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Reactive Oxygen Species are Implicated in Physiological Angiogenesis *in vivo* and *in vitro*

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S u m m a r y. Reactive Oxygen Species (ROS) have been increasingly recognized as important components in a plethora of cellular mechanisms. Still, only few data exist concerning their involvement in physiological angiogenesis. Aim of the present work was to elucidate the implication of ROS in angiogenesis in vivo, using the model of chicken embryo chorioallantoic membrane (CAM) and in vitro, using human umbilical vein endothelial cells (HUVEC). We suggest that ROS and particularly O_2^- and H_2O_2, seem to be important for angiogenesis in vivo and in vitro.

INTRODUCTION

Angiogenesis, which is observed durng physiological and pathological conditions, is the genesis of new capillaries from preexisting vasculature in response to angiogenic stimuli (1). Recent evidence suggests that reactive oxygen species (ROS) promote proliferation and migration of vascular smooth muscle (VSMC) and endothelial cells (EC) (2). Furthermore, expression of many angiogenic genes including those for vascular endothelial growth factor, fibroblast growth factor, platelet-derived growth factor and receptors such as Flt-1, Flk-1, Ang-1 and Ang-2 are likely to be regulated by redox signalling (3,4).

In order to clarify the role of ROS in physiological angiogenesis in vivo, we investigated whether H_2O_2, several ROS scavengers and oxidase inhibitors affect angiogenesis in the *in vivo* chicken embryo chorioallantoic membrane (CAM) model of angiogenesis. Moreover, we investigated the effect of the same substances on the proliferation and migration of human umbilical vein endothelial cells (HUVEC).

METHODS

The *in vivo* chicken embryo CAM angiogenesis model was used, as previously described (5). Human umbilical vein endothelial cells (HUVEC) were isolated from human umbilical cords and cultured as previously described (6). Proliferation and migration of the cells were studied as previously described (6). The significance of variability between the results from various groups and the corresponding controls was determined by unpaired t-test or ANOVA.

RESULTS

Superoxide dismutase (SOD, removes O_2^-) and tempol, a membrane permeable SOD mimetic, decreased the number of CAM vessels in a dose dependent manner. 4-(2-aminoethyl)-benzenesulfonyl fluoride (AEBSF) and apocynin (NADPH inhibitors) also caused a dose-dependent decrease in the number of CAM vessels. In contrast, allopurinol, a xanthine oxidase inhibitor, had no effect on angiogenesis *in vivo*.

H_2O_2 up-regulated angiogenesis, while catalase (detoxifies H_2O_2 to H_2O) had a small, non-significant inhibitory effect on angiogenesis. Sodium pyruvate, a membrane permeable H_2O_2 scavenger, reduced significantly the number of vessels in a dose dependent manner.

The effect on the number of CAM vessels, in all the above cases, was not due to toxicity, as verified on CAM paraffin sections stained with eosin-hematoxylin or treated with a kit for *in situ* detection of apoptosis.

Tempol, AEBSF, apocynin and sodium pyrouvate decreased the number of HUVEC in a concentration dependent manner. H_2O_2 increased, while SOD, catalase and allopurinol had no effect

on HUVEC proliferation. All the tested scavengers and the NADPH inhibitors, except allopurinol, significantly inhibited migration of HUVEC.

CONCLUSIONS

- Both in vitro and in vivo studies indicate that $O_2^{\cdot-}$ and H_2O_2, even at the basal levels, are implicated in the angiogenic response of the vascular tissue.
- NADPH oxidase activity is required for endothelial cell proliferation and migration *in vitro* and angiogenesis in vivo.
- We suggest that ROS and particularly $O_2^{\cdot-}$ and H_2O_2, seem to be important for angiogenesis *in vivo* and *in vitro*.

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