EXPERIMENTAL RESEARCH

The Role of Lysophosphatidic Acid in Radiation Injury: Pilot Study

Radyasyon Hasarında Lizofosfatidik Asitin Yeri: Ön Çalışma

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Abstract : Lysophosphatidic acid is the simplest phospholipid known. It is produced by platelets, endothelial cells, leukocytes, some cancer cells, astrocytes, and injured fibroblasts. Lysophosphatidic acid is thought to play a role in platelet aggregation and fibrosis. Since in radiation injury, platelet aggregation on endothelial cell surfaces and fibrin formation are the main events, lysophosphatidic acid might play a major role in radiation injury.
Key Words: Lysophosphatidic acid, phospholipids, radiation

Özet : Lizofosfatidik asit bilinen en basit fosfolipid olup trombositlerden, endotel hücrelerinden, lökositlerden, bazı kanser hücrelerinden, astrositlerden ve zedelenmiş fibroblastlardan salgılanır. Lizofosfatidik asitin trombosit agregasyonu ve fibroziste görev aldığı düşünülmektedir. Radyasyon yaralanmasında, endotel hücre yüzeylerinde trombosit agregasyonu ile fibrin oluşumu ana olaylar olduğundan lizofasfatidik asitin radyasyon yaralanmasında önemli bir rol oynaması olasıdır.
Anahtar Sözcükler : Fosfolipidler, lizofosfatidik asit, radyasyon

INTRODUCTION

Although lysohophatidic acid (LPA) (Figure 1a) is known as a membrane phospholipid its role is completely different from other phopholipids. LPA is not only a key intermediate in phospholipid metabolism but also a signalling molecule, probably acting through different pathways (6, 7, 10, 11). The most prominent effect is its aggregating activity on platelets (6, 7). Jalink et al. reported that LPA was also released from injured fibroblasts (10). Binding of fibronectin to cells is enhanced in a dose-dependent manner by LPA (3, 16).

Following radiation, the earliest effect is erythema due to dilatation of capillaries. Radiation promotes endothelial cells to produce chemotactic factors leading to adherence of polymorphonuclear leukocytes (PMN) to endothelial cell monolayers (5). Capillary obliteration by fibrin and platelets is observed at 2-6 months after radiation. Next, after 6 months, endothelial proliferation, thickening of the basement membrane, and the replacement of the capillary lumen by collagen is observed. Platelet aggregation on the endothelial cell surface, areas of focal necrosis, lymphocyte and macrophage infiltration, and focal calcification may be seen (13).

Many theories of the pathogenesis of radiation injury and necrosis have been discussed in the literature. A possible role of microvascular injury and disruption of blood brain barrier (17), an allergic reaction with resulting alterations in proteins and increase in vascular permeability which causes a progressive damage to endothelium resulting in generalized failure of the microcirculation (4), expression of specific cytokines and immunoregulatory molecules cause cytotoxicity (13),

increase in vascular permeability leading to edema and fibrin deposition, later replaced by collagen fibers resulting in fibrosis (15) are the main explanations suggested for radiation injury.

It is clear that the underlying mechanism is still unknown. But in all steps of radiation injury, adhesion and fibrin formation are the main events which suggests close relationship between the cytokins, surface adhesion molecules, and membrane phospholipids. The aim of this pilot study is to show the possible role of LPA in radiation injury.

MATERIALS AND METHODS

Study Design and Animals

The experimental protocol was approved by the University of Virginia Animal Research Committee. 4 male New Zealand White Rabbits, (3.6 to 3.9 kg) were randomly allocated to one of 2 groups.

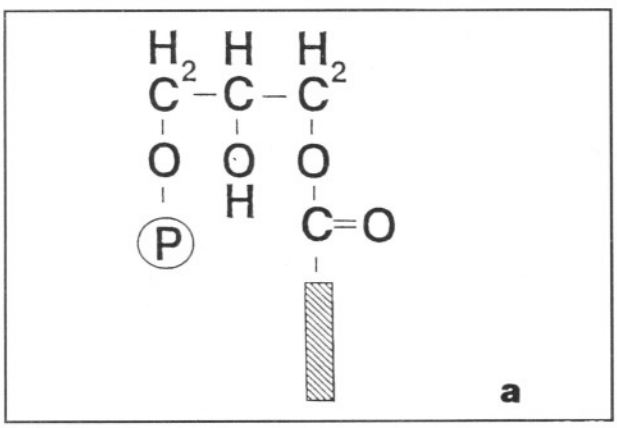
Rabbits were anesthetized by intramuscular injection with a mixture of ketamine (Ketaset, 50 mg/kg) and xylazine (Rompun, 10 mg/kg), and intubated with an endotracheal tube. A 23-gauge butterfly needle was inserted percutaneously into the cisterna magna. After withdrawel of 1.0 ml of cerebrospinal fluid (CSF) for determination of pretreatment basal LPA level, rabbits were observed for respiratory distress for 15 minutes. In the first group (n:2), after positioning of a rabbit skull frame, animals were transferred to the magnetic resonance imaging study. Stereotactic radiosurgery planning was made by using Leksell Gamma Plan 3.00. The irradiation was done by gamma knife (Elekta AB, Stockholm, Sweden). The collimator was 4 mm size and the center maximum dose was 140 Gy. This dose derived from human radiosurgery experience to aim at radiation necrosis within a few weeks. The target was the right fronto-parietal cortex, 5 mm from the midline. Most of the irradiation energy was delivered to 4 mm diameter volume of the brain and peripheral dose of such volume was 70 Gy. Next day CSF samples were obtained and repeated cisternal punctures were done once a week following gamma therapy.

The second group (n:2) did not get any radiation therapy but CSF samples were taken at the same time as from the treated rabbits for control LPA assessment.

LPA Bio-assay

A blinded investigator determined concentrations of LPA in the CSF samples using the Xenopus laevis oocyte bioassay.

LPA activates an endogenous G protein-coupled receptor in these cells, which leads to activation of phospholipase C and generation of inositol trisphosphate (IP3) (Figure 1 b). The IP3 acts on its receptor on intracellular Ca^{2+} stores, and induces an increase in intracellular Ca^{2+} concentration. This change in Ca^{2+} concentration can be measured conveniently using the endogenously present Ca^{2+}-activated Cl^- channel as a reporter. The amplitude of the resulting Ca^{2+}-activated Cl^- current ($I_{Cl(Ca)}$) is integrated, and the



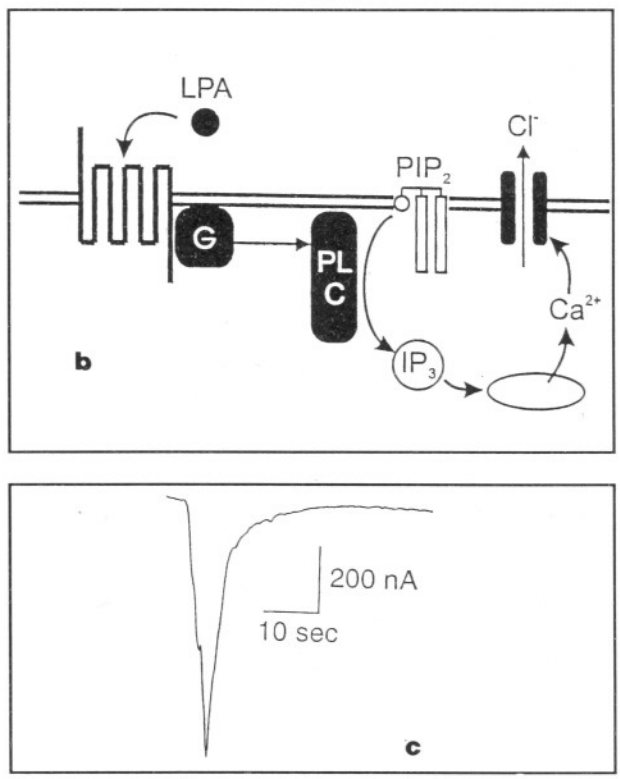


Figure 1, a) Molecular structure of lysophosphatidic acid, b) LPA activates an endogenous G-protein coupled receptor in oocytes which leads to activation of phospholipase C and generation of inositol triphosphate, c) a typical current induced by LPA application.

resulting charge movement in microCoulombs (μC) is proportional to the LPA concentration in the sample. This measure is specific for LPA, as no other G protein coupled receptors we know to be expressed in the oocyte. Figure 1 c shows a typical current induced by LPA application.

RESULTS

General Observations

Weekly repeated cisternal puncture and CSF withdrawal continued for 4 months. During that time rabbits were observed carefully for any kind of injury due to puncture and side effects of radiation. The physiological parameters (heart rate, blood pressure, blood gas analysis) of both treated and control groups were completely in normal range but in the first week after the gamma treatment, rabbits were fatigued, drowsy and decreased appetite. This improved after the first week and the animals remained neurologically and physiologically normal.

LPA-Bioassay

Oocyte responses to CSF samples from control animals (n: 2) were less then 1.5 microCoulombs over the 96 h of the experiment (Figure 2). This indicates

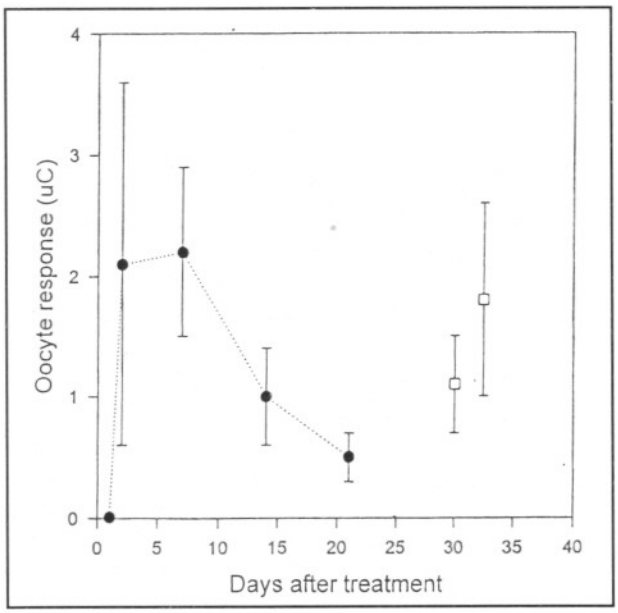


Figure 2 . Lysophosphatidate levels increased in 48 hours after the gamma-radiation.

that multiple cisternal punctures for CSF sampling did not influence CSF LPA concentrations. In contrast, responses to CSF from the radiation-treated group were increased more than 2 microCoulombs, 12-24 hours after performing the radiation and gradually decreased in the following days (Figure 2).

DISCUSSION

The earliest effect after high radiation therapy is seen in a few hours as erythema due to dilatation of capillaries. The sizes of the gaps between the endothelial cells increases as the radiation dose is increased (12). Secretion of chemotactic factors leads to adherence of PMN to endothelial cell layers (5).

Intermediate changes are observed at 2-6 months. Thrombi, consisting of fibrin and platelets, often obliterate capillaries. Leakage of plasma protein into the walls of the arterioles may lead to hyalinization (9). LPA enhances binding of fibrinogen to glycoprotein IIb-IIIa which causes platelet aggregation thus forming plugs in the vessels (16).

Late changes are observed at 6 months and later. Endothelial proliferation obliterates capillaries, the capillary lumen is replaced by collagen, the basement membrane is thickened, and telangiectatic vessels can be seen. Arteries became tortuous, with regions of dilatation and constriction. Increased amounts of acellular material, including collagen, are deposited in the intima and media, cell depletion leads to insudation of vessel walls by fibrin, which is then replaced by collagen resulting in vessel walls thickening and luminal narrowing (13, 14). Brouty-Boye et al. observed the production of large amounts of oncofetal fibronectin in human breast fibroblasts isolated from post-radiation fibrotic tissues (2). LPA is one of the most potent mitogens for fibroblasts known. Binding of fibronectin to cells is enhanced by LPA in a dose-dependent manner (3, 16). LPA is produced by platelets, endothelial cells, leukocytes, and injured fibroblasts (6, 7, 10). LPA might play a major role in post-radiation fibrosis. In our study we observed an early LPA elevation 24-48 hours after radiation which subsided progressively.

There is an apparent microvascular injury due to the increase in vascular permeability which leads to impairment in the microcirculation, vaso-occlusion and complete tissue destruction (17). Fibrinoid necrosis and thrombosis are the endstage lesions (4). Increases in vascular permeability lead to edema and deposition of fibrin in the interstitial spaces and blood vessel walls, which is later replaced by collagen fibers, resulting in the fibrosis (15).

This mechanism is controlled by a complicated signaling and mediator system. The effect of cytokines on lymphocytes, fibroblasts, and tumor

cells is by binding to cell surface receptors to initiate signaling pathways to induce fibroblast proliferation, recruitment of inflammatory cells, and activation of endothelial cells (8).

Radiation-induced fibrosis is poorly understood. Most characteristic features in fibrotic lesions are infiltrating inflammatory cells, atypical fibroblasts, abundant deposition of extracellular matrix components which were caused by continuous signals originating mainly from cytokines and growth factors (1).

Radiation-induced platelet aggregation and fibrosis are the main events and are probably controlled by numerous mediators and signaling systems. Adhesion molecules and phospholipids might play a major role in the effects of radiation both in the early and late phases.

In this pilot study we obtained significant early elevation of LPA compared with the control group which proves its prominent role in radiation injury. In the follow-up period of 3 months we did not observe another elevation of LPA levels which might indicate late radiation injury. The future aspects of this study is to use LPA bioassay for early radiation injury determination in cerebrospinal fluid from patients after radiation therapy in suspected cases.

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