Mitochondria Are Direct Targets of the Lipoxygenase Inhibitor MK886

A STRATEGY FOR CELL KILLING BY COMBINED TREATMENT WITH MK886 AND CYCLOOXYGENASE INHIBITORS*

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We have investigated the mitochondrial and cellular effects of the lipoxygenase inhibitor MK886. Low concentrations (1 μM) of MK886 selectively sensitized the permeability transition pore (PTP) to opening, whereas higher concentrations of MK886 (10 μM) caused depolarization through combination of an ionophoretic effect with inhibition of respiration. MK886 killed prostate cancer PC3 cells only at the higher, toxic concentration (10 μM), whereas the lower concentration (1 μM) had no major effect on cell survival. However, 1 μM MK886 alone demonstrably induced PTP-dependent mitochondrial dysfunction; and it caused cell death through the mitochondrial pathway when it was used in combination with the cyclooxygenase inhibitor, indomethacin, which had no effects per se. Treatment with 1 μM MK886 plus indomethacin sensitized cells to killing by exogenous arachidonic acid, which induces PTP opening and cytochrome c release (Scorrano, L., Penzo, D., Petronilli, V., Pagano, F., and Bernardi, P. (2001) J. Biol. Chem. 276, 12055–12060). Combination of MK886 and cyclooxygenase inhibitors may represent a viable therapeutic strategy to force cell death through the mitochondrial pathway. This approach should be specifically useful to kill cells possessing a high flux of arachidonic acid and its metabolites like prostate and colon cancer cells.

Mitochondria are increasingly recognized as essential organelles in the process of cell death (see Ref. 1 for a recent review). Mitochondria can decide the fate of the cell through the release of apoptogenic proteins such as cytochrome c (2), apoptosis inducing factor (3), Smac-DIABLO (4, 5), and endonuclease G (6); and mitochondrial dysfunction caused by the PT1 may precipitate a bioenergetic crisis with ATP depletion and Ca2+ dysregulation that can cause cell death irrespective of whether caspase have been activated (7–9). The PT may also be instrumental in the release of the apoptogenic proteins, and this may be particularly important for large proteins that do not possess a selective permeability pathway (1). The PT is modulated by a variety of factors involved in intracellular signaling (10), and among these arachidonic acid (11) is particularly interesting in the context of tumor cell biology.

Arachidonic acid is released by activated phospholipases A2 and then converted into prostaglandins, prostacyclins, and thromboxanes by COX, and into leukotrienes by LOX (12–14). The role of arachidonic acid in tumor formation/progression is not easy to address because two opposing effects may overlap. Indeed, arachidonic acid may favor cell survival through its COX and LOX metabolites (15) and instead promote cell death in its free acid form (16). An important link between arachidonic acid metabolism and the mitochondrial proapoptotic pathway is suggested by the findings that apoptosis induction by arachidonic acid involved activation of caspase-3, a process that is amplified by release of mitochondrial cytochrome c (2) and Smac/DIABLO (4); and that Bax, which kills cells via the mitochondrial pathway (17–22), is essential for the apoptotic response of cells to nonsteroidal anti-inflammatory drugs (23). Our previous results are consistent with the idea that mitochondria are key targets for added and, possibly, endogenous arachidonic acid released by cytosolic phospholipase A2 upon its activation by tumor necrosis factor α (11).

Studies on the role of 5-LOX metabolites in cancer cell survival have revealed a strong proapoptotic effect of micromolar concentrations of the 5-LOX inhibitor 3-[3-tert-butylsulfanyl-1-(4-chlorobenzyl)-5-isopropyl-1H-indol-2-yl]-2,2-dimethylpropionic acid, commonly known as MK886, an indole-based multifunctional derivative (Refs. 24–30; see Ref. 31 for review). Interestingly, MK886-induced death of cancer PC3 cells was preceded by mitochondrial depolarization, but the mechanistic basis for this observation remains unclear (28). To clarify the mechanism of mitochondrial depolarization by MK886, and its

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§ The abbreviations used are: PT, permeability transition; PTP, permeability transition pore; LOX, lipoxygenase; COX, cyclooxygenase; MK886, 3-[3-tert-butylsulfanyl-1-(4-chlorobenzyl)-5-isopropyl-1H-indol-2-yl]-2,2-dimethylpropionic acid; MOPS, 4-morpholinepropanesulfonic acid; CsA, cyclosporin A; CsH, cyclosporin H; TMPD, tetramethyl-p-phenylene diamine; DNP, dinitrophenol; FCCP, carbonyl cyanide-p-trifluoromethoxyphenyl hydrzone; FLAP, 5-lipoxygenase-activating protein; ROI, region of interest; TMRM, tetramethylrhodamine methyl ester.

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possible relationship with arachidonic acid metabolism, induction of the PT and cell death, we have investigated the effects of MK886 on isolated mitochondria and intact cells. We show that MK886 has prominent effects on mitochondria that may represent the basis for its cytotoxicity. Indeed, low concentrations (1 μM) of MK886 selectively sensitized the permeability transition pore (PTP) to opening, whereas higher concentrations (10 μM) caused depolarization through combination of an ionophoretic effect with inhibition of respiration. MK886 killed prostate cancer PC3 cells only at the higher concentration, whereas the lower concentration had no major effect on cell survival. However, 1 μM MK886 demonstrably induced PTP-dependent mitochondrial dysfunction, and it caused cell death through the mitochondrial pathway when it was used in combination with the COX inhibitor, indomethacin, at concentrations of the latter that had no effects per se. Combination of low concentrations of MK886 with a COX inhibitor may represent a viable therapeutic strategy to force cell death through the mitochondrial pathway, and this approach should be specifically useful to kill cells possessing a high flux of arachidonic acid and its metabolites like prostate and colon cancer cells.

MATERIALS AND METHODS

Liver mitochondria were prepared by standard centrifugation techniques from albino Wistar rats weighing ~300 g (32). Mitochondrial swelling was followed as the change of light scattering of the mitochondrial suspension at 545 nm. The experiments were performed with a Hitachi PerkinElmer 650-40 fluorescence spectrophotometer with excitation and emission slits of 1 nm. Oxygen consumption was determined polarographically using a Clark oxygen electrode. All assays were performed at 25 °C in instruments equipped with thermostatic control and magnetic stirring.

PC3 human prostate cancer cells were grown in RPMI 1640 medium, supplemented with 2% glucose and 10% fetal calf serum plus 50 units/ml penicillin and 50 μg/ml streptomycin in a humidified atmosphere of 95% air and 5% CO2 at 37 °C in a Forma tissue culture water-jacketed incubator. The RPMI medium and the supplements were purchased from Sigma.

For fluorescence microscopy with TMRM, PC3 cells (50 × 103) were seeded on glass cover slips in six-well plates. Following one of the various treatments described in the figure legends, cells were washed and incubated in Hanks’ balanced salt solution in presence of 20 nM TMRM and either 1 μM CsH or 1 μM CsA for 30 min at 37 °C. Treatment of control cells with CsH is necessary because the extent of cell and hence mitochondrial loading with potentiometric probes is affected by the activity of the plasma membrane multidrug resistance P-glycoprotein, which is inhibited by both CsH and CsA, whereas only CsA inhibits the PTP (33).

Recordings were started after equilibration of TMRM for 5 min at room temperature. Cell fluorescence images were acquired with an Olympus IMT-2 inverted microscope equipped with a xenon light source (75 watts), a 12-bit digital cooled CCD camera (Micronmax, Princeton Instruments), excitation and emission wavelength filters, 525 ± 25 nm to 590 longpass (TMRM). Images were acquired with a 40×, 1.3 numeric aperture oil immersion objective (Nikon) at 2-min intervals (exposure time, 80 ms), and analyzed using Metamorph software (Universal Imaging). Clusters of several mitochondria were photographed in each well at 60× magnification at 37 °C with a 40×/1.3 oil immersion objective. Data were recorded every 60 s for 10 min using MetaMorph software. In the experiments of Fig. 6, cells were grown in 96-well microtiter plates (8.5 × 103 cells/well), treated as described in the figure legends and viability was assessed based on the resazurin (Sigma) assay as described in Ref. 34. Briefly, the incubation medium was removed by aspiration, and serum-free RPMI 1640 growth medium containing 10% (v/v) resazurin was added (0.1 ml/well). The ratio of reduced to oxidized resazurin (which reflects the metabolic activity of viable cells) was detected at 540/620 nm. We verified that the ratio increased linearly with the number of cells in the range of cell densities used in the experiments.

RESULTS

In the experiments of Fig. 1 (panel A), mitochondria energized with glutamate plus malate (triangles), with succinate in the presence of rotenone (circles), or with ascorbate plus TMPD (squares) were treated with increasing concentrations of MK886, and oxygen consumption was measured with a Clark oxygen electrode. It can be seen that MK886 caused a concentration-dependent increase of respiration, which reached a plateau at slightly less than 10 μM MK886, irrespective of the substrate being oxidized. At concentrations of 10 μM or higher, on the other hand, MK886 instead caused inhibition of respiration with glutamate, malate, or succinate as the substrates, but not with ascorbate plus TMPD (panel A). The inhibitory effect of MK886 on respiration was then studied in mitochondria treated with the uncoupler DNP. The experiments of Fig. 1 (panel B) show that MK886 inhibited respiration in the same concentration range causing uncoupling, and that inhibition was not observed when ascorbate plus TMPD were the substrates. These experiments demonstrate that MK886 has direct effects on mitochondria, where it causes uncoupling, and respiratory inhibition at complex III (and possibly at complex I) but not at complex IV. These relatively high concentrations of MK886 (above 5 μM), which are typically employed in studies of cytotoxicity, also permeabilized mitochondria to K+, Na+, and Ca2+, but not to choline or sucrose (results not shown). It should be noted that these experiments were carried out in the presence of CsA, to prevent PTP opening and the accompanying changes of respiration that range from uncoupling to respiratory inhibition depending on the substrates being used (see Ref. 10 for discussion).

We next assessed whether MK886 had effects on the PTP. Because the PTP is voltage-dependent (35), MK886 concentrations causing uncoupling and respiratory inhibition (and therefore depolarization) cannot be used, because these would cause PTP opening through depolarization. We therefore chose a low concentration of MK886 (1 μM), which had negligible effects on respiration (Fig. 1) and on energy coupling, as demonstrated by the lack of effects on the resting membrane potential and on the profile of membrane depolarization obtained by uncoupler titrations (results not shown). We tested whether 1 μM MK886 would sensitize PTP opening by low concentrations of uncoupler, a method that allows to assess shifts in the PTP voltage threshold (36). In the experiments of Fig. 2 (panel A), mitochondrial volume was monitored as the absorbance of the mitochondrial suspension at 545 nm, and the fraction (Po) of mitochondria that responded to the various treatments with PTP opening was determined as previously described (36). Mitochondria were first loaded with a small amount of Ca2+, which per se did not cause any effects on PTP opening (trace a). The subsequent addition of 60 nM FCCP caused PTP opening in a small fraction of the mitochondria (trace b), indicating that after addition of the uncoupler the membrane potential was still more negative than the threshold voltage required for PTP opening in the majority of mitochondria. Treatment with 1 μM MK886 dramatically affected the mitochondrial response to 60 nM FCCP, which was now followed by PTP opening in nearly all mitochondria (trace c) and...
could be completely prevented by CsA (trace d). Fig. 2 (panel B)
reports the quantitative assessment of the dependence of PTP
opening on the concentration of FCCP in the absence (open
symbols) or presence (closed symbols) of 1 μM MK886. It can be
seen that MK886 sensitized PTP opening by uncoupler, which
could be observed at FCCP concentrations between 40 and 60
nM. These experiments indicate that MK886 is a PTP sensitizer
at a concentration that causes negligible effects on mitochon-
drial coupling and respiration.

We next carried out a series of determinations on the effects
of MK886 on the membrane potential maintained by mitochon-
dria in PC3 cells in situ based on the accumulation of the
potentiometric probe TMRM (33). Fig. 3 (panel A) documents
that the addition of 1 μM MK886 had negligible effects on
TMRM fluorescence irrespective of whether CsA was present or
not (closed and open symbols, respectively). When the concen-
tration was raised to 3 μM (panel B), MK886 caused a decrease
of TMRM fluorescence that is consistent with in situ mitochon-

Cell Death by MK886 and COX Inhibitors

![Diagram](image)

**Fig. 3. Effects of MK886 on TMRM fluorescence in PC3 cells.** PC3 cells grown on coverslips in six-well plates were rinsed with Hanks’ balanced salt solution and loaded with 20 nM TMRM, and fluorescence was monitored as described under “Materials and Methods.” The graphs report the changes of TMRM fluorescence intensity over mitochondrial ROI. In the experiments denoted by open squares, cells were loaded in presence of 1 μM CsA, whereas, in the experiments denoted by filled squares, cells were loaded in presence of 1 μM CsA. The arrows indicate the addition of MK886 (1 μM in panel A, 3 μM in panel B, and 10 μM in panel C) or FCCP (2 μM). Values are mean ± S.E. of 10 different experiments.

MK886 concentration of MK886. A further increase of the MK886 concentration to 10 μM caused a rapid mitochondrial depolarization that was instead insensitive to CsA (panel C). These experiments demonstrate that MK886 affected mitochondria in situ in a manner that is consistent with its effects in isolated mitochondria. At low concentrations it sensitized the PTP to opening, whereas at higher concentrations it caused direct mitochondrial depolarization, possibly through a combination of its ionophoretic and inhibitory effects on respiration. Because cell death is generally observed only at the higher concentrations of MK886 (27, 28), it is legitimate to ask whether mitochondrial dysfunction rather than LOX inhibition is the major event in cell death induced by MK886. We confirmed that 10 μM MK886 depolarizes mitochondria in situ (Fig. 3, panel C) (28), and that it rapidly causes the death of PC3 cells (results not shown). We then investigated the effects of 1 μM MK886, a concentration that did not directly affect energy coupling (Fig. 1) and did not cause measurable effects on the mitochondrial membrane potential in situ (Fig. 3), but inhibits LOX in PC3 cells, IC₅₀ for inhibition of LOX being ~3 nM (37).

In the experiments of Fig. 4, PC3 cells were incubated in serum-free medium for up to 6 days, a condition that did not affect the survival of control cells (black bars). It can be seen that cell survival was not significantly affected by treatment with 1 μM MK886 (gray bars), indicating that inhibition of 5-LOX as such was not sufficient to kill PC3 cells under these conditions. Likewise, the COX inhibitor indomethacin was not cytotoxic (striped bars), suggesting that prostaglandins and other COX metabolites were not essential survival factors for PC3 cells. However, a clear cytotoxic effect was observed when 1 μM MK886 was added together with indomethacin (hatched bars). Similar results were obtained in complete medium containing 10% fetal calf serum, but the cytotoxic concentrations of MK886 were higher, possibly because of binding to serum components (results not shown).

To explore the status of mitochondrial function during treatment with MK886 and indomethacin, we studied mitochondrial TMRM accumulation at 2, 4, and 6 days into the various treatments. The striking resistance to serum starvation of PC3 cells (Fig. 4) was matched by maintenance of a normal accumulation of TMRM by mitochondria for up to 6 days (Fig. 5, panels A, A’, and A’, open symbols). TMRM accumulation was not significantly affected by treatment with CsA (panels A, A’, and A’, closed symbols) nor by the addition of oligomycin (panels A, A’, and A’, arrowheads marked O). This latter finding indicates that throughout the period of serum starvation the mitochondrial membrane potential was maintained by respiration rather than by hydrolysis of glycolytic ATP. Finally, the expected probe release readily followed addition of the uncoupler FCCP (panels A, A’, and A’, arrowheads marked F).

When 10 μM indomethacin was added to the serum-free medium, TMRM accumulation and its response to CsA or oligomycin were indistinguishable from those of untreated cells at 2 and 4 days of incubation (Fig. 5, panels B and B’, respectively; symbols are the same as in panels A). After 6 days, on the other hand, TMRM accumulation was somewhat lower in the absence of CsA (panel B’), suggesting that increased PTP flickering might have ensued, causing in turn a lower steady-state mitochondrial membrane potential. Interestingly, addition of oligomycin was followed by mitochondrial depolarization (panel B’, arrowhead O), suggesting that mitochondria were maintaining the membrane potential by ATP hydrolysis, but overall cell survival was still unaffected despite the impending mitochondrial dysfunction (compare with Fig. 4).

In the presence of 1 μM MK886, mitochondrial dysfunction was readily detectable at 2 and 4 days of incubation (Fig. 5, panels C and C’, respectively). Indeed, TMRM accumulation was lowered, and could be increased by CsA (closed symbols in both panels); and oligomycin caused mitochondrial depolarization. After 6 days of treatment with MK886, the accumulation of TMRM decreased further and became unresponsive to CsA, but cells were still able to survive (compare with Fig. 4).

When both 1 μM MK886 and 10 μM indomethacin were added together, mitochondrial accumulation of TMRM was already decreased after 2 days (Fig. 5, panel D), indicating early onset of mitochondrial dysfunction that was also detectable at 4 and 6 days of treatment (panels D’ and D”, respectively). It should be recalled that treatment with 1 μM MK886 and 10 μM indomethacin caused relevant cell death at all time points (Fig. 4). These assays therefore underestimate mitochondrial dysfunction because they can only be performed on the survivors (i.e. the most resistant cells). It appears legitimate to conclude that, after treatment with the combination of 1 μM MK886 and 10 μM indomethacin, mitochondrial dysfunction is an early event that precedes overt cell death. It should be mentioned that the...
cellular effects of MK886 could not be mimicked by the LOX inhibitor caffeic acid, which also had no detectable effects on isolated mitochondria (results not shown).

We finally tested the effects of MK886 and indomethacin on the viability of cells that had been treated with concentrations of arachidonic acid ranging between 1 and 10 μM. The experiments of Fig. 6 document that the combination of 1 μM MK886 and 10 μM indomethacin caused cell death within 24 h of the addition of 5 μM arachidonic acid, which was otherwise devoid of effects on cell survival. A similar effect was observed at 10 μM arachidonic acid, although the response was less clear-cut because of the onset of cytotoxicity by arachidonic acid alone. These results indicate that treatment with MK886 plus indomethacin sensitizes PC3 cells to the cytotoxic effects of added arachidonic acid.

DISCUSSION

MK886 is unique among inhibitors of 5-LOX in that it acts on an arachidonic acid transfer protein, FLAP, which delivers the substrate to LOX (38). MK886 induces cell death in several models (27–29), but it is clear that cytotoxicity does not depend on LOX inhibition because cell killing by MK886 can also be observed in cells that do not express FLAP (39). In the present report, we have clarified the mitochondrial and cellular effects of MK886. Our findings explain the mechanisms underlying the cytotoxicity of MK886 and provide a rationale for the use of MK886 in combination with COX inhibitors to force cell death in prostate cancer PC3 cells.

The Mitochondrial Effects of High Concentrations of MK886—At relatively high concentrations (above 5 μM), MK886 caused mitochondrial depolarization through combination of an uncoupling (protonophoric) effect with inhibition of respiration. As mentioned under “Results,” MK886 permeabilized mitochondria to small cations like K+, Na+, and Ca2+ but not to choline or sucrose. In principle, this effect could be the result of opening of endogenous ion-conductive pathways or to an ionophoretic effect of MK886 itself. To address this issue, we have prepared sealed egg yolk phospholipid liposomes containing the Ca2+ indicator Quin-2. The addition of concentrations

![Fig. 4. Effects of indomethacin and MK886 on survival of PC3 cells.](image)

![Fig. 5. Effects of oligomycin and CsA on mitochondrial TMRM accumulation in PC3 cells treated with indomethacin and MK886.](image)
of MK886 ≥ 5 μM was followed by permeabilization of liposomes to Ca\(^{2+}\) (but not to Quin-2), indicating that MK886 itself is responsible for ion transport, i.e., that it behaves as an ionophore (results not shown).

The basis for respiratory inhibition by MK886 remains unclear. As shown in Fig. 1, respiration supported by ascorbate plus TMPD was unaffected by MK886, indicating that cytochrome c oxidase is resistant to the inhibitory effects of the drug. On the other hand, inhibition of respiration was observed both with succinate and glutamate/malate as the substrates, strongly suggesting that complex III is a target of inhibition by MK886. Irrespective of the detailed mechanisms of respiratory inhibition, the combination of the ionophoretic and inhibitory effects of MK886 disrupts mitochondrial energy conservation and ion homeostasis. Furthermore, ion permeabilization may cause plasma membrane depolarization and Ca\(^{2+}\) overload, which have indeed been detected following the addition of 20 mM MK886 to Fluo-3-loaded PC3 cells (results not shown). These effects of MK886 can easily explain the cytotoxicity of this drug and are entirely consistent with the finding that MK886 can induce the death of cells that do not express FLAP (39). Thus, through a combination of its ionophoretic and mitochondrial effects, MK886 at relatively high concentrations can kill cells independently of its inhibitory effects on LOX.

The Mitochondrial Effects of Low Concentrations of MK886—A low concentration of MK886 (1 μM) had no detectable effects on mitochondrial respiration (Fig. 1). However, this concentration of MK886 sensitized the PTP to opening by Ca\(^{2+}\) plus uncoupler in isolated mitochondria (Fig. 2), and a slightly higher concentration (3 μM) caused CsA-sensitive depolarization of mitochondria in situ in PC3 cancer cells (Fig. 3, panel B). Long-term treatment of PC3 cells with 1 μM MK886 did not cause overt cell death (Fig. 4), but it induced onset of CsA-sensitive mitochondrial dysfunction (Fig. 5), indicating that 1 μM MK886 increased the PTP open time in situ. It is legitimate to ask whether this effect of MK886 may depend on blockade of arachidonic acid metabolism via 5-LOX, given that the IC\(_{50}\) for inhibition of FLAP by MK886 is ~3 nM (37); and (ii) that arachidonic acid, levels of which might have increased as a result of LOX inhibition, is a powerful inducer of the PTP (11). We have tested this possibility by treating cells with caffeic acid, which inhibits LOX but has no detectable effects on mitochondrial function (results not shown). Treatment of PC3 cells with 5 μM caffeic acid alone or in combination with indomethacin did not cause either mitochondrial dysfunction or cell death, suggesting that sensitization of the PTP is a direct consequence of the interactions of MK886 with mitochondria rather than a consequence of LOX inhibition. It therefore appears that inhibition of LOX alone is not sufficient to kill PC3 cells, and that the PTP-inducing effects of 1 μM MK886 in situ are not sufficient to cause cell death, but may be instrumental when COX is inhibited by indomethacin (see below).

Induction of Cell Death by Low Concentrations of MK886 plus Indomethacin—Treatment with 1 μM MK886 plus 10 μM indomethacin sensitized PC3 cells to the cytotoxic effects of low concentrations of added arachidonic acid (Fig. 6). This finding provides an important clue into the basis for the cytotoxicity of this drug combination in the absence of added arachidonic acid as well. Indeed, we suspect that inhibition of both LOX and COX may have caused an increase of intracellular arachidonic acid, and that the sensitizing effect of MK886 on the PTP (Fig. 2) may have added to the inducing effect of arachidonic acid, thus stabilizing the PTP open time and causing critical mitochondrial dysfunction, precipitating in turn cell death. Support for this hypothesis is also provided by the finding that cell death could also be elicited by a combination of 1 μM MK886 and 10 μM NS398, a selective inhibitor of COX-2 (40–43) (results not shown). These findings may have significant implications for cancer therapy.

Arachidonic Acid and Cancer—Polyunsaturated fatty acids can enter key metabolic pathways without activation to their CoA esters. The most important for apoptotic signaling is arachidonic acid, which is released by activated phospholipases A\(_2\) and then converted into prostaglandins, prostacyclins, and thromboxanes by COX, and into leukotrienes by LOX (12, 13). A link has been established between fat content in the diet and risk of prostate cancer, which would largely depend on the supply of arachidonic acid and its transformation in LOX and COX metabolites (44). Consistently, (i) arachidonic acid stimulates the growth of prostate cancer cells (28), possibly through overproduction of 5- and 15-LOX metabolites acting as anti-apoptotic autocrine factors (45); and (ii) COX-2 is specifically
up-regulated in a variety of cancer cells (40, 46–50), and causes mammary tumors when overexpressed under the control of the murine mammary tumor virus promoter (51). Thus, in cells where LOX and COX are very active arachidonic acid may represent the source of powerful anti-apoptotic agents, and thus favor tumor progression. On the other hand, free arachidonic acid is a potential pro-apoptotic agent. Cells deficient in cytosolic phospholipase A2 or in Δ6-desaturase (a key enzyme in the biosynthesis of arachidonic acid) were resistant to cell death induced by tumor necrosis factor α (52, 53); inhibition of COX-dependent transacylase caused accumulation of non-esterified fatty acids and apoptosis (30, 54); and overexpression of COX-2 or of fatty acyl-CoA ligase protected from the killing effects of added arachidonic acid (55). Importantly, arachidonic acid induces cell death through the PTP11 (11) and activation of caspase-3, a process that is amplified by release of mitochondrial cytochrome c (2) and Smac/DIABLO (4); and Bax, which kills cells via the mitochondrial pathway (17–22), is essential for the apoptotic response of cells to nonsteroidal anti-inflammatory drugs (23). Arachidonic acid also stimulates the production of ceramide (30, 56–59), which may feed back on mitochondria causing or potentiating PTP opening.

Our results provide a rationale for killing of malignancies characterized by a high flux of arachidonic acid through LOX and COX. Indeed, and despite the presence of these enzymes in normal tissues as well, selective cytotoxicity may be achieved through combination of PTP sensitization by MK886 and the effects of endogenous arachidonic acid, which should be proportional to its cellular flux. In this respect it is reassuring that nonsteroidal anti-inflammatory drugs reduce the risk of colon cancer (60) irrespective of whether the effect depends more on the elevation of intracellular free arachidonic acid or rather on the decreased production of its COX and LOX metabolites (15, 55, 59, 61). The present studies also illustrate how clarification of the mechanisms through which drugs affect mitochondrial function in situ may lead to a substantial improvement of pharmacological strategies against cancer.