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SCIENCE DIRECT®
Microbes and Infection 7 (2005) 391–398
Microbes and Infection
www.elsevier.com/locate/micinf

Original article

A plant polyphenol-rich extract restores the suppressed functions of phagocytes in influenza virus-infected mice

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Received 12 May 2004; accepted 16 November 2004

Available online 16 March 2005

Abstract

Influenza infection was induced in white ICR mice by intranasal (i.n.) inoculation of the virus A/Aichi/2/68 (H3N2). The number, migration and phagocyte indices of alveolar and peritoneal macrophages (pMØ) and of blood polymorphonuclear leukocytes (PMNs), as well as the inhibition of the PMN adherence in the presence of a specific antigen were followed for 9 days after infection. The effect of the i.n. application of a polyphenol-rich extract, designated as polyphenolic complex (PC), isolated from the medicinal plant *Geranium sanguineum* L., on the inspected immune parameters was studied in parallel with the virological parameters of the infection, e.g. rate of mortality, mean survival time (MST), infectious lung virus titre and consolidation of the lungs. It was found that the application of PC induced a continuous 2- to 2.5-fold rise in the number of both peritoneal and alveolar macrophages (aMØ) in the infected and healthy controls. The migration of both peritoneal and aMØ increased 1.5- to 2-fold in the group of infected PC-treated animals and four to fivefold in the control group, the maximum being on day 9. PC stimulated phagocyte activities of blood PMNs in both infected and healthy mice. The leukocyte adherence inhibition (LAI) index decreased in the infected and PC-treated animals. The restoration of the suppressed functions of phagocytes in influenza virus-infected mice (VIM) was consistent with a prolongation of MST and reduction in mortality rate, infectious virus titre and lung consolidation. The immunoenhancing properties of PC apparently contribute to the overall protective effect of the plant preparation in the lethal murine experimental influenza A/Aichi infection.

Keywords: Influenza virus infection; Plant polyphenols; Macrophages; Phagocytosis

1. Introduction

Influenza infection is a common respiratory disease, causing high morbidity in the general population, a high mortality in at-risk groups, as well as considerable costs of hospitalisation and treatment and losses in productivity [1]. Despite recent success in the development of new antiviral agents, the need for effective therapies for influenza virus infection continues to exist. The search for viral inhibitors of plant origin is a promising approach in the development of new therapeutic agents. A large number of extracts and pure substances have been tested, and a selective antiviral effect has been proved for some of them (for review see [2,3]). Often, the virus inhibitory effect has been attributed to the presence of polyphenol compounds [2,4].

Earlier research proved that a semi-standardised polyphenol-rich extract, designated as polyphenolic complex (PC), obtained from the medicinal plant *Geranium sanguineum* L., inhibited the reproduction of influenza viruses type A and B in vitro and in ovo and protected mice from mortality in the experimental influenza virus infection [5]. The anti-influenza virus effect of the preparation in cell cultures was specific and selective [6]. However, investigations showed that the in vitro virus inhibitory activity was fairly modest, and this was in contrast with the significant protec-

Abbreviations: aMØ, alveolar macrophages; CH, control healthy; cRPMI, complete RPMI-1640 medium; i.n., intranasal; LAI, leukocyte adherence inhibition; PC, polyphenolic complex; PBS, phosphate-buffered saline; pMØ, peritoneal macrophages; PMNs, polymorphonuclear leukocytes; PI, phagocyte index; p.i., post infection; SS, statistical significance; $TCID_{50}$, 50% tissue culture infectious doses; VC, virus control; VIM, virus-infected mice.

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doi:10.1016/j.micinf.2004.11.013

tion in vivo. Thus, the therapeutic effect of PC needed explanation. We presumed that it might be attributed to a combination of more than one biological activity—selective antiviral effect, non-selective immunomodulating action and some non-specific biological and pharmacological interactions known in natural polyphenols, such as protein binding, radical scavenging and antioxidant activities. Following this line of investigation, we established an in vitro stimulating effect of PC on the phagocytic activity of murine polymorphonuclear leukocytes (PMNs) and peritoneal macrophages (pMØ), as well as a beneficial effect of the extract on spontaneous NO production [7].

The aim of the present work was to investigate the effect of the plant preparation on the number and functions of macrophages and PMNs from PC-treated healthy and influenza virus-infected mice (VIM) and in this way to provide evidence for the implication of its immunomodulatory potential for the overall protective effect in lethal murine experimental influenza A/Aichi infection.

2. Materials and methods

2.1. Plant material

G. sanguineum L. (Geraniaceae) has been introduced into the experimental field of the Institute of Botany, Bulgarian Academy of Science, Sofia and a voucher specimen was deposited in the herbarium of the Institute (SOM 5/86). Ground air-dried aerial roots, collected during the flowering period, were defatted with petroleum ether and treated with EtOH to fully extract the polyphenol compounds. The extract was lyophilised (yield 16%); the obtained preparation (PC) was a dark red powder, odorless, soluble in water. The extract was prepared and kindly provided by Dr. S. Ivancheva, Institute of Botany, Bulgarian Academy of Sciences. The polyphenol content of PC was controlled by thin layer chromatography and by quantitative determination of tannins, flavonoids and catechins [8].

2.2. Mice

Male and female (16–18 g), inbred ICR mice were obtained from the Experimental Animal Station, BAS, Sofia. They were quarantined 24 h prior to use and maintained on standard laboratory chow and tap water ad libitum for the duration of the studies. Experiments with animals were indispensable in investigations concerning the treatment of influenza infection and the evaluation of immunological parameters. The number of experimental animals was reduced as much as possible, depending on statistical significance (SS). Refinement of the tests with animals was achieved by careful planning of multifactor experiments. The animals were bred under standard conditions, accepted by the Bulgarian Veterinary Health Service. Specialised personnel took care of their welfare.

2.3. Viral infection

The infection was induced under light ether anaesthesia by intranasal (i.n.) inoculation of A/Aichi/2/68 (H3N2) (A/Aichi), adapted to mouse lungs, with infectious titre 10^7 50% tissue culture infectious doses per ml ($TCID_{50}$ ml^{-1}) and haemagglutination titre 4096. This virus causes haemorrhagic pneumonia in mice. The strain is from the collection of the Institute of Microbiology, Bulgarian Academy of Science, Sofia. To induce a lethal infection, mice were challenged with 10 LD_{50} (50% lethal dose) of the virus in a volume of 0.05 ml phosphate-buffered saline (PBS)/mouse. The virus was maintained by passages in mouse lungs and fertile eggs, and virus stock was kept at –80 °C.

2.4. Experimental design

Mice were separated in four experimental groups: *group 1*—influenza virus-infected and PC-treated (VC + PC); *group 2*—influenza virus-infected and PBS-treated—virus control (VC); *group 3*—mock-infected and PC-treated (PC); *group 4*—mock-infected and PBS-treated—control healthy (CH). PC was inoculated by i.n. instillation 3 h before infection at a dose of 10 mg kg^{-1} in 0.05 ml PBS under light ether anesthesia. Two additional groups of 10 animals each (VC and VC + PC) were observed daily for 14 days after viral challenge. After the end of the experiments, surviving mice were sacrificed under ether anesthesia.

2.5. Peritoneal macrophages (pMØ)

pMØ were collected from at least six mice on days 2, 6 and 9 post infection (p.i.) by three washings of peritoneal cavities with 5 ml cold Hank's solution. The phenotype of the cells was determined by light microscopy of cell populations immediately after washings of peritoneal cavities. The cell suspensions were centrifuged at 1200 × *g* at 0 °C and re-suspended in RPMI-1640 (Fluca) medium, supplemented with 10% fetal calf serum, 100 IU ml^{-1} penicillin, 100 μg ml^{-1} streptomycin, 2 mM L-glutamine and 2 mM HEPES, and adjusted to different concentrations for the tests described below.

2.6. Alveolar macrophages (aMØ)

The aMØ were collected on the same days p.i. by five washings of tracheal–broncho–alveolar cavity of at least six mice with 1 ml cold Hanks' balanced salt solution (HBSS) according to Holt [9]. The phenotype of the cells was determined by light microscopy of cell populations immediately after washings of broncho–alveolar cavities. The cells were washed and re-suspended in complete RPMI-1640 medium (cRPMI) in concentrations necessary for the assays described below.

2.7. Migration of aMØ and pMØ

Migration of both kinds of macrophages was estimated according to Leu et al. [10]. Briefly, macrophages in concen-