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Original Contribution

A noncanonical NF- κ B pathway through the p50 subunit regulates Bcl-2 overexpression during an oxidative-conditioning hormesis response



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ABSTRACT

Cells can respond to damage and stress by activating various repair and survival pathways. One of these responses can be induced by preconditioning the cells with sublethal stress to provoke a prosurvival response that will prevent damage and death, and which is known as hormesis. Bcl-2, an antiapoptotic protein recognized by its antioxidant and prosurvival functions, has been documented to play an important role during oxidative-conditioning hormesis. Using an oxidative-hormetic model, which was previously established in the L929 cell line by subjecting the cells to a mild oxidative stress of 50 μ M H₂O₂ for 9 h, we identified two different transductional mechanisms that participate in the regulation of Bcl-2 expression during the hormetic response. These mechanisms converge in activating the nuclear transcription factor NF- κ B. Interestingly, the noncanonical p50 subunit of the NF- κ B family is apparently the subunit that participates during the oxidative-hormetic response.

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Mammalian cells can respond to damage and stress by activating various repair and survival pathways. Preconditioning the cells to sublethal stress is known to induce a prosurvival response that prevents damage and death. Hence, as a consensus terminology to unify the main mechanism that preconditioning and adaptive responses have in common, the term hormesis has been proposed, suggesting that exposure to low levels of stress will activate existing cellular and molecular pathways that will enhance the ability of the cell and organism to withstand more severe stress [1–3].

Every year many papers are published describing very different molecules that protect cells against oxidative insults, hypoxic-ischemic damage [4], oxygen and glucose deprivation [5], xenobiotic toxicity, etc. Most of the molecules analyzed improve cellular survival by increasing Bcl-2 expression [6,7]. There are also reports in which preexposure to sublethal stress creates an

antiapoptotic environment that prevents cell death. This preconditioning treatment generates tolerance or adaptation to oxidative stress by induction of the overexpression of survival genes such as Bcl-2 [8,9]. Interestingly, studies with transformed cells have shown that molecules that inhibit NF- κ B and the Akt/phosphatidylinositol 3-kinase (PI3K) pathway, such as xanthone derivatives, α -mangostin [10], curcumin [11], green tea polyphenols [12], ellagic acid (a common component of berries) [13], fucoidan (a sulfated polysaccharide) [14], osthole [15], and bromelain (obtained from pineapple) [16], are reported to have therapeutic applications in the treatment of cancer because they eliminate cellular defense mechanisms, decrease Bcl-2 levels, and induce cell death.

Bcl-2 has been recognized for its cytoprotective, antioxidant, and antiapoptotic functions [17–19]. Bcl-2 is also known to increase reduced glutathione levels [20] and superoxide dismutase and proteasome activity [21]. In addition to its protective activity, Bcl-2 has been demonstrated to have a cell cycle inhibitory function by retarding mammalian cell proliferation [22] and by its ability to induce cellular senescence [23].

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One of the more studied and earliest described transduction pathways during cell survival is PI3K/Akt/NF- κ B/Bcl-2. Several works have recognized the efficiency of the survival mechanism to inhibit apoptosis induced by numerous stimuli such as UVB radiation, H₂O₂ [24], low-density lipoprotein oxidation [25,26], and others. This pathway has also been inhibited as a strategy against several cancer types, with good results [27–29].

NF- κ B is a transcription factor consisting of five different subunits (RelA, p65, RelB, cRel, p52, and p60), which are able to interact with one another and form homo- or heterodimers, putting forward their capability to interact with various promoters, thus transactivating multiple genes [30,31]. NF- κ B is induced by stimuli such as ROS, UV radiation, proinflammatory cytokines, lymphokines, and growth factors [32–35].

Therefore we propose that most of these pro-survival or protective molecules have a common mechanism of action by activating a hormetic response by modifying the cellular redox state. Recently we established a model to study the oxidative-conditioning hormesis response (OCH) by conditioning the cell line L929 for 9 h with 50 μ M H₂O₂ [36]. Cells subjected to OCH and then reexposed to severe oxidative insult (H₂O₂ 200–400 μ M) presented a significantly improved survival rate of 70–80%, which was abrogated when Bcl-2 was inhibited or silenced [36]. Hence the aim of this work was to determine the transductional events that regulate Bcl-2 overexpression as part of the mechanism of action during the oxidative-conditioning hormetic response.

Experimental procedures

Chemicals

All chemicals and reagents were of the highest analytical grade and most of them were purchased from Sigma (St. Louis, MO, USA). Reagents obtained from other suppliers are detailed in the text.

Cell culture

Mouse L929 lung fibroblasts were cultured at 37 °C in an atmosphere of 95% air and 5% CO₂ as described elsewhere [37].

Cellular viability and oxidative-conditioning hormesis

L929 cells were seeded at 1×10^5 cells/well into 24-well plates (Corning, Acton, MA, USA) and were treated with 50 μ M H₂O₂ for 9 h, to induce the OCH response, and it was compared in some experiments with a nonhormetic condition of 200 μ M H₂O₂ as described previously [36]. To analyze PI3K, protein kinase C (PKC), and Akt involvement in Bcl-2 overexpression during the hormetic response, cells were incubated for 30 min with their inhibitors, wortmannin, 100 μ M (PI3K); chelerythrine, 5 μ M (PKC); and Akt inhibitor II, 10 μ M (Akt), before the OCH treatment.

Cell survival

Cell survival was quantified by trypan blue assay, counting the living cells in a hemocytometer under a phase-contrast optical microscope, as described previously [23].

Western blot analysis

Kinase activation was evaluated by determining the total and phosphorylated forms as an early event in Bcl-2 overexpression during OCH (15, 30, 45, 60, 90 min). Bcl-2 was also evaluated after the OCH treatment in cells pretreated for 30 min with their inhibitors, wortmannin, 100 μ M (PI3K); chelerythrine, 5 μ M (PKC);

and Akt inhibitor II, 10 μ M (Akt). To demonstrate that specificity to NF- κ B, Bcl-2 expression was evaluated after the hormetic treatment in cells that were pretreated 1 h with 30 μ M SN50, a cell-permeative specific inhibitor of NF- κ B translocation.

Whole-cell lysates were prepared using RIPA buffer (50 mM Tris-HCl, pH 8.0, 120 mM NaCl, 0.5% NP-40, 100 mM NaF, 0.2 mM NaVO₃, 1 μ g/ml aprotinin, 1 mM phenylmethanesulfonyl fluoride, 1 μ g/ml leupeptin). Cell homogenates were incubated at 4 °C for 5–10 min and then centrifuged at 22,000g, 4 °C, for 20 min. Protein concentration was determined in the supernatant using a commercial Bradford reagent (Bio-Rad, Hercules, CA, USA) [39]. Total proteins were separated by 13% SDS-PAGE and transferred to polyvinylidene difluoride membranes (Invitrogen) and probed with specific antibodies: anti-Bcl-2, anti-PI3K, anti-p-PI3K, anti-Akt, anti-p-Akt, anti-PKC- α , or anti-p-PKC- α (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Actin was used as a loading control. Membranes were washed three times with Tris-buffered saline-Tween and incubated with a horseradish peroxidase-conjugated anti-mouse IgG secondary antibody (Pierce, Rockford, IL, USA) for 1 h. After three washes, the blots were developed using a commercial chemiluminescence reagent (Supersignal; Pierce).

Electrophoretic mobility-shift assay (EMSA)

Nuclear extracts were prepared with Igepal CA-630 according to Gómez-Quiroz et al. [38]. Protein concentration was determined in the supernatant using a commercial Bradford reagent (Bio-Rad) [39]. NF- κ B DNA-binding activity was assayed using consensus oligonucleotides. The NF- κ B sequence 5'-AGTTGAGGGGACITTC-CAGGC-3' (Promega, Madison, WI, USA) was used as a probe and was labeled with T4 polynucleotide kinase (USB, Cleveland, OH, USA) and [γ -³²P]ATP (3000 Ci/mmol; MP Biomedical, Irving, CA, USA) and purified using Bio-Spin 30 chromatography columns (Bio-Rad). The reaction mixture contained nuclear protein extract (20 μ g) in 5 μ l of incubation buffer (50 mM Tris-HCl, pH 7.5, 200 mM NaCl, 5 mM EDTA, 5 mM β -mercaptoethanol, 20% glycerol), 1 μ g dl-dC, and ³²P-labeled probe.

For the supershift assay, a 200 nM concentration of polyclonal anti-p65, anti-p52, or anti-p50 antibodies (Santa Cruz Biotechnology) was added to the reaction mixture for 30 min. The reactions were electrophoresed on 6% polyacrylamide native gels. The gels were exposed in a Storage Phosphor Screen (Amersham Bioscience, Arlington Heights, IL, USA) and after 24 h were analyzed in a variable-mode imager (Typhon 9400; Amersham Bioscience) using the software ImageQuant TL (Amersham Bioscience).

Immunofluorescence experiments

Treated L929 cells were fixed with 4% paraformaldehyde and then incubated in blocking buffer (2% bovine serum albumin, 0.2% nonfat milk, and 0.2% Triton X-100 in PBS). Cells were washed and incubated with the primary antibody anti-p50 (Santa Cruz Biotechnology, dilution 1:50), followed by another incubation with the secondary antibody (Alexa 680-conjugated anti-rabbit, dilution 1:200) and with DAPI (10 μ M), to stain the nucleus. Slides were mounted with fluorescence mounting medium (Dako Cytomation, Glostrup, Denmark). Images were obtained with a multi-photon confocal LSM 780 NLO microscope (Carl Zeiss, Jena, Germany).

Data analysis

Data are reported as the means \pm SD for at least three independent experiments performed in triplicate. ANOVA followed by

the Tukey test was used to compare data. A 0.05 level of probability was used as a minimum criterion of significance.

Results

PI3K, Akt, and PKC regulate Bcl-2 expression during the hormetic response

Previously we reported the role played by Bcl-2 during the hormetic response induced by mild oxidative stress [36]. To determine the signaling mechanism that regulates Bcl-2 expression, here we evaluated the participation of the main kinases known for their contribution to cell survival against oxidative stress.

PI3K, Akt, and PKC- α phosphorylation was monitored at short time points after OCH treatment (50 μ M H₂O₂) (Fig. 1). When PI3K and p-PI3K were evaluated, a fast activation was observed by the first 15 min after OCH (more than 5-fold increase compared with the untreated cells, $p < 0.001$), and then another activation of the same magnitude was observed at 120 min ($p < 0.001$; Fig. 1A). In the case of Akt phosphorylation, an increase of more than 7-fold was observed in p-Akt (Ser 473) after 60 min of OCH ($p < 0.001$), but this phosphorylation decreased to 2-fold against the control at 120 min ($p < 0.05$), as shown in Fig. 1B. PKC- α presented a completely different pattern of activation, with intermittent augmentation of p-PKC- α . At 15 min p-PKC- α increased 3.5-fold against the control ($p < 0.01$), whereas at 45 and 60 min the increment was 5- and 3-fold, respectively ($p < 0.01$); last, at 120 min the phosphorylation rose by more than 14 times ($p < 0.001$; Fig. 1C).

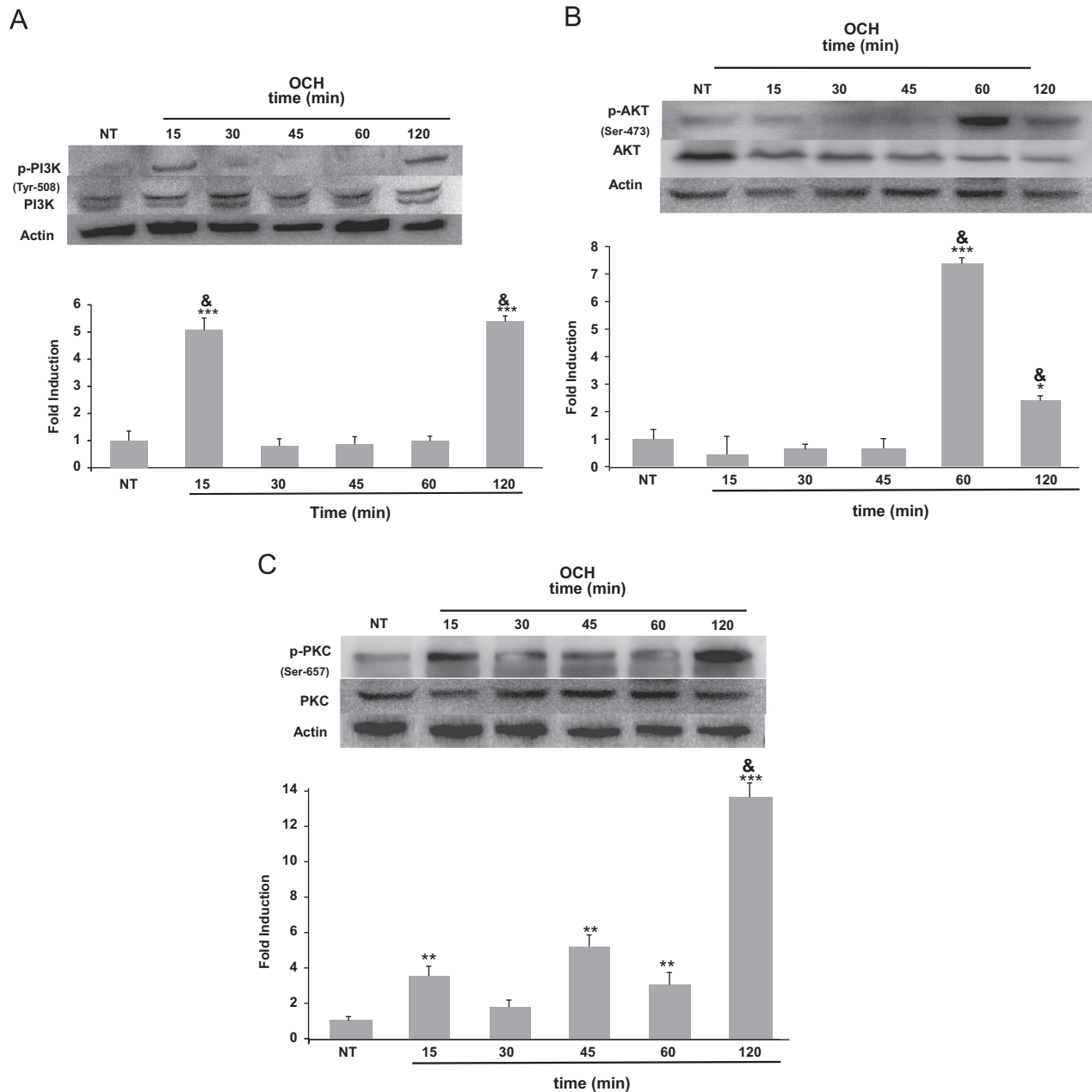


Fig. 1. Canonical kinase phosphorylation after OCH treatment. Representative blots of 1929 cells after 9 h treatment with 50 μ M H₂O₂ (OCH) as described under Experimental procedures. The densitometric analysis shows the relative expression rate between the phosphorylated and the total protein for (A) PI3K/p-PI3K, (B) Akt/p-Akt, and (C) PKC/p-PKC. The results were normalized against the actin control. Each point represents the mean \pm SD of three determinations performed in independent experiments. Statistical significance with respect to controls, * $p < 0.05$, ** $p < 0.001$, and *** $p < 0.001$, or among treatments, & $p < 0.05$.

To demonstrate the direct participation of those kinases in OCH-induced Bcl-2 regulation, cells were incubated for 30 min with the corresponding inhibitor for each kinase (wortmannin for PI3K, chelerythrine for PKC, and Akt inhibitor II for Akt), and Bcl-2 protein levels were evaluated by Western blotting at 9 h. Our results showed a 60% increase in Bcl-2 levels after the OCH treatment ($p < 0.05$), whereas when cells were pretreated with the kinase inhibitors no increment in Bcl-2 was observed. When

the cells were subjected to a nonhormetic oxidative condition of 300 μM H_2O_2 , Bcl-2 levels decreased 90% compared to untreated cells (Fig. 2A). To verify the kinases' participation in the survival response during OCH, cellular viability was evaluated under the same conditions mentioned before. As expected, cells treated for 9 h with 50 μM H_2O_2 (OCH) were 100% viable, and no difference with control cells was observed, whereas cells pretreated with the kinase inhibitors showed decreased viability by 60% ($p < 0.01$)

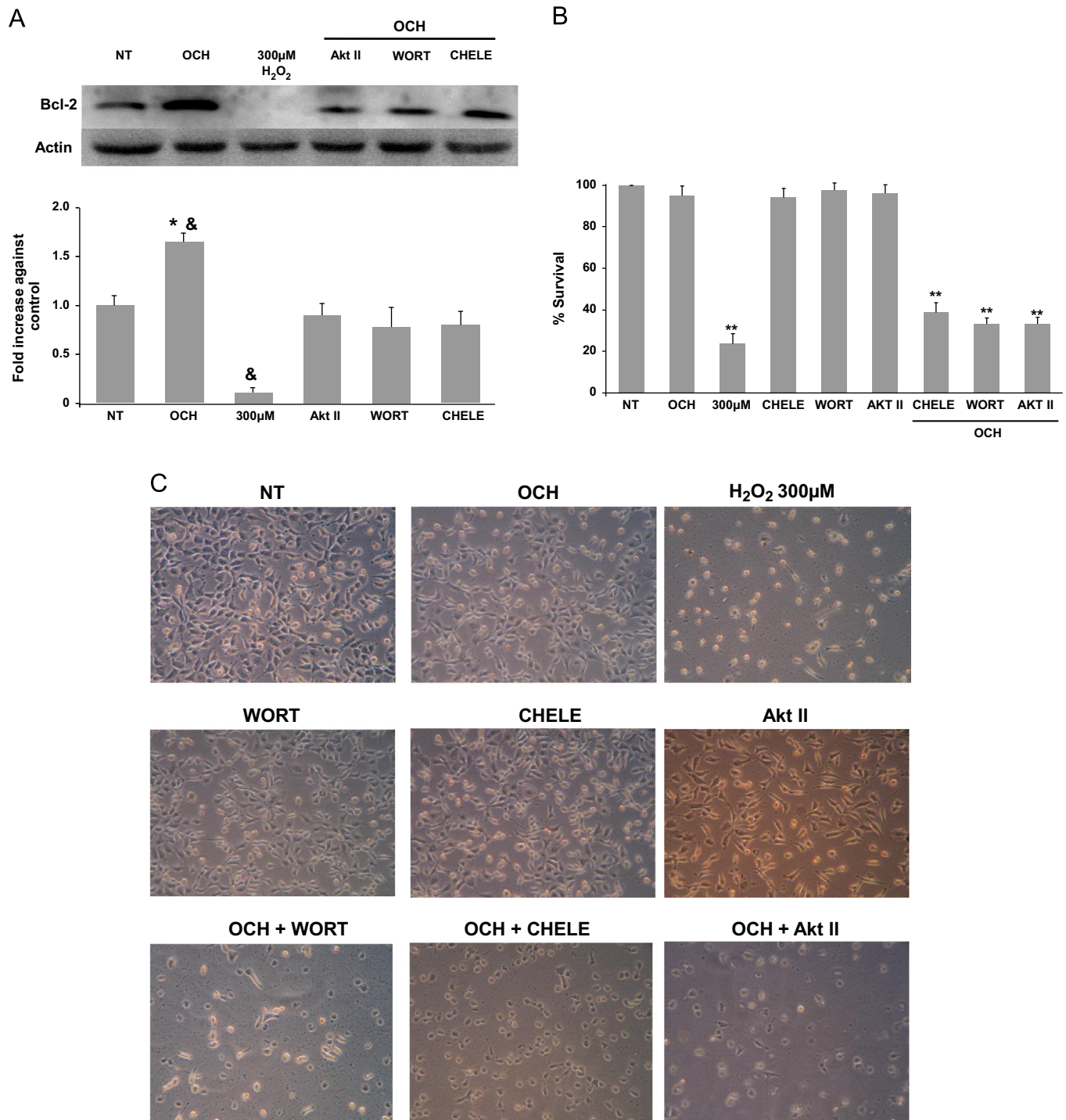


Fig. 2. PI3K/Akt and PKC regulate Bcl-2 expression and survival during OCH treatment. (A) Bcl-2 representative blot for OCH treatment using the specific kinase inhibitors as described under Experimental procedures: wortmannin for PI3K, Akt inhibitor II for Akt, and chelerythrine for PKC. 300 μM H_2O_2 was used an oxidative control. Densitometric analysis was normalized against actin. (B) Cell survival was determined after OCH and compared to survival of cells that were pretreated with the kinase inhibitors as described under Experimental procedures. (C) Optical microscopy showing cellular morphology after OCH and compared to that observed in cells that were pretreated with the kinase inhibitors as described under Experimental procedures (original magnification 100 \times). Each point represents the mean \pm SD of three determinations performed in independent experiments. Statistical significance with respect to controls, * $p < 0.05$ and ** $p < 0.001$, or among treatments, & $p < 0.05$.

after OCH. A 75% decrease in viability was observed at 300 μ M H_2O_2 (Fig. 2B). The cellular morphology, analyzed by optic microscopy, agrees with the viability results. The cultures pretreated with the kinase inhibitors showed visible damage and cell demise (Fig. 2C).

NF- κ B regulates Bcl-2 expression

Bcl-2 is a highly regulated gene; its promoter encompasses two response elements for NF- κ B, a redox-regulated transcription factor that is known to be activated during oxidative stress response and is associated with cell survival. To determine if NF- κ B activation induces Bcl-2 expression after OCH, cells were pretreated for 30 min with 30 μ M SN50, a cell-permeative specific inhibitor of NF- κ B translocation, before the OCH treatment. Bcl-2 levels were determined after 9 h, and as expected, Bcl-2 levels increased after OCH, but this increase was abrogated when SN50 was used (Fig. 3). The same effect was observed when the cells were treated with 300 μ M H_2O_2 ($p < 0.05$), thus confirming the role of NF- κ B in increasing Bcl-2 in the survival response during OCH.

PI3K/Akt and PKC activate NF- κ B during hormesis response

Our results are consistent with other reports that have described several transduction pathways known to regulate Bcl-2 expression, such as PI3K/Akt–NF- κ B and PKC- α –NF- κ B. However, it was still important to demonstrate that NF- κ B was being activated and actually binding to the DNA during the hormetic response developed by OCH treatment. Fig. 4A shows a representative NF- κ B EMSA at 1 and 2 h of OCH stimulus. NF- κ B DNA binding was increased fivefold at 2 h compared to untreated cells ($p < 0.01$). To verify the participation of PI3K, Akt, and PKC- α in NF- κ B activation during OCH, their activity was abrogated by pretreating the cells with their respective pharmacological inhibitors. Cells were then subjected to the OCH for 2 h and NF- κ B activation was evaluated. The results in Fig. 4B show that when the kinases were inhibited, NF- κ B binding to DNA significantly decreased ($p < 0.01$),

suggesting that PI3K, Akt, and PKC- α activate NF- κ B in response to OCH treatment and that all of them participate in Bcl-2 expression regulation (Fig. 2A).

p65 does not participate in Bcl-2 transcriptional regulation but p50 does

NF- κ B is a transcription factor that can be composed of various subunits such as p65, p52, p50, RelA, and RelB, which can interact with one another forming homo- or heterodimers. Of these, the most studied in processes related to cell survival and antioxidant response is subunit p65. To confirm p65 participation during OCH, an EMSA and a supershift assay against p65 were performed. Surprisingly, as presented in Fig. 5, the p65 band did not show a substantial delay in its electrophoretic mobility during OCH treatment, whereas a significant retardation is observed during TNF- α treatment, which was used as a positive control. Hence, to find out which NF- κ B subunit was involved in Bcl-2 expression enhancement during the antioxidant hormetic response, we performed supershifts against the three most important NF- κ B subunits: p50, p52, and p65. Fig. 6A shows that p50 was the subunit that retarded its electrophoretic mobility, and two bands with different molecular weights were observed in the EMSA, suggesting that the low-weight band might represent p50 homodimers, whereas the high-weight band might correspond to p50 heterodimers with another NF- κ B subunit, such as RelA or RelB (Fig. 6A). Finally, to corroborate p50 participation as part of the hormetic response, its nuclear translocation during OCH treatment was determined with confocal microscopy, as illustrated in Fig. 6B. The upper row shows the untreated cell photomicrographs, in which p50 (stained in red) can be observed in the cytoplasm, whereas in the OCH-treated cells (lower row), p50 can be seen in the nucleus, hence confirming p50 participation and translocation.

Discussion

In this work we have established two different signaling mechanisms that participate in the regulation of Bcl-2 expression during the hormetic response. The hormetic model or OCH was previously established in the L929 cell line [36], subjecting the cells to a mild oxidative stress (50 μ M H_2O_2) for 9 h.

The first mechanism involves PI3K, Akt, NF- κ B, and Bcl-2 and has been described before as part of the survival and antioxidant response against several toxic stimuli in various cell types [27,39,41]. The PKC signaling pathway, and mostly the PKC- α isoform, regulates a wide range of vital biological functions and processes, such as cell proliferation, apoptosis, differentiation, migration, and adhesion [42], and it is also involved in oxidative stress and the inflammatory responses [43]. PKCs contain unique structural features that are susceptible to oxidative modifications. The N-terminal regulatory domain contains zinc-binding, cysteine-rich motifs that are readily oxidized by hydrogen peroxide; when oxidized, the regulatory domain autoinhibitory function is compromised and, consequently, cellular PKC activity is stimulated [44]. PKC- α phosphorylates NF- κ B/p65 in U1242 glioblastoma multiforme cells [45] and confers resistance against cisplatin-induced apoptosis in prostate cancer cells; this interaction elicits a mechanism resulting in Bcl-2 posttranslational stabilization and elevated expression [46]. Our results show an oscillating pattern response with time for PKC- α , which might be explained by the fact that PKC- α is known to regulate various oxidative stress processes, such as the induction of heat shock proteins, particularly HSP70 [47]; JNK activation to inhibit apoptosis during the reoxygenation process [48]; and the antioxidant

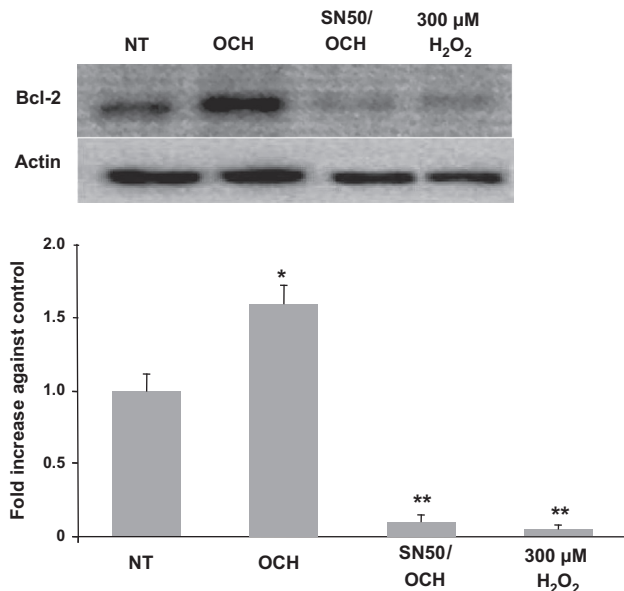


Fig. 3. NF- κ B is involved in regulating Bcl-2 expression. A representative Bcl-2 Western blot of OCH treatment is shown. NF- κ B activation was inhibited using SN50 as described under Experimental procedures. Densitometric analysis was normalized against the actin control. Each point represents the mean \pm SD of three determinations performed in independent experiments. Statistical significance with respect to controls, * $p < 0.05$ and ** $p < 0.001$.

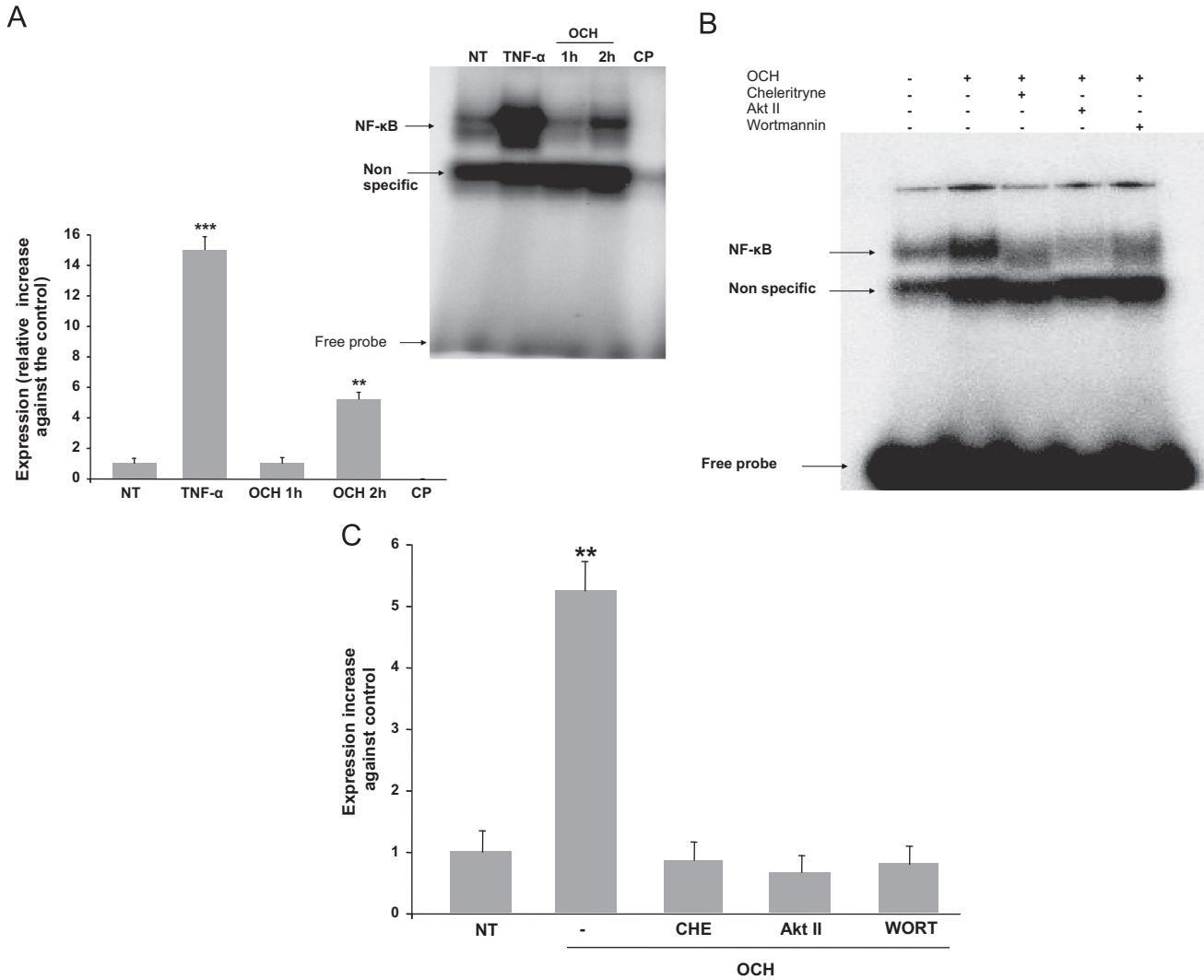


Fig. 4. NF- κ B activation in response to OCH treatment. (A) NF- κ B representative EMSA and densitometry during OCH treatment. TNF- α (10 ng/ml for 15 min) was used as a positive control. (B) PI3K, Akt, and PKC participation in activation of NF- κ B during OCH. The specific kinase inhibitors were used as described under Experimental procedures (wortmannin for PI3K, Akt inhibitor II for Akt, and chelerythrine for PKC). Each point represents the mean \pm SD of three determinations performed in independent experiments. Statistical significance with respect to controls, ** p < 0.001 and *** p < 0.001.

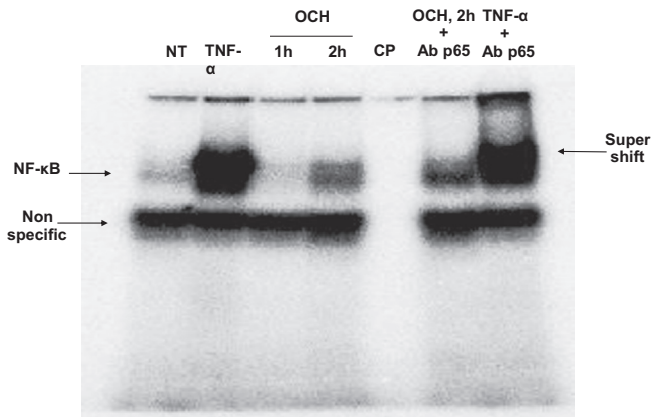


Fig. 5. p65 subunit does not participate as part of NF- κ B during OCH. A p65 representative EMSA showing the lack of activation of this subunit during OCH treatment is shown. TNF- α (10 ng/ml for 15 min) was used as a positive control.

response through Nrf2 [49,50]. However, it would be interesting to deeply explore what is inducing this oscillating pattern.

In our hormetic model, there is an increase in PI3K/Akt activation, which correlates with an enhancement in NF- κ B activation

and a further increment in Bcl-2 expression; this was confirmed when the pharmacological inhibitors for PI3K and Akt (wortmannin and Akt inhibitor II, respectively) were used, because the prosurvival response was abrogated. Wortmannin and Akt inhibitor II have been successfully used in preconditioning studies and have shown a high effectiveness at decreasing PI3K and Akt activity [51–54]. However, these inhibitors are not isoform specific, hence to determine the inhibition of kinase-specific isoforms more studies are needed.

The second mechanism, which involves PKC/NF- κ B/Bcl-2, was earlier described by Choi and co-workers [55] as an essential signaling cascade participating in preconditioning myocardial cells against stroke, in a process involving ROS. In the same way, we demonstrated that both NF- κ B activation and Bcl-2 expression increased in a PKC- α -dependent manner and were abrogated when chelerythrine, a PKC-specific inhibitor, was used. However, because chelerythrine inhibits all PKC isoforms [51,52,56,57], it cannot be assured that the effect seen here is only and specifically due to PKC- α isoform inhibition.

Interestingly, both pathways converged in NF- κ B, a transcription factor known to participate during cellular responses to oxidative challenges. NF- κ B, as well as its repressor I κ B, encompasses biochemical elements that respond to changes in redox

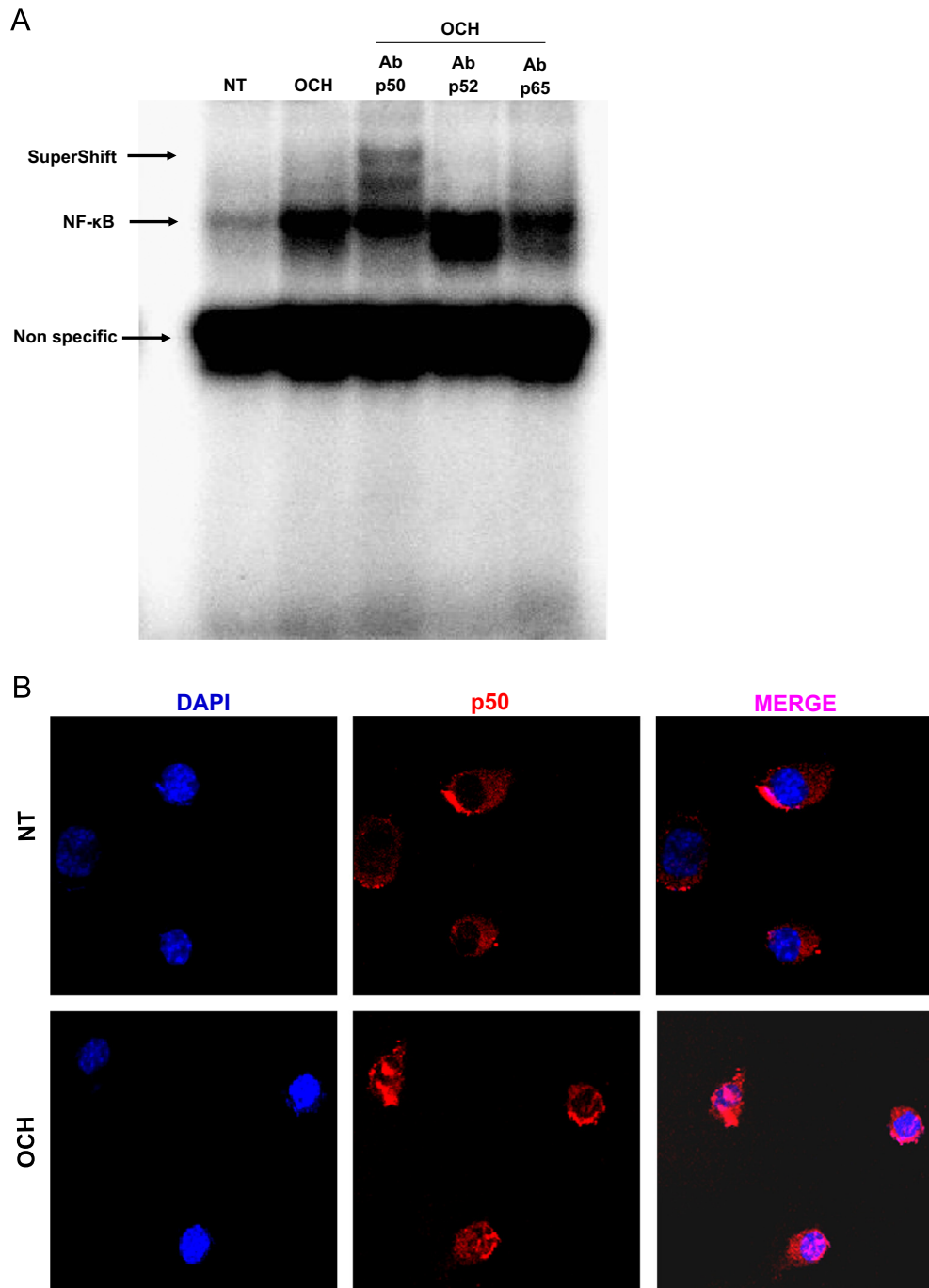


Fig. 6. p50 subunit participation as part of NF-κB during OCH. (A) Representative EMSA for several NF-κB subunits. Antibodies against p52, p50, and p65 were used. NT, not treated. (B) Confocal microscopy showing p50 in the nucleus during OCH. The nucleus was stained with DAPI (10 μM) and a secondary antibody, Alexa 680, was used for p50. The upper row shows the untreated cells photomicrographs, in which p50 (stained in red) can be observed in the cytoplasm; whereas in the OCH-treated cells (lower row), p50 can be seen in the nucleus. Images were obtained with a multiphoton confocal LSM 780 NLO microscope.

state [58,59]. Furthermore, it has been reported that NF-κB is involved in the regulation of genes associated both with survival [60,61] and with antioxidant response [62,63].

Our results show that NF-κB takes part in Bcl-2 expression regulation because when this transcription factor was inhibited with SN50, Bcl-2 expression dropped off. This result is supported by several reports in which the various response elements for NF-κB in Bcl-2 promoter have been described [64,65].

Even though NF-κB's most studied or canonical pathway, known to be involved in survival and anti-inflammation responses, entails a p65–p50 heterodimer [66], our results did not head in that direction. Using electrophoretic mobility-shift

assays and immunocolocalization by confocal microscopy, we identified the components of the transcription factor NF-κB that participate in the hormetic response induced by H₂O₂. Our results confirmed p50 subunit involvement, either in homodimeric or in heterodimeric form, as shown by the two high-molecular-weight bands in the supershift assay in Fig. 6A. Moreover, our data showed that there was no activation of p65 and p52 subunits. These results suggest that Bcl-2 expression enhancement during OCH is not regulated by the canonical NF-κB pathway.

Recently, a noncanonical NF-κB pathway has been proposed that is atypical because the active dimer is a p50–p50 homodimer [67]. Our results support this proposal and it is supported by other

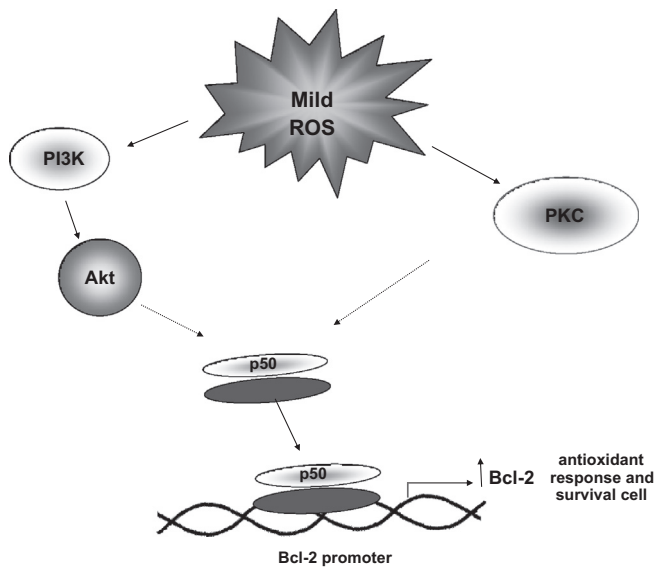


Fig. 7. Bcl-2 expression during OCH. The mild oxidative treatment activates two different signaling pathways: PI3K/Akt/NF- κ B and PKC/NF- κ B. Both pathways participate in regulating the expression of a prosurvival protein: Bcl-2. The cellular response is mediated by p50, a member of the NF- κ B family, which is able to put together homo- and heterodimers and translocate into the nucleus to bind to its response element in Bcl-2. Bcl-2 enhancement during the hormetic response is known to be of paramount importance during cellular survival. ROS, reactive oxygen species.

reports suggesting that the kinases responsible for I κ B degradation are inhibited by oxidative stress [68–71]. The former implies that NF- κ B subunits that are regulated by I κ B, such as p65, RelB, and cRel, do not participate in oxidative-related processes. Furthermore, it has been reported that the NF- κ B canonical pathway does not take part in Bcl-2 transcriptional regulation in response to oxidative stress and that the mechanism involved in Bcl-2 expression is a noncanonical one, with p50 as a main player [72]. Nevertheless, the possible function of these p50 heterodimers still needs to be explained. There are at least two possible explanations. One is that p50 might be required to interact with other members of the NF- κ B family; although in our hands none of those members were detected with the conventional supershifts, possibly because of their low concentration. The second possibility is that the upper part of the p50 supershift complex might be formed by p50 heterodimers along with a coactivator protein needed for the transcription process. One of the preferred candidates for the coactivator is the Bcl-3 protein, which was proposed by Cristofanon and his group [72] as a fundamental part of a transcriptional complex that regulates Bcl-2 expression in response to oxidative stress, but this remains to be confirmed in our experimental model.

All the previous suggests that in response to an oxidative hormetic challenge cells might respond by activating two different signaling pathways, which conclude in the overexpression of a broad-spectrum survival protein such as Bcl-2, as represented in Fig. 7. Interestingly, our results showed that the hormetic response involves transcription factors that participate in both the prosurvival and the antioxidant mechanisms, and these results agree with some recent reports in which Bcl-2 augmentation is regulated by Nrf2, a transcription factor known for its role during antioxidant and phase II responses [73], putting forward the idea that the cellular response to toxins, damage, and stress might simultaneously have prosurvival and antioxidant components.

In conclusion, the PI3K/Akt/NF- κ B/Bcl-2 pathway participates in the mechanism that activates the hormetic response induced by mild oxidative stress, and p50 is the particular NF- κ B subunit that

regulates Bcl-2 expression (Fig. 7). It still remains to be determined which other subunits or molecules might interact with p50 to form the heterodimers that were observed in our assays. This is important because the results presented here might contribute to the design of new therapeutic strategies to counteract cellular damage when oxidative stress is involved during cancer, aging, and neurodegenerative diseases.

Acknowledgments

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