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Research Report

# Protective mechanism of sulindac in an animal model of ischemic stroke ☆

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ABSTRACT

*Background and Purpose:* The present study analyzed whether administration of sulindac, a non-steroidal anti-inflammatory drug (NSAID) would prevent, attenuate or repair ischemia induced brain injury and reverse functional impairment in a focal ischemia model of stroke. *Methods:* Male Sprague-Dawley rats (weight 250–300 g) were subjected to middle cerebral artery occlusion (MCAO). Sulindac was given 2 days before and 24 h after ischemia at 0.2 mg/day with daily injections until sacrifice on day 3 or day 11. Infarct size was measured by TTC staining and western immunoblot was employed. *Results:* TTC analysis of brain slices indicated a decrease in infarct size in sulindac treated animals. Western blot results indicated that sulindac induced expression of Hsp 27, a marker of cell stress, in the ischemic penumbra and core on days 3 and 11. Hsp 27 is important as a protective molecular chaperone. Increases were also found in the protective molecules Akt and Bcl-2 in the ischemic penumbra and core following sulindac administration. *Conclusion:* Our data indicate that administration of sulindac results in decreased infarct size and that there is a central role for the molecular chaperone Hsp 27, the pro-survival kinase Akt and the anti-apoptotic component Bcl-2 in mediating these protective effects.



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## 1. Introduction

Sulindac is a non-steroidal anti-inflammatory drug (NSAID) that is capable of inhibiting cyclo-oxygenases (COX) 1 and 2 (Vane, 1971). In addition to its known anti-inflammatory activity there have been numerous studies in recent years on the ability of sulindac and its metabolites to act as potential anti-cancer agents, based on their ability to slow the progression of colorectal polyps to colon cancer, as well as their ability to kill colon and other cancer cells (Marchetti et al., 2009; Taketo, 1998).

Mammalian cells can respond to a variety of stresses such as heat, cold, oxidative stress, metabolic disturbance, and environmental toxins through necrotic or apoptotic cell death, while increased expression and phosphorylation of heat shock proteins such as Hsp 27 can protect cells against cellular stress. Heat shock proteins (Hsp) commonly exhibit molecular chaperone activity and also interact with a wide variety of proteins to exert specific effects. In the central nervous system, Hsp are induced in response to many injuries including stroke, neurodegenerative disease, epilepsy, and trauma. Hsp are highly conserved and under physiological conditions act as molecular chaperones and/or have anti-apoptotic activities. Two of the major heat shock proteins in the brain are the 70 kDa Hsp (Hsp 70) and the 27 kDa Hsp (Hsp 27) (Stetler et al., 2009). Over expression of Hsp 27 has been shown to reduce cortical damage after cerebral ischemia (Van der Weerd et al., 2010). Hsp 27 is characterized by its inducibility in both glial cells and neurons following a wide range of noxious stimuli including ischemia, epileptic seizure and hyperthermia (Van der Weerd et al., 2010).

Recent studies suggest that sulindac protects normal cells against oxidative damage. Previous studies on the heart suggested that sulindac protection against ischemic damage occurs through an ischemic preconditioning mechanism.

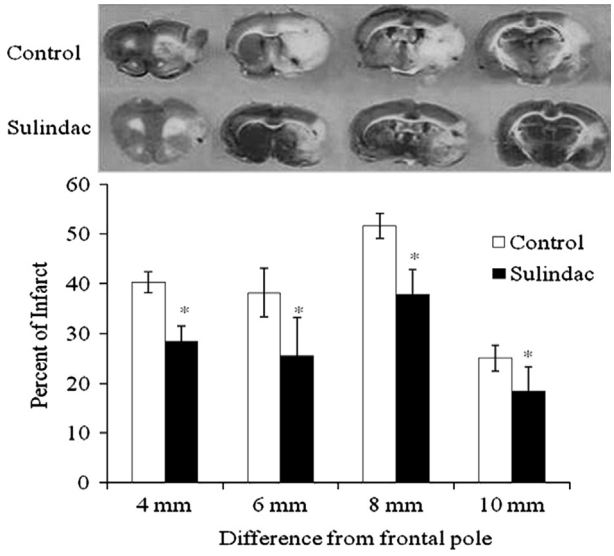


**Fig. 1 – TTC analysis of brain slices indicated a decrease in infarct size in sulindac treated animals at 4 mm, 6 mm, 8 mm and 10 mm from the anterior pole ($P<0.01$; 2 way ANOVA). Data represent infarct volume as percent of ipsilateral hemisphere volume and values are means ± SE of 10 experiments for MCAO and MCAO plus Sulindac.**

Sulindac was found to induce iNOS and Hsp 27 in a protein kinase C (PKC) dependent manner. It has been widely proposed that compounds that could precondition cells to oxidative stress may have important therapeutic value, since oxidative damage appears to play a major role in age related diseases (Moench et al., 2009).

In the current study we employed a rodent model of transient focal ischemia because this is very similar to human stroke in terms of pathophysiology. The middle cerebral artery (MCA) is the most commonly affected blood vessel in human occlusive/ischemic stroke (Virtanen et al., 2003) and the MCA is the artery most commonly targeted in rodent stroke models (Macrae, 2011).

In the present study, we have examined the protective effect of sulindac elicited by ischemia/reperfusion in the rat brain subjected to MCA occlusion (MCAO) and we provide evidence that sulindac is highly protective and exerts this pro-survival effect through pathways associated with pharmacological preconditioning.

## 2. Results

### 2.1. *Analysis of TTC staining*

Analysis of TTC staining of brain slices indicated a significant decrease in infarct size in sulindac treated animals at 4 mm, 6 mm, 8 mm and 10 mm from the anterior pole (Fig. 1) ($P<0.01$; 2 way ANOVA).

### 2.2. *Sulindac induces Hsp 27 expression in an ischemic model of stroke*

A previous study has indicated that sulindac induces Hsp 27 in heart as part of a late phase preconditioning mechanism. This was demonstrated using an *ex vivo* Langendorff myocardial ischemia model (Moench et al., 2009). Our current study indicates that sulindac treated animals express enhanced levels of Hsp 27 in penumbra and core of the ischemic area of the stroke model (left side) on day-3 (Fig. 2A) and day-11 (Fig. 2B) after vessel occlusion. Quantification of western blots by densitometry showed approximately a 2–3 fold increase in Hsp 27 expression on day-3 (Fig. 2A) and a 9 fold increase in Hsp 27 expression on day-11 (Fig. 2B) after sulindac treatment.

### 2.3. *Activation of Hsp 27 and Akt*

In several cell types Hsp 27 has been shown to modulate apoptosis by control of Akt activation. A previous investigation has identified Hsp 27 as an Akt substrate that dissociates from Akt upon phosphorylation (Rane et al., 2003). This study demonstrated that disruption of the interaction between Hsp 27 and Akt impairs Akt activation, leading to an enhanced rate of constitutive neutrophil apoptosis (Rane et al., 2003; Rane and Klein 2009). Furthermore, activation of Akt by Hsp 27 has been found to be necessary for cell survival. In the present study Akt is activated after ischemia. The sulindac treated groups show greater than 3-fold Akt activation in the penumbra of the ischemic model of stroke compared to the