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Sulindac enhances the killing of cancer cells exposed to oxidative stress

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Sulindac is an NSAID that has been shown to have anti-cancer activity not related to its ability to inhibit COX 1 and 2. Recently, there have been a large number of studies attempting to elucidate its mechanism of action. Our laboratory has shown that sulindac protects normal cells against oxidative stress. Furthermore, it was found that under similar conditions in which normal cells were protected against oxidative damage, several different cancer cell lines showed enhanced killing when treated with sulindac and an oxidizing agent. It was apparent that there was a basic difference in how normal and cancer cells reacted to oxidative stress after exposure to sulindac. Subsequent studies have shown that sulindac protects normal cardiac cells against oxidative damage resulting from ischemia/reperfusion by a preconditioning mechanism. Since cancer cells, because of a defect in their respiratory chain, obtain less energy from mitochondrial respiration than normal cells, it seemed reasonable that mitochondrial dysfunction might play a major role in the enhanced killing of cancer cells by sulindac and oxidative stress. A recent report studying the effect of sildenafil (viagra), a known preconditioning agent on normal and cancer cells has provided insight into sulindac's mechanism of action. Our results suggest that the enhanced killing of cancer cells by sulindac and oxidative stress also involves early steps in a preconditioning response resulting in ROS formation. In normal cells this leads to survival by a preconditioning pathway, but cancer cells react by initiating a pathway leading to apoptosis.

Sulindac selectively enhances the killing of cancer cells exposed to oxidative stress

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Abstract

Sulindac was one of the early non steroidal anti-inflammatory drugs (NSAIDS) that has also been show to have anti-cancer activity, unrelated to its NSAID properties. We have recently shown that sulindac can enhance the killing of cancer cells exposed to agents causing oxidative stress, such as H_2O_2 and tert-butyl-hydroperoxide (TBHP). In contrast normal cells were not affected and some normal cells, such as lung and cardiac cells were protected against oxidative damage. The enhanced killing of cancer cells involves mitochondrial dysfunction resulting in reactive oxygen species (ROS) formation, loss of mitochondrial membrane potential and death by apoptosis. It appeared that the selective killing of cancer cells under these conditions might be related to the "Warburg Effect". Warburg, more than 5 decades ago, noted that cancer cells preferred to obtain their energy from glycolysis, rather than respiration, even in the presence of sufficient oxygen and postulated that there was a defect in mitochondrial respiration in cancer cells as compared to normal cells. To obtain further evidence that mitochondrial respiration was involved in the sulindac effect on cancer cells, we tested dichloracetic acid (DCA), a compound that activates pyruvic dehydrogenase (PDH) and increases mitochondrial respiration and the production of ROS. The combination of sulindac and DCA was also shown to selectively kill cancer cells, but not normal cells. We have also recently demonstrated that the ability of sulindac to protect cardiac cells against oxidative damage involves a preconditioning mechanism. To determine whether another preconditioning agent could replace sulindac in killing cancer cells, we tested sildenafil (viagra) in our system. Sildenafil could partially replace sulindac in the presence of DCA or TBHP using skin squamous cancer cells. This suggests that an isoform of PKC may be involved in the enhanced killing of cancer cells by sulindac and oxidative stress. We have shown that inhibitors of PKCδ prevented the effect of sulindac in this system.

Background

The structure of sulindac and its metabolites are shown in Figure 1. Sulindac is a mixture of two epimers (R and S) and is a prodrug. It can be reduced by the methionine sulfoxide reductase (Msr) enzymes, MsrA and MsrB1 to sulindac sulfide, the active NSAID. Sulindac can also be oxidized by the cytochrome P450 system to sulindac sulfone, which is not a COX inhibitor.

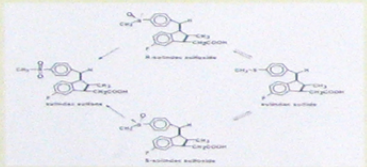

Figure 1- Metabolism of sulindac

Figure 2 shows earlier published results on the effect of sulindac in enhancing the killing of 3 cancer cell lines, colon, lung and skin, in the presence of TBHP. Under the conditions used sulindac and TBHP alone had little effect, but the combination showed marked enhanced killing [1].

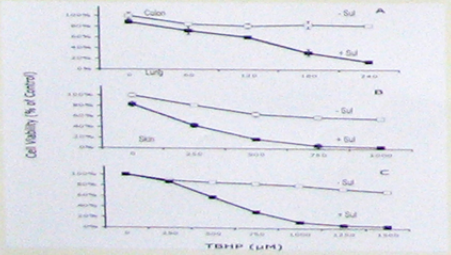

Figure 2 – Sulindac enhances killing of cancer cells exposed to TBHP

In contrast there was no enhanced killing of comparable normal cells and in the case of normal lung cells (Figure 3) sulindac protected these cells against oxidative stress caused by TBHP [1].

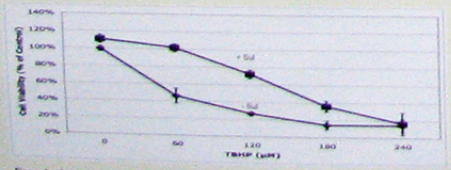

Figure 3 – Sulindac protects normal lung cells against oxidative stress

Mitochondrial dysfunction was involved in the cancer killing since under the conditions used there was a marked increase of ROS in lung cancer cells in the presence of both sulindac and TBHP, but little ROS produced with either sulindac or TBHP alone. In addition, other studies showed a loss of mitochondrial membrane potential in lung cancer cells in the presence of sulindac and TBHP [1].

Cancer cells are known to prefer glycolysis to respiration. Normal cells obtain >95% of their energy from respiration and less than 5% from glycolysis, whereas cancer cells only obtain 50-60% of their energy from respiration and the rest from glycolysis. Mitochondrial respiration in cancer cells is thought to be under stress and we wanted to test whether a compound that stimulated respiration would also cause killing in cancer cells in the presence of sulindac.

DCA (Figure 4) is known to increase respiration by inactivating an inhibitor of pyruvate dehydrogenase, resulting in an increase in the level of acetyl-CoA and a decrease in lactate formation.

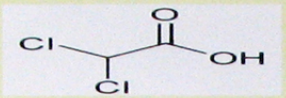

Figure 4 – Structure of dichloroacetic acid (DCA)

This is summarized in Figure 5.

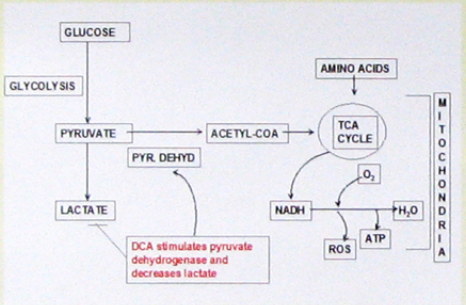

Figure 5 – DCA stimulates respiration by activating pyruvate dehydrogenase

Results

As shown in Figure 6 the combination of sulindac and DCA also resulted in enhanced killing of lung and skin cancer cells, but not the comparable normal cells.

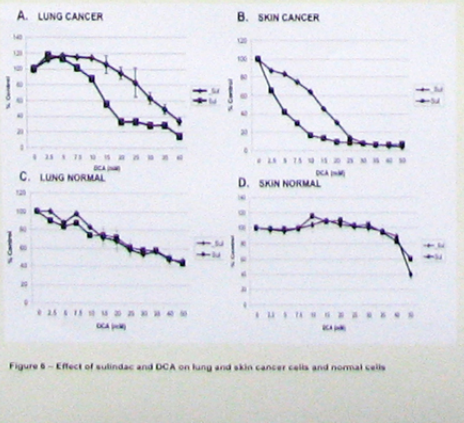

Figure 6 – Effect of sulindac and DCA on lung and skin cancer cells and normal cells

As expected and shown in Figures 7 and 8 the combination of sulindac and DCA caused a large increase in ROS and loss of mitochondrial membrane potential (increase in green fluorescence).

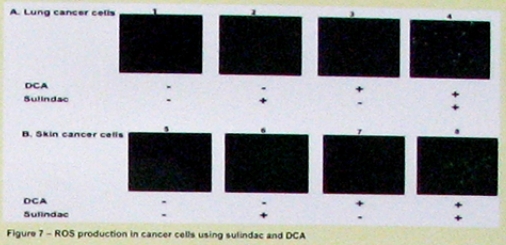

Figure 7 – ROS production in cancer cells using sulindac and DCA

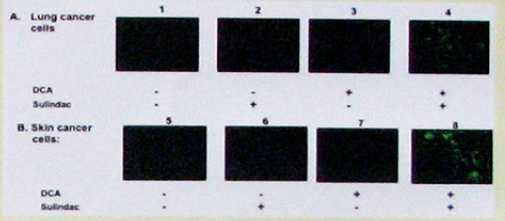

Figure 8 – Effect of sulindac and DCA on mitochondrial membrane potential in cancer cell

Studies with ROS scavenger agents such as N-acetylcysteine showed that the killing effect was dependent on ROS production (Figure 9).

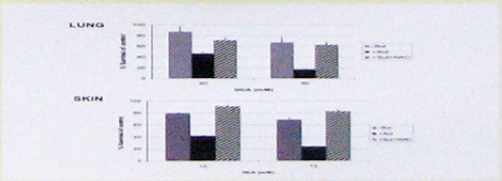

Figure 9 – N-Acetylcysteine (NAC) can reverse sulindac killing

Sulindac sulfone could replace sulindac, when used in combination with either TBHP or DCA, indicating that the effect of sulindac was not due to its NSAID activity. Other studies have shown that the protection of normal cells by sulindac was due to a preconditioning mechanism that involved ROS formation and PKC activation, presumably PKCε [2]. To determine whether there was any involvement of a preconditioning pathway in the enhanced cancer killing we tested sildenafil (viagra) a known chemical preconditioning agent [3], in place of sulindac with both TBHP and DCA using skin and lung cancer cells. The results with DCA are shown in Figure 10. It can be seen that sildenafil showed a partial effect as compared to sulindac in the enhanced killing of skin and lung cancer cells in the presence of DCA. Increasing the concentrations of sildenafil did not further improve the enhanced killing of lung and skin cancer cells using DCA and TBHP treatment. However, the fact that sildenafil behaves similarly to sulindac in the killing of cancer cells exposed to oxidative stress, suggests that some components of preconditioning pathway may also be involved in the sulindac effect.

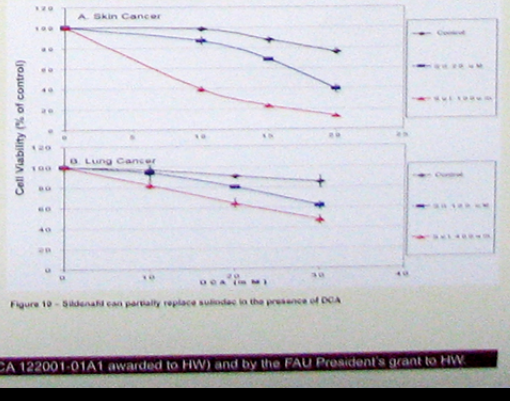

Figure 10 – Sildenafil can partially replace sulindac in the presence of DCA

Sildenafil and sulindac are known to activate PKC in cardiac cells to initiate a preconditioning response. Current evidence suggests that isoforms of PKC can act in protective as well as death pathways in cells subjected to oxidative stress. PKCεhas been identified as playing a major role in ischemic preconditioning while PKCδ has been implicated in cell death pathways.

Therefore, we looked more closely at the role of PKCε and PKCδin the sulindac/oxidative stress cancer killing mechanism. As shown in Figure 11A the broad spectrum PKC inhibitor, cherylethrin, led to a marked inhibition of the enhanced killing of cancer cells in the presence of sulindac and TBHP. However, a specific PKCε inhibitor (V1-V2 peptide) had little or no effect in the system (Figure 11B), while an inhibitor of PKCδ (rottlerin) showed a 70% inhibition of the the killing by sulindac (Figure 11C).

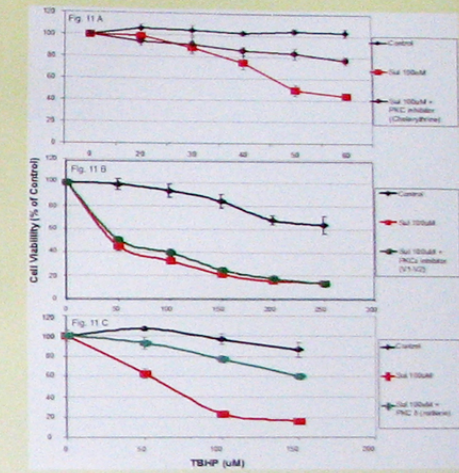

Figure11 – The effect of PKC inhibitors in the killing of skin cancer cells in the presence of sulindac and TBHP

Summary

Figure 12 summarizes our results regarding how sulindac protects normal cells against oxidative stress but enhances the killing of cancer cells exposed to agents that cause oxidative stress. The common features of both pathways are the role of mitochondria and ROS. In normal cells this initiates a preconditioning response that involves PKCε leading to protection against oxidative stress. In cancer cells this leads to activation of PKCδ and initiates apoptosis.

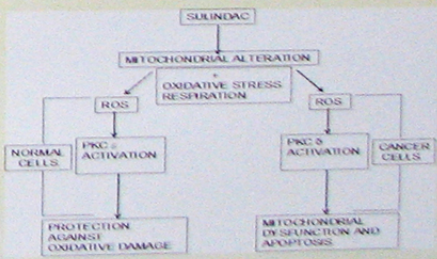

Figure 12 – Proposed pathway for the enhanced killing of cancer cells under sulindac and oxidative stress

References

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2- Moench, I., Prentice, H., Rickaway, Z., and Weissbach, H. Sulindac confers high level ischemic protection to the heart through late preconditioning mechanisms. *PNAS*. 106, 19811- 19816 (2009).

3-Kukreja, R. Pharmacological preconditioning with sildenafil: Basic mechanisms and clinical implications. *Vascular Pharmacology* 42, 219-232 (2005)

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