Role of Oxyradicals in Gastric Injury

formation and glutamate toxicity, other mechanisms such as protein degradation and DNA damage may also contribute in part to the pathophysiological involvement of free radicals in ischemic neuronal death.

REFERENCES


3. Role of Neutrophil-derived Oxygen Radicals in Ischemic Mucosal Injury and the Mechanism of Formation in the Rat Stomach

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We reported in 1985 that oxygen-derived free radicals (FR) mediate the formation of gastric injury induced by hemorrhagic hypotension and reinfusion of shed blood (ischemia-reinfusion (I-R) injury) in the rat (1). Due to many studies in the last several years (2, 3), it is widely accepted that FR are involved in the pathogenesis of I-R injury in animals. With regard to the source of FR, some investigators (2, 3) have emphasized that a burst of FR production occurs in the xanthine oxidase system in cells during the reinfusion period when abundant molecular oxygen is reintroduced, leading to the formation of I-R injury. However, the role of FR produced by the NADPH oxidase system in neutrophils, another major source of FR production, has not been completely investigated. In addition, the site of onset of gastric damage remains to be defined. It is also unclear if the FR responsible for I-R injury are generated during the ischemic period. Here, the involvement of neutrophil-derived FR and chemical mediators in I-R injury and the mechanism of the formation of gastric damage are discussed.

Methods

Male SD rats were fasted overnight and anesthetized i.p. with pentobarbital sodium and were given 0.1N HCl in the stomach. Animals were then subjected to 20 min hemorrhagic hypotension (BP, 20–30 mmHg) by bleeding from the carotid artery and 20 min reinfusion of shed blood as described previously (1). After killing the rat, the stomach was removed and fixed in formalin. In the study of leukotrienes (LT), 60 min hemorrhagic hypotension (BP <40 mmHg) and 60 min reinfusion were given without acid instillation. The extent of histologic gastric damage was estimated on HE-stained tissue specimens and expressed as lesion scores (LS) using previously described criteria (4). Blood for the measurement of luminol-dependent chemiluminescence (CL) was drawn from the portal vein immediately before killing rats.

Results

Chemiluminescence (unit = relative light unit, RLU) in blood measured by photometer (Monolight TM 401, U.S.A.) and expressed as the ratio of [peak CL/neutrophils number in blood sample] was significantly greater in I-R rats than in controls with no I-R (I-R, 17.3±3.3×10⁻⁴; control, 6.6±0.8×10⁻⁴ RLU, p<0.05) (5).

The increase in CL induced by I-R was significantly inhibited by anti-neutrophil monoclonal antibody (ANA, supplied by Prof. Sendo of Yamagata University) which was administered i.p. 12 h prior to hemorrhagic hypotension at a dose of 3 ml for the purpose of diminishing the number of neutrophils to 1/10–20 of untreated rats. The total CL activity in blood samples was significantly lower in rats treated with ANA than in controls receiving normal rat serum (ANA, 26±6; control, 235±46...
Furthermore, gastric lesions were significantly reduced in rats treated with ANA (ANA vs control: corpus LS, 1.2±0.2, 2.0±0.2, p<0.01; antral LS: 0.8±0.1, 1.5±0.2, p<0.05) (6). These findings were similar in i.v. administration (5 min prior to bleeding) of 10 mg/kg of CV-3988 (Takeda Pharmaceuticals, Japan), a platelet-activating factor (PAF) antagonist, when compared to controls receiving saline (CV-3988 vs control: CL/neutrophils number, 5.3±0.7×10^4, 17.3±3.3×10^4 RLU, p<0.01; corpus LS, 0.9±0.3, 1.8±0.3, p<0.01, antral LS, 0.9±0.3, 2.0±0.3, p<0.01) (5).

AA-861 (Takeda, Japan), a 5-lipoxygenase inhibitor, and YM-638 (Yamanouchi Pharmaceuticals, Japan), a sulfidopeptide LT antagonist, which were each given orally to separate groups of I-R rats at a dose of 100 mg/kg at 1 h prior to hemorrhagic hypotension, significantly decreased lesion scores compared to controls receiving vehicle (AA-861 vs YM-638 vs control: corpus LS, 0.4±0.1 (p<0.01), 0.6±0.1 (p<0.05), 1.4±0.2; antral LS, 0.6±0.1 (p<0.05), 0.7±0.1 (p<0.05), 1.3±0.2) (7).

Gastric damage evaluated histologically was induced by both ischemia alone and by I-R with similar severity as reported previously (8).

SOD activity of gastric mucosa measured by the nitrite method, which is reported to increase with FR generation in E. coli exposed to paraquat, was elevated to the same extent in rats receiving ischemia alone as in those subjected to I-R; this elevation was significantly greater than that in controls not receiving I-R (ischemia vs I-R vs control: corpus, 123.0±4.8 (p<0.05), 127.4±3.6 (p<0.05), 96.6±4.4 NU/mg protein; antrum, 71.6±2.8 (p<0.05), 81.4±6.8 (p<0.05), 62.1±3.1 NU/mg protein) (9).

Discussion

The results indicate that I-R stimulates FR production in neutrophils; PAF may mediate FR generation from neutrophils and neutrophil-derived FR, PAF and LT are factors in the I-R injury. Also, the results suggest that FR are generated even during the ischemic period. Neutrophil-derived FR and chemical mediators such as PAF and LT may all be present in the circulation system since the sources of their production are thought to be platelets, neutrophils and endothelial cells, etc. The present observations suggest that these substances reciprocally augment their intensity and result in a chain reaction facilitating further production by stimulating other inactivated cells, culminating in tissue damage. In particular, FR derived from activated neutrophils may attack the target cells, that is, endothelial cells of the microcirculation system in gastric mucosa, leading to the onset of cell injury. The damaged endothelial cells allow in turn activated neutrophils to leak into parenchymal tissue to aggravate tissue injury. In this mechanism, PAF and LT are considered to play roles such as to adhere neutrophils to the surface of endothelial cells facilitating extravasation. In addition, gastric injury seems likely to occur even during the ischemic period. In conclusion, oxygen radicals of neutrophils may trigger the onset of the formation of ischemic or ischemia-reinfusion injury in the microcirculation system of rat gastric mucosa. In this mechanism, PAF and LT play important roles as chemical mediators to aggravate the tissue injury.

References