HYPOTHESIS

Stimulation of apoptosis by sulindac
and piroxicam

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ABSTRACT

1. Sulindac, cis-5-fluoro-2-methyl-1-(p-methylsulphinylbenzylidene)indene-3-acetic acid, inhibits growth of colon polyps and cancers. This effect has been attributed to inhibition of prostaglandin synthesis but more recent observations indicate that, in vitro, cells that do not have cyclo-oxygenase nor RNA for synthesis of such enzymes are affected by sulindac. Therefore the presumptive effect is probably not correct.

2. It has also been found that sulindac stimulates apoptosis. It is herein postulated that in tumour cells such effects may be due to interaction of the anionic form of the drug with protons in the intermembrane space of mitochondria to disrupt the potential across the inner mitochondrial membrane and thereby initiate apoptosis. Normal cells are not affected.

INTRODUCTION

Clinical and in vitro observations have shown that sulindac, a non-steroidal anti-inflammatory drug (NSAID) homologue of indomethacin, induced regression of colon polyps [1–10] and cultured cancer cells. The effect is reversible on discontinuance of the drug. Indomethacin also inhibits growth of cultured cells in vitro and in vivo [11,12]. Originally it was logically presumed that the inhibition of prostaglandin synthesis was in some way responsible, but this possibility was excluded when it was observed that concentrations in excess of those necessary to inhibit prostaglandin synthesis were required: prostaglandins did not reverse the effect of indomethacin; and no prostaglandin synthesis was found in the rat hepatoma cell line under study [12]. More recently it has been confirmed that inhibition of epithelial proliferation by acidic NSAIDs occurs in other cell lines that neither synthesize prostaglandins nor have mRNA for cyclo-oxygenase [4].

Sulindac is a small, flat molecule with an indene ring on which are acetic acid, fluorine, vinylbenzene [phenyl-ethylene (or styrene)] and methyl sulphinyl in the para position of the benzene [13] (Figure 1). It is aromatic with delocalized π electron clouds above and below the indene and benzene rings with contributions from substituent sulphur and oxygen atoms. Overall it is negatively

Figure 1 Representation of the structure of sulindac and piroxicam

Both are acidic, aromatic and have sulphur atoms that can contribute electrons to accentuate and maintain negative charge initiated by hydrogen ion dissociation from the carboxyl acetic acid appendage of sulindac and the enolic hydroxyl adjacent to a carbon–carbon double bond of piroxicam.

Key words: apoptosis, mitochondria, sulindac, piroxicam, tumour.
Abbreviation: NSAID, non-steroidal anti-inflammatory drug.
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Figure 2 The various compartments of mitochondria and the asymmetric distribution of protons and electrons across the inner membrane

The inner membrane is not permeable to anions or protons by diffusion. High pH means low hydrogen ion concentration in the matrix and about 10 times more proton concentration in the inner space and cytoplasm. (See Lodish et al. [25] for detailed description and illustrations.)

Charged and capable of interaction with cations [14]. It moves freely through plasma membranes.

Sulindac is ingested as an inactive prodrug sulphoxide with a partially oxidized sulphur atom that is reduced [13, 15–17] or oxidized [18, 19] in the liver to establish and maintain an approximate 50:50 equilibrium between the oxidized and reduced derivatives in plasma. There is an enterohepatic circulation and also cyclic renewal of the reduced form carried out in the liver presented with the oxidized forms by the circulating blood [13]. It is therefore a means of systemic electron transport which maintains exposure of peripheral tissues to the reduced and oxidized forms of the drug. In the blood the two main derivatives of sulindac, the oxidized sulphone and the reduced sulphide, are about 95% bound to albumin and the remainder is free. The \( pK_a \) of sulindac is 5.8 ± 0.5 [13] so that at the pH of cytoplasm (7.2–7.4) it is mostly ionized and present as a base in the Lewis sense. When acetic acid on the indene ring of sulindac dissociates a hydrogen ion, a negatively charged carboxylate anion remains (\( \text{X-COO}^- \)), where X represents the remainder of the molecule [20]. These anions should be capable of ionic interaction with positively charged entities such as lysine and arginine and with protons accumulated in the intermembrane space of mitochondria (discussed below).

Piroxicam is another NSAID that inhibits growth of colon polyps and cancers [21–23], sometimes with remarkably small doses [24] (Figure 1). It is acidic by virtue of an enolic hydroxyl on a heterocyclic ring adjacent to a carbon–carbon double bond [20]. Its \( pK_a \) is 5.1 [22]. When the hydrogen ion is dissociated, a negatively charged anion remains. A benzene ring is fused providing an aromatic planar heterocycle with nitrogen and sulphur atoms, both of which contribute delocalized \( \pi \) electrons to accentuate the negative charge of the \( \pi \) electron clouds above and below. It is postulated that these similarities to sulindac with its charged aromatic structures provide a partial explanation for the functional similarities as discussed below.

This review looks at an alternative interpretation of the several demonstrations that acidic NSAIDs stimulate apoptosis [4–10]. It is postulated that such effects may be due to inhibition of mitochondrial function as the proximate cause of apoptosis of mutant cells. Some of the previous work in mitochondrial physiology is reviewed and its relevance to current problems of cancer therapy explained, particularly induction of apoptosis.

INNER MITOCHONDRIAL MEMBRANE FUNCTION

The inner mitochondrial membrane is the main barrier between cytosol and mitochondrial matrix (Figure 2). It is relatively impermeable to inward movement of both anions and protons [25], but protons generated in the matrix during electron transport are extruded outwards into the intermembrane space at multiple sites in the membrane to establish and maintain a proton concentration gradient across the inner mitochondrial membrane. The same chemical processes produce electrons that accumulate in the matrix so that there is an electric potential of some 200 mV (matrix negative). Protons flow back into the matrix through the F\(_{0}\)F\(_{1}\)-ATPase, a complex of catalytic proteins driven by the proton concentration gradient and the electrochemical potential across the inner membrane – the protomotive force. Such movement of protons inwards across the inner membrane is coupled to ATP synthesis [25].

Plasma and outer mitochondrial membranes are more permeable to whole molecules than to ions or protons that have a charge [25]. Therefore, when anions such as...
those that dissociate from sulindac or piroxicam in cytoplasm and the intermembrane space of mitochondria, take on protons, the resulting intact molecules preferentially diffuse out into cytoplasm and out of the cell, creating a carrier-mediated diffusion for protons. This is a physiological means of dealing with metabolically derived weak acids such as lactate [26–28].

**INNER MITOCHONDRIAL TRANSMEMBRANE POTENTIAL, SULINDAC AND APOPTOSIS**

Expression of APC gene in cultured colon carcinoma cells induces apoptosis [10], and it has been suggested that some acidic NSAIDs, sulindac in particular, might fulfill a similar role [10,4–9], but the mechanism of such an effect has not been established. It is herein proposed that when negatively charged anionic sulindac or piroxicam permeate the mitochondrial intermembrane space, the proton gradient across the inner membrane is dissipated and a catastrophic lesion forms which initiates apoptosis. It is believed that this is a practical method that can be imposed for disposition of protons outwards and disruption of the transmembrane potential (ΔΨ) [29–31].

Mitochondrial ΔΨ controls permeability of the inner membrane to small molecular solutes including protons so that when it is inhibited, ATP synthesis is reduced, proteases are activated, nuclear DNA is fragmented and mitochondrial contents pass to cytoplasm, e.g. cytochrome c [29]. There is a permeability transition pore that functions as a sensor of voltage, matrix pH, thiol, adenine nucleotide and divalent cation concentrations. The ΔΨ is indispensable for normal mitochondrial function and coupling of electron transport to proton movement down the gradient across the inner membrane—the protomotive force [29–31]. Cells induced to undergo apoptosis by various means have been found to have a ΔΨ reduction that precedes the effects on DNA [29]. ΔΨ disruption and subsequent nuclear apoptosis cannot be dissociated. Mitochondria undergoing permeability transitions also appear to release an apoptogenic protein (apoptosis inducing factor) [29]. There is a substantial literature concerning pharmacological modulation of mitochondrial permeability transition and the composition and function of permeability transition pores. Collapse of the ΔΨ is an early and irreversible aspect of the overall phenomenon [29].

APC protein, a prototypic suppressor for colon polyps and cancers, associates with β-catenin and Tcf-4 (T-cell transcription factor) and down-regulates transcription mediated by β-catenin with Tcf-4 [32,33]. β-Catenin is a transcription activator when complexed with only Tcf but this function is down-regulated when APC protein is in the complex. Mutant APC gene product in colon and rectal tumours is defective in this regard. Without wild-type APC product the β-catenin–Tcf complex is a constitutive transcription activator [32]. Therefore, the end effect of wild-type APC in colon tumour cells is similar to that of exposure to acidic NSAIDS which is postulated to disrupt the ΔΨ across the inner mitochondrial membrane and initiate apoptosis. Healthy normal cells regulated (suppressed) by wild-type APC would not be affected whereas mutant cells without the protective effect of APC protein might be subject to apoptosis initiated by anionic derivatives of sulindac and piroxicam. The relatively recent demonstrations that sulindac stimulates apoptosis in cultured colon cancer cells while sparing normal cells, and that APC has a similar specific effect [10], emphasizes that there is still much to learn and that we may have erred in attributing the effect of sulindac to inhibition of prostaglandin synthesis. I am proposing a simplification based on long-standing physiology and biochemistry.

**PROSPECTS**

Identification of a way to manipulate mitochondrial function and stimulate apoptosis of benign and malignant mutant tumour cells without harm to normal cells appears to provide a powerful, yet easily tolerated form of adjuvant chemotherapy which could be administered in conjunction with surgery and also for malignant tumours, standard chemotherapy and radiation. There is some evidence that larger more effective doses of acidic NSAIDs can be administered to patients for long periods if the upper gastroduodenal side-effects are controlled with omeprazole [34]. Resistance to NSAIDs given as a single agent for colon polyps occurs rarely if at all. It is emphasized that benign and metastatic lesions that have responded to NSAIDs are not cured. When such drugs are omitted, most but not all benign and metastatic lesions recur. In other words, the metabolic derangement that is responsible for tumour growth remains and may require more or less continuous therapy. A standard optimal dose for patients has not been established and the clinical aspects of the proposal remain to be explored.

**REFERENCES**


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