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Acute cardio-respiratory effects in rats of PS4α, an antineoplastic peptidoglycan from *Mycobacterium vaccae*

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Abstract

PS4α is a high molecular weight peptidoglycan extracted from *Mycobacterium vaccae*, which has demonstrated considerable antineoplastic activity in-vivo without apparent toxicity. Available for testing in only small quantities, a sensitive in-vivo method for measuring pulse and breathing rates in cannulated rats was applied to this compound at doses of 5, 50 and 500 μg kg^{-1}. Various parameters (mean arterial pressure, maximum transpulmonary pressure, compliance, heart rate, minute volume, respiratory rate and tidal volume) were followed for up to 1 h and demonstrated no significant deviation in the baseline values obtained before injection. This compound at doses up to 500 μg kg^{-1} had no apparent acute toxicity in rats, but chronic effects at this and higher doses have to be determined by more conventional toxicological methods before proceeding to evaluate PS4α as an antineoplastic agent.

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Introduction

Mycobacteria are a group of aerobic non-motile non-sporulating acid-fast bacteria that encompasses some of the world's most serious pathogens including tuberculosis and leprosy. With the exception of *M. leprae*, which has yet to be cultivated in-vitro, mycobacteria can be assigned into two broad groups based primarily on their growth rates (Wayne & Kubica 1986). The slow growers, including *M. tuberculosis* and *M. bovis*, may need several weeks to form grossly visible colonies on solid media whereas fast growing strains, under optimal nutrient and temperature conditions, will produce colonies in only a few days. Fast growing mycobacteria are mainly saprophytes and have received less attention than the pathogenic slow growers.

Based on a literature review, Pearl (1929) noted an inverse relationship between cancer and tuberculosis, patients with tuberculosis rarely having cancer. This appears to be due to a direct effect of the invading organism on the immune system. Attenuated *M. bovis* (bacillus Calmette Guérin, BCG vaccine) is utilized clinically as an immunostimulant in the treatment of human bladder cancer. Antineoplastic materials have been isolated from the human Ayoma substrain of *M. tuberculosis* (Suzuki et al 1986) and from the Tice and the Connaught substrains of BCG vaccine (Lou et al 1994; Garrido et al 1997). Antitumour activity has also been reported for hot water extracts from *M. vaccae* (Groves et al 1995) and *M. phlei* (unpublished).

More recently the structure of material from *M. vaccae*, PS4A, extracted with hot water from cell cultures, has been identified as consisting of a peptidal backbone

to which are attached poly (glucose) and poly (3-O-methylmannan) moieties (Tian et al 2000). Only fractions with molecular weights in excess of 50 kDa were biologically active against a murine S180 sarcoma model (Tian & Groves 1999). Although antibodies to PS4A have been raised in rabbits (Tian & Groves 1999), no evidence of toxicity was noted during any of the animal testing but conventional toxicity testing protocols were not feasible owing to the difficulty of preparing sufficient material on a laboratory scale. An improved extraction procedure similar to that developed for BCG by Garrido et al (1997) was applied to *M. vaccae*. In this process the organisms were extracted at ambient room temperature in 8 M urea, resulting in an improved yield of a higher molecular weight material, PS4α, which demonstrated significantly improved antineoplastic activity in the murine S180 sarcoma model. The measured molecular weight of PS4α covered the range 500 kDa to 20 MDa, which may be due in part to hydrophobic interactions similar to those observed for PS1 (Farrugia et al 1998). Antineoplastic activity to at least 1 pg/mouse (50 pg kg^{-1}) has been measured (Tian & Groves 1999). Accordingly, following a procedure based on that recently reported by Villar et al (1998) for PS1, we have tested PS4α for acute toxicity. This method, described in detail by Dosaka-Akita et al (1993), recorded the response in cannulated rats to blood gases and other parameters such as pulse and respiration rates as the drug was administered intravenously. As noted by Villar et al (1998), this type of procedure appeared to be suitable for preliminary toxicity testing when only modest amounts of drug were available for evaluation.

Materials and Methods

Materials

PS4α was isolated in this laboratory from *M. vaccae* cultures by the procedure described by Garrido et al (1997). Briefly, organisms from the National Collection of Type Cultures, Washington, D.C., were cultured on solid media for seven days and used to inoculate liquid media followed by further incubation at 37°C for seven days. Cells were collected and washed with phosphate-buffered saline by centrifugation and lyophilized. The lyophilized cultures were then dispersed with stirring in 8 M urea at ambient room temperature for 24 h, filtered (0.2-μm membrane) and the filtrate dialysed overnight against distilled water using a 3.5 kDa molecular weight cut-off membrane. The retentate was then passed through a 10 kDa molecular weight cut-off ultrafilter (Amicon), the retentate frozen at −20°C and lyophilized to yield a white powder, PS4α.

Animals

Male Sprague-Dawley rats (300–350 g; PanLab, Barcelona, Spain) were housed in viral antigen-free (VAF) rooms in the University of Valencia Experimental Research Laboratories. The rats were maintained on a freely available standard laboratory diet with free access to water for eight days after arrival, according to VAF protocols and University of Valencia Care Committee guidelines. The rats were housed at controlled temperature (23 ± 1°C), humidity (50 ± 10%) and light (06.00–18.00 h).

Surgery

The rats were anaesthetized with 25% urethane and surgically implanted with a polyethylene catheter (PE 60, Silastic Brand Medical Grade Tubing, Dow Corning Corporation, Midland, MI; 0.51 mm i.d., 0.94 mm o.d.) into the carotid artery. Another cannula was implanted into the jugular vein according to the procedure described by Bhargava et al (1998). A tracheotomy was carried out to maintain the animal on artificial ventilation.

Instrumentation

The instrumentation differed from that described by Villar et al (1998) in that a simultaneous recording of transpulmonary pressure, air flow rates and derivatives and tidal volume were made using a Lung Function Recording System (Mumed Systems Ltd, London, UK). The technique for measuring airway resistance and dynamic lung compliance was first described by Neergaard & Wirtz (1927) and adapted for small animals by Amdur & Mead (1958). Both methods rely upon the comparison of transpulmonary pressure, air flow rate and its derivatives, and tidal volume at particular points in the respiratory cycle, and can be used in spontaneous and anaesthetized ventilated animals. The arterial blood pressure signal was used to calculate systolic, diastolic and mean arterial pressure. Compliance, intrapulmonary pressure, minute volume and tidal volume were measured for 5 min before,

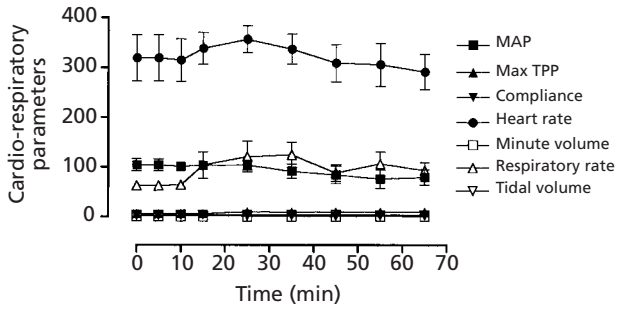

Figure 1 Cardio-respiratory parameters measured before and during 60 min after the intravenous injection of 500 μg kg^{-1} PS4α at 5 min of baseline recording. The cardio-respiratory parameters were: mean arterial pressure, MAP (mmHg); maximum transpulmonary pressure, Max TPP (cm H_2O); compliance (mL (cm H_2O)$^{-1}$); heart rate (beats min^{-1}); minute volume (mL min^{-1}); respiratory rate (breaths min^{-1}); and tidal volume (mL).

during and after intravenous administration of 5, 50 or 500 μg kg^{-1} PS4α in saline.

Results and Discussion

Data are summarized for the highest dose, 500 μg kg^{-1}, in Figure 1. All the parameters at the three doses of drug tested did not demonstrate any significant change. It may be concluded that there was no indication of an acute effect being produced by PS4α on the cardio-respiratory system of the rat at a dose at least tenfold higher than that required for antineoplastic activity. However, substantially more acute toxicity studies are required for PS4α in addition to chronic toxicity evaluation over a much longer period of time and in more than one animal species. Nevertheless, although slightly different from the procedure described by Villar et al (1998), the method described here would also appear to be a valuable initial screening technique when the availability of test material is limited.

References

Amdur, M. O., Mead, J. (1958) Mechanism of respiration in unanaesthetized guinea-pigs. *Am. J. Physiol.* **192**: 364–368

Bhargava, H. N., Villar, V. M., Cortijo, J., Morcillo, E. J. (1998) Analgesic and thermic effects, and cerebrospinal fluid and plasma pharmacokinetics, of intracerebroventricularly administered morphine in normal and sensitized rats. *J. Pharm. Pharmacol.* **50**: 197–203

Dosaka-Akita, K., Tortella, F. C., Holaday, J. W., Long, J. B. (1993) The kappa opioid agonist U-50,488H antagonizes respiratory effects of mu opioid receptor agonists in conscious rats. *J. Pharmacol. Exp. Ther.* **264**: 631–637

Farrugia, I. V., Dadey, E. J., Ashline, K., Groves, M. J. (1998) Comparative measurement of the molecular weight of an antineoplastic glucan from BCG vaccine. *J. Pharm. Pharmacol.* **50**: 1205–1211

Garrido, J. L., Klegerman, M. E., Reyes, H. R., Groves, M. J. (1997) Antineoplastic glycans in the cellular integument of *Mycobacterium bovis*, BCG vaccine, connaught substrain. *Cytobios* **90**: 47–65

Groves, M. J., Klegerman, M. E., Ciftci, K., Tian, X. X. (1995) Isolation, characterization and formulation of an antineoplastic glycan from cultures of *Mycobacterium vaccae*. *J. Pharm. Pharmacol.* **47**(Suppl. B): 1082

Lou, Y., Klegerman, M. E., Muhammad, A. S., Dai, X., Groves, M. J. (1994) Initial characterization of an antineoplastic, polysaccharide-rich extract of *Mycobacterium bovis* BCG, Tice substrain. *Anticancer Res.* **14**: 1469–1476

Neegaard, K. V., Wirtz, K. (1927) Die messung der Stromumgswiderstande in der Atemwege des Menschen, insbesondere bei Asthma und Emphysem. *Z. Klin. Med.* **105**: 51–59

Pearl, R. (1929) Cancer and tuberculosis. *Am. J. Hyg.* **9**: 97–159

Suzuki, F., Brutkiewicz, R. B., Pollard, R. B. (1986) Importance of Lyt 1+ T-cells in antitumor activity of an immunomodulator, SSM, extracted from human-type tubercle bacilli. *J. Natl. Cancer. Inst.* **77**: 441–447

Tian, X. X., Groves, M. J. (1999) Formulation and Biological activity of antineoplastic proteoglycans derived from Mycobacterium vaccae in chitosan nanoparticles. *J. Pharm. Pharmacol.* **51**: 151–157

Villar, V. M., Groves, M. J., Tian, X. X., Reed, A., Morcillo, E. J., Cortijo, J., Buehler, P., Gulati, A. (1998) Acute cardio-respiratory effects of PS1, an antineoplastic glucan, in rats. *Pharm. Pharmacol. Commun.* **4**: 447–449

Wayne, L. G., Kubica, G. P. (1986) Genus Mycobacterium. In: Sneath, P. H. A., Mair, N. S., Sharpe, M. E., Holt, J. G. (eds) *Bergey's Manual of Systematic Bacteriology*. Williams and Wilkins, Baltimore, MD, pp 1436–1457