M., Di Lisa, F., and Bernardi, P. (2004) J. Biol. Chem. 279, 25219–25225

22. Bonaldo, P., Brahetta, P., Zanetti, M., Piccolo, S., Volpin, D., and Bressan, G. M. (1998) Hum. Mol. Genet. 7, 2135–2140

23. Irwin, W. A., Bergamin, N., Sabatelli, P., Reggiani, C., Megghian, A., Merlini, L., Brahetta, P., Columbaro, M., Volpin, D., Bressan, G. M., Bernardi, P., and Bonaldo, P. (2003) Nat. Genet. 35, 267–273

24. Kluhn, P. C., Soriano, M. E., Irwin, W., Penzo, D., Scorrano, L., Bitsch, A., Neumann, H. G., and Bernardi, P. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 10014–10019

25. Decker, K., and Keppler, D. (1974) Rev. Physiol. Biochem. Pharmacol. 71, 77–100

26. Leist, M., Gantner, F., Kunstle, G., and Wendel, A. (1998) Rev. Physiol. Biochem. Pharmacol. 133, 109–155

27. Wiegmann, K., Schütze, S., Machleidt, T., Witte, D., and Kronke, M. (1994) Cell 78, 1005–1015

28. Dressler, K. A., Mathias, S., and Kolesnik, R. N. (1992) Science 255, 1715–1718

29. De Maria, R., Lenti, L., Malisan, F., d’Agostino, F., Tomassini, B., Zeuner, A., Rippe, M. R., and Testi, R. (1997) Science 277, 1652–1655

30. Schütze, S., Potthoff, K., Machleidt, T., Berkovic, D., Wiegmann, K., and Kronke, M. (1992) Cell 71, 765–776

31. Garcia-Ruiz, C., Coléll, A., Mari, M., Morales, A., and Fernandez-Checa, J. C. (1997) J. Biol. Chem. 272, 11369–11377

32. Arora, A. S., Jones, B. J., Patel, T. C., Bronk, S. F., and Gores, G. J. (1997) Hepatology 25, 986–993

33. Scorrano, L., Petronilli, V., Di Lisa, F., and Bernardi, P. (1999) J. Biol. Chem. 274, 22581–22585

34. Kristal, B. S., and Brown, A. M. (1999) J. Biol. Chem. 274, 23169–23175

35. Garcia-Ruiz, C., Coléll, A., Paris, R., and Fernandez-Checa, J. C. (2000) FASEB J. 14, 841–858

36. Petrus, B. J., Bielawska, A., Subramanian, P., Wjesinghe, D. S., Maseyka, M., Leslie, C. C., Evans, J. H., Freiberg, J., Roddy, P., Hannun, Y. A., and Chalfant, C. E. (2004) J. Biol. Chem. 279, 11320–11326

37. Jayadev, S., Linaric, C. M., and Hannun, Y. A. (1994) J. Biol. Chem. 269, 5757–5763

38. Mari, M., Coléll, A., Morales, A., Paredes, C., Varela-Nieto, I., Garcia-Ruiz, C., and Fernandez-Checa, J. C. (2004) J. Clin. Investig. 113, 895–904

39. Costantini, F., Petronilli, V., Colonna, R., and Bernardi, P. (1995) Toxicology 99, 77–88

40. Fontaine, E., Ichas, F., and Bernardi, P. (1998) J. Biol. Chem. 273, 25734–25740

41. Yin, X. M., Wang, K., Gross, A., Zhao, Y., Zinkel, S., Kloche, B., Roth, K. A., and Kornmeyer, S. J. (1999) Nature 400, 886–891

42. Malagasc-Cazenave, S., Séguin, B., Lévéque, S., Garcia, V., Carpentier, S., Atié, M. F., Brouchet, A., Gouazé, V., Andrieu-Abadie, N., Barreira, Y., Benist, H., and Lavade, T. (2004) J. Biol. Chem. 279, 18648–18655

43. Zhao, Y., Li, S., Childs, E. E., Kuharsky, D. K., and Yin, X. M. (2001) J. Biol. Chem. 276, 27432–27440

44. Adam, D., Wiegmann, K., Adam-Klages, S., Ruff, A., and Kronke, M. (1996) J. Biol. Chem. 271, 14617–14622

45. Adam-Klages, S., Adam, D., Wiegmann, K., Struve, S., Kolanus, W., Schneider-Mergener, J., and Kronke, M. (1996) Cell 86, 937–947

46. Garcia-Ruiz, C., Coléll, A., Mari, M., Morales, A., Calvo, M., Enrich, C., and Fernandez-Checa, J. C. (2003) J. Clin. Investig. 111, 197–208

47. Bernardi, P. (1999) Physiol. Rev. 79, 1127–1155

48. Leist, M., and Jäättela, M. (2001) Nat. Rev. Mol. Cell. Biol. 2, 589–598

49. Petronilli, V., Penzo, D., Scorrano, L., Bernardi, P., and Di Lisa, F. (2001) J. Biol. Chem. 276, 12030–12034

50. Ankarronna, M., Dypbukt, J. M., Bonfeco, E., Zhivotovsky, B., Orrenius, S., Lipton, S. A., and Nicotera, P. (1995) Neuron 15, 961–973

51. Gramaglia, D., Gentile, A., Battaglia, M., Ranzato, L., Petronilli, V., Fassetta, M., Bernardi, P., and Rasola, A. (2004) Cell Death Differ. 11, 342–353

52. Andreeva, L., Heads, R., and Green, C. J. (1999) Int. J. Exp. Pathol. 80, 355–365

53. Walsh, C. T., Zydowsky, L. D., and McKeon, F. D. (1992) J. Biol. Chem. 267, 13115–13118

54. Crompton, M., Ellinger, H., and Costi, A. (1988) Biochem. J. 255, 357–360

55. Broekemeier, K. M., Dempsey, M. E., and Pfeiffer, D. R. (1989) J. Biol. Chem. 264, 7826–7830

56. Connerr, C. P., and Halestrap, A. P. (1992) Biochem. J. 284, 381–385

57. Nicolli, A., Basso, E., Petronilli, V., Wenger, R. M., and Bernardi, P. (1996) J. Biol. Chem. 271, 2185–2192

58. Connerr, C. P., and Halestrap, A. P. (1996) Biochemistry 35, 8172–8180

59. Krauskopf, A., Lhote, P., Mutter, M., Deulour, J. F., Ruegg, U. T., and Bueler, T. M. (2003) J. Biol. Chem. 278, 41685–41690

60. Nguyen, N. S., Cottet-Maire, F., Bueler, T. M., Lo R. A., Krauskopf, A. S., Armstrong, J. M., Vickers, A. E., Mace, R., and Ruegg, U. T. (1999) Proc. Radiol. Biol. Med. 27, 1267–1275

61. Wasaki, S., Sakaida, I., Uchida, K., Kayano, K., and Okita, K. (1997) Liver 17, 107–114

62. Kawakami, T., Sato, S., and Suzuki, K. (2000) Hepatol. Res. 20, 294–297

63. Crouser, E. D., Julian, M. W., Blaho, D. V., and Pfeiffer, D. R. (2002) Crit. Care Med. 30, 276–284

64. Feldmann, G., Haouzi, D., Moreau, A., Durand, S. A., Bringuer, A., Berson, A., Mansouri, A., Fau, D., and Pessayre, D. (2000) Hepatology 31, 674–683

65. Yoshida, M., Sekiya, K., Inoue, K., and Fujita, R. (1995) J. Gastroenterol. 30, 67–73

Downloaded from http://www.jbc.org/ by guest on July 24, 2018
Desensitization of the Permeability Transition Pore by Cyclosporin A Prevents Activation of the Mitochondrial Apoptotic Pathway and Liver Damage by Tumor Necrosis Factor-α

Maria Eugenia Soriano, Luca Nicolosi and Paolo Bernardi

J. Biol. Chem. 2004, 279:36803-36808.
doi: 10.1074/jbc.M405297200 originally published online June 16, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M405297200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 65 references, 28 of which can be accessed free at http://www.jbc.org/content/279/35/36803.full.html#ref-list-1