Induction of Apoptosis by Celecoxib in Cell Culture: An Uncertain Role for Cyclooxygenase-2

To the Editor:

A recent report by Kern et al. (1) described molecular mechanisms of apoptosis in human hepatocellular carcinoma cell lines in response to treatment with the selective cyclooxygenase-2 (COX-2) inhibitor (coxib), celecoxib. In their study, the authors treated Huh7 and HepG2 hepatocellular carcinoma cell lines with up to 100 μmol/L of celecoxib and found that this treatment led to tumor cell death via the activation of death receptor signaling and apoptosis originating from mitochondria; critical to these processes was the down-regulation of myeloid cell leukemia-1 (Mcl-1) protein, an antiapoptotic member of the Bcl-2 family. The authors concluded that the observed effects were mediated via the inhibition of COX-2.

In my laboratory, we have done similar experiments with the same and other cell lines that point to the opposite conclusion, i.e., that the effects of celecoxib are independent of any involvement of COX-2.

It has been well established that in cell cultures, coxib concentrations of 0.1 to 1.0 μmol/L are generally sufficient to rapidly and effectively block prostaglandin E2 production in different types of cells, including tumor cells with varying levels of overexpressed COX-2 (for example, see ref. 2). Therefore, one would expect that those drug effects that are dependent on COX-2 inhibition should be detectable at very low micromolar concentrations, and, furthermore, that they should be mimicked by other COX-2 inhibitors, such as the coxib rofecoxib or some of the traditional COX-1/COX-2 inhibitory nonsteroidal anti-inflammatory drugs. In contrast, structural analogues of celecoxib that lack the ability to inhibit COX-2 (3) should not be able to duplicate the COX-2–dependent effects of celecoxib.

We treated Huh7 and HepG2 cell lines with celecoxib, rofecoxib, indomethacin (a traditional COX-1/COX-2 inhibitor with an IC50 towards COX-2 that is comparable to celecoxib), or with 2,5-dimethyl-celecoxib (DMC, a close structural analogue of celecoxib that lacks COX-2–inhibitory activity; ref. 4). As shown in Fig. 1A, celecoxib and DMC, but not rofecoxib or indomethacin, greatly reduced the viability of these cells; celecoxib and DMC of <100 μmol/L killed the vast majority of the cell population, whereas rofecoxib and indomethacin—even at 250 μmol/L—did not potently stimulate cell death. When the levels of Mcl-1 protein in drug-treated cells were analyzed, it became apparent that only celecoxib and DMC, but not rofecoxib or indomethacin, were able to down-regulate this antiapoptotic protein (Fig. 1A).

We repeated the above approach in a number of tumor cell lines with varying levels of COX-2 expression and obtained essentially the same results, i.e., that celecoxib and DMC, but not rofecoxib and indomethacin, potently down-regulated Mcl-1 protein expression and caused growth arrest and apoptosis. For example, as shown in Fig. 1, BxPC-3 and MIA-PaCa-2 pancreatic carcinoma cells responded nearly identically to these drugs, even though COX-2 protein is absent in the former and was expressed at very high levels in the latter (5).

Therefore, in consideration of the above-presented rationale for using these four drugs in parallel, in combination with their application to COX-2–positive and COX-2–negative cell lines, one would conclude that the observed effects of celecoxib on cell death and Mcl-1 are independent of COX-2—in agreement with

Figure 1. Huh7, BxPC-3, and MIA-PaCa-2 cells were treated in parallel with either celecoxib, rofecoxib, indomethacin, DMC, or DMSO (vehicle only). A, cells were exposed to various concentrations of these drugs for 48 h, and cell survival was determined with the conventional MTT assay. B, cells were treated with 100 μmol/L of each drug for 4 h, and Mcl-1 protein levels were examined by Western blot analysis (Huh7 cells, top). With the use of a cell death ELISA, it was further confirmed that celecoxib and DMC, but not rofecoxib and indomethacin, potently stimulated apoptosis at 75 μmol/L (data not shown). All these experiments were also done with HepG2 cells, which yielded very similar results (data not shown). Experiments done by Adel Kardosh.
numerous other reports that have presented COX-2–independent growth-inhibitory effects of this coxib.

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References

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doi:10.1158/0008-5472.CAN-06-3414
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