MANGANESE-CONTAINING SUPEROXIDE-DISMUTASE DEFICIENCY IN POLYMORPHONUCLEAR LEUKOCYTES OF ADULTS WITH RHEUMATOID ARTHRITIS

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Abstract—Superoxide dismutase (SOD) is known to regulate the level of superoxide radicals inside cells. The purpose of this work was to investigate the role of SOD activity in tissue damage produced by superoxide radicals. SOD was measured in polymorphonuclear cells of patients with rheumatoid arthritis and controls. The distinct SOD activities, including manganese-containing and copper-zinc-containing enzymes, were evaluated in cytoplasm and mitochondria of human granulocytes. Except for the comparison between total SOD and cytoplasmic copper-zinc SOD, no correlation was found among the different SOD levels. Moreover, a significant decrease was observed only for cytoplasmic manganese-containing enzyme in granulocytes of adults with rheumatoid arthritis. These data confirm the necessity of evaluation of various SOD classes and suggest the interest of biochemical tests in granulocytes for early diagnosis and better comprehension of tissue damage due to inflammation.

INTRODUCTION

For many years, extensive research has been done on the mechanisms of involvement of the superoxide anion ($O_2^-$) in various biological systems. The release of this superoxide free radical during phagocytosis leads to tissue damage. The role of superoxide dismutase (SOD) in regulating the level of superoxide radicals inside cells is well established. The purpose of this work was to investigate the role of SOD activity in tissue damage produced by superoxide radicals. SOD was measured in polymorphonuclear cells of patients with rheumatoid arthritis and controls. The distinct SOD activities, including manganese-containing and copper-zinc-containing enzymes, were evaluated in cytoplasm and mitochondria of human granulocytes. Except for the comparison between total SOD and cytoplasmic copper-zinc SOD, no correlation was found among the different SOD levels. Moreover, a significant decrease was observed only for cytoplasmic manganese-containing enzyme in granulocytes of adults with rheumatoid arthritis. These data confirm the necessity of evaluation of various SOD classes and suggest the interest of biochemical tests in granulocytes for early diagnosis and better comprehension of tissue damage due to inflammation.

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damage. Protection against $O_2^-$ is thus provided by a superoxide dismutase (SOD) which catalyzes the following reaction (1-3):

$$O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

Many distinct types of SOD, including manganese-containing and copper-zinc-containing enzymes, have been isolated and characterized (4,5). All these isoenzymes are able to scavenge $O_2^-$ by catalyzing its dismutation. In 1974, the level of superoxide radical was implicated in the inflammatory process and a relationship between the SOD level and its antiinflammatory action was then reported. Indeed, superoxide radicals degrade hyaluronic acid, collagen, and proteoglycans in vitro (6). Recently, Rister has found a SOD deficiency in granulocytes of children with rheumatoid arthritis, but this author gives no indication of the two distinct classes of SOD: copper–zinc– (SOD1) and manganese-containing enzymes (SOD2) (7). The present report studies the enzymic activities of total SOD, SOD1, and SOD2 present in cytoplasm and granules of polymorphonuclear cells of adults with rheumatoid arthritis.

**MATERIALS AND METHODS**

*Population Studied.* Thirty milliliters of venous blood were drawn from 43 adult volunteers and 23 patients with rheumatoid arthritis and selected according to clinical, radiologic, and biological criteria of the American Rheumatic Association.

*Isolation of Polymorphonuclear Cells (PMNs).* Human PMNs were isolated from heparinized blood according to the Boyum’s Ficoll–Contrix gradient at 14 g/100 ml (Pharmacia Fine Chemical). The final pellet was adjusted at a concentration of $5 \times 10^7$ PMN/ml. The cells were typically greater than 95% PMNs and greater than 98% viable, as judged by trypan blue exclusion (8).

*Subcellular Fractionation of PMNs.* Isolated cells were then diluted in 0.5 ml of 20 mM sodium carbonate–bicarbonate buffer (pH 10) containing 0.1 mM EDTA and sonicated at $4^\circ$ C for 50 sec. The lysates were centrifuged 10 min at 200g to remove unbroken cells. The supernatant was then centrifuged at 20,000g for 30 min and used for enzymic assays: total SOD, copper–zinc (SOD1), and cytoplasmic manganese-containing enzyme (SOD2c). Electron microscopy confirmed the lack of granules in the supernatant, as suggested by Salin et al. (9).

The resulting pellet obtained after lysate centrifugation was resuspended and treated with 0.2 ml of 0.2% Triton X-100 for 10 min to lyse the granules. This solubilized pellet fraction was assayed for content of granule manganese-containing enzyme (SOD2g).

*Superoxide Dismutase Assays.* This assay relies on the ability of SOD to inhibit the $O_2^-$ mediated reduction of ferricytochrome $c$, according to the method described by Salin and McCord (9). Spectrophotometrical reduction of cytochrome $c$ was monitored after providing superoxide anion by the xanthine–xanthine oxidase system. The incubation medium for the total SOD measurement, in 3 ml final volume, was: 20 mM sodium carbonate buffer pH 10, 0.1 mM EDTA, 0.5 $\mu$M ferricytochrome $c$, $5 \times 10^{-7}$ mM xanthine, 0.022 units xanthine oxidase, and 20,