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Fever and inflammatory cytokine response in rats injected subcutaneously with viral double-stranded RNA analog, polyinosinic:polycytidylic acid (Poly-I:C)

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ABSTRACT

The objective of this study was to determine whether viral double-stranded RNA analog, polyinosinic:polycytidylic acid (Poly-I:C), is pyrogenic in rats when administered subcutaneously, thus determining whether rats can serve as an experimental model to investigate the regulation of local and systemic inflammatory responses to a Toll-like receptor(TLR)-3 agonist. Rats implanted intraperitoneally with temperature-sensitive radiotelemeters were injected subcutaneously in the skin of the tail with saline, 100 μg kg^{-1} Poly-I:C, or 1000 μg kg^{-1} Poly-I:C. In a separate group of rats blood and tail-skin samples were taken 3 and 24 h after injections to assess changes in local and systemic inflammatory cytokine release. Injection of 1000 μg kg^{-1} Poly-I:C induced an acute fever, which lasted for approximately seven hours. The fever was associated with elevated local tissue concentrations of interleukin(IL)-1β, IL-6, and cytokine-induced neutrophil chemoattractant (CINC)-1, and elevated plasma concentration of CINC-1. Cytokine concentrations had returned to background concentrations by 24 h after the injection. Injection of 100 μg kg^{-1} Poly-I:C failed to induce fever, but did induce significant increases in the local tissue, but not circulating, concentration of CINC-1 within 3 h of the injection. Tissue CINC-1 concentrations had returned to background concentrations by 24 h after the injection. In conclusion, when administered at great enough concentrations, subcutaneously injected Poly-I:C is pyretic in rats, and the pyresis is associated with elevated concentrations of local and systemic inflammatory mediators. Thus the rat can be used to study signaling pathways induced by localized, subcutaneous administration of this TLR-3 agonist that mimics viral infection.

1. Introduction

The synthetic double-stranded RNA analog polyinosinic:polycytidylic acid (Poly-I:C) is a useful tool for studying signaling pathways employed by the innate immune system in response to stimulation of Toll-like receptor(TLR)-3 during viral infections (Akira et al., 2006). Systemic and intracerebroventricular injection of Poly-I:C induces robust and reproducible fever in a variety of laboratory animals, including rabbits (Kimura et al., 1994; Krueger et al., 1988; Rotondo et al., 1987, 1988; Soszynski et al., 1991), mice (Hiramoto et al., 1991; Homan et al., 1972), rats (Chuang et al., 1990; Fortier et al., 2004; Galic et al., 2009; Matsumura et al., 2007), and guinea pigs (Cooper et al., 1988; Homan et al., 1972; Voss et al., 2007, 2006). Yet, the response to localized inoculation with the pyrogen is less pronounced. When Voss et al. (2006) characterized the pyrogenic and inflammatory response to subcutaneous or intramuscular inoculations with Poly-I:C in guinea pigs they found only mild pyrogenic and cytokine-inducing responses to intramuscularly administered Poly-I:C, and no febrile or cytokine response to Poly-I:C injected into a Teflon chamber that was implanted subcutaneously (Voss et al., 2006).

Route-specific variation in fever and cytokine response to pyrogens also has been reported for bacterial pyrogens. For example, the fever and cytokine response to localized peripheral injection of Gram-negative bacterial pyrogen, lipopolysaccharide (LPS), in guinea pigs is reduced compared to when the same dose is injected systemically, but unlike Poly-I:C administration, subcutaneous injection of LPS still induced fever and increased local and systemic cytokine release (Ross et al., 2000; Rummel et al., 2005, 2004). In rabbits, the fevers induced by intramuscular and subcutaneous administration of LPS were significantly smaller in magnitude and duration than when the pyrogen was injected intravenously, and for

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the Gram-positive bacterial pyrogen, *Staphylococcus aureus* cell walls, the intramuscular and subcutaneous routes of administration completely failed to induce fever, while intravenous administration induced robust fever (Cartmell et al., 2002). Thus, within a species of experimental animal, pyrogenicity is influenced by the type of pyrogen used and by the route of administration.

Assessing the fever and inflammatory response to an antigen when administered via different routes provides valuable information on the various signaling pathways employed by the innate immune system in response to an antigen (Cartmell et al., 2000; Goldbach et al., 1997; Ross et al., 2000; Rummel et al., 2004), so failure to induce a fever in guinea pigs with subcutaneous Poly-I:C limits the scope of experimental approaches that can be employed to study Poly-I:C, and hence Toll-like receptor-3, signaling pathways. Like in the guinea pig, Poly-I:C is pyrogenic when injected systemically in rats (Fortier et al., 2004; Matsumura et al., 2007), but its pyrogenicity when administered by a subcutaneous route in rats has not been tested. Thus, our aim was to determine whether Poly-I:C is pyrogenic in rats when administered subcutaneously, thus determining whether rats can serve as an experimental model to investigate the regulation of local and systemic inflammatory responses to a TLR-3 receptor agonist, and the contribution signaling pathways induced by the agonist make to fever. We report that subcutaneous inoculation of rats with a high dose of Poly-I:C produces a febrile response, and the presence of a fever is associated with local and systemic inflammatory cytokine release. The model we describe demonstrates that subcutaneous injection of Poly-I:C in rats can serve as an experimental tool to investigate the regulation of local and systemic inflammatory responses to a TLR-3 receptor agonist, and the contribution these inflammatory changes make to fever during simulated viral infection compared to bacterial infection.

2. Materials and methods

2.1. Animals

We used 70 male Sprague-Dawley rats that weighed 230–250 g at the start of the experiments. Rats were housed individually in cages at an ambient temperature of ~23 °C and on a 12:12 h light/dark cycle (lights on at 07:00). Food and water were available *ad libitum*. All the experimental procedures were cleared by the Animal Ethics Screening Committee of the University of the Witwatersrand (Clearance no. 04/45/03).

2.2. Drug preparations and injection

Solutions of Poly-I:C (Sigma-Aldrich, Kempton Park, South Africa) were prepared by dissolving appropriate amounts of the immunogen in sterile, pyrogen-free saline, such that 100 μg kg^{-1} and 1000 μg kg^{-1} Poly-I:C could be administered subcutaneously in a 100 μl volume. All injections were administered midway down the length of the tail, on the dorsal surface, in rats temporarily restrained in transparent plastic restrainers. We injected the pyrogen into the rat tail because the tight skin on the tail provides a convenient location to inject a small volume of solution without the solution rapidly re-distributing in the subcutaneous space, thus helping to ensure a high localized concentration of the injected substance. Immediately after injection, rats were returned to their cages.

2.3. Cytokine assays

We measured concentrations of IL-6 and CINC-1 using a rat-specific enzyme-linked immunosorbent assay (ELISA; National Institute of Biological Standards and Control, UK), as previously described (Safieh-Garabedian et al., 1995). Briefly, 100 μl of recombinant rat cytokine standard or sample was added to each well of microtitre plates, which had been pre-coated overnight with sheep anti-rat polyclonal antibody, and incubated overnight at 4 °C. The following day, sheep anti-rat biotinylated polyclonal antibodies were added (1:2000 dilution), and the samples incubated at room temperature (~22 °C) for one hour. Then, 100 μl of streptavidin-poly-horseradish peroxidase (1:10,000 dilution; Euroimmun, Cape Town, South Africa) was added to each well at room temperature, and after 30 min the plates were washed and the color reagent *o*-Phenylenediamine dihydrochloride (40 μg, 100 μl $well^{-1}$; Sigma-Aldrich, Kempton Park, South Africa) added. The reaction was terminated with H_2SO_4 (1 M, 150 μl $well^{-1}$) and the optical density measured at 490 nm. Each sample was measured in duplicate. Cytokine concentrations were expressed as pg ml^{-1} for assays of plasma, and pg mg^{-1} for tail tissue. IL-6 and CINC-1 cytokines were analyzed using the appropriate sheep anti-rat polyclonal and biotinylated antibodies for each cytokine, and there was no cross-reactivity between the cytokine ELISAs.

IL-1β was assayed using a commercially available rat-specific IL-1β ELISA kit (R&D Systems, Minneapolis, MN, USA). The assay was completed according to the manufacturer's instructions.

2.4. Measurement of body temperature

Rats anaesthetized with ketamine (80 mg kg^{-1}) and xylazine (40 mg kg^{-1}) had sterile, wax-coated temperature-sensitive radiotelemeters (VM-FH; Mini-Mitter, Bend, OR, USA) implanted intraperitoneally seven days before the start of experimentation. Before implantation, telemeters were calibrated in a water bath against a high accuracy quartz-crystal thermometer to an accuracy of 0.1 °C (Quat 100; Heraeus, Hanau, Germany). The output frequency of each telemeter was monitored by a receiver plate (RTA 500; Mini-Mitter, Bend, OR, USA) placed beneath each rat's cage, and transmitted via a peripheral processor (Datacol-3; Mini-Mitter, Bend, OR, USA) to a personal computer, where frequencies were converted to temperatures and recorded. Body temperature was recorded every five minutes throughout the experiment.

2.5. Experimental protocol: pyrogenic response to Poly-I:C

Two groups of 6 rats each were used to test the pyrogenicity of subcutaneously injected Poly-I:C in rats. Depending on their group, rats were injected subcutaneously either with saline and 100 μg kg^{-1} Poly-I:C, or saline and 1000 μg kg^{-1} Poly-I:C. Therefore, each rat was injected twice, once with saline and once with Poly-I:C (either the high or low dose), with the injections administered in random order, at least two weeks apart.

2.6. Experimental protocol: inflammatory response to Poly-I:C

In a separate series of experiments, using different rats to those used to test the pyrogenicity of Poly-I:C (*2.5. Experimental protocol: pyrogenic response to Poly I:C*), rats were injected subcutaneously either with saline ($n=12$), 100 μg kg^{-1} Poly-I:C ($n=12$), or 1000 μg kg^{-1} Poly-I:C ($n=12$). Three hours later six rats from each group were anesthetized with isoflurane and blood and tail tissue samples were taken for cytokine analysis and then killed by injection of pentobarbital sodium (Euthanase; Kyron Laboratories, Johannesburg, South Africa). Twenty-four hours after injection, the remaining six rats from each group were similarly anesthetised and blood and tail tissue samples taken for cytokine analysis.