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Increased Susceptibility of Aged Rats to Haemorrhage and Intravascular Hypercoagulation Following Endotoxin Administered in a Generalized Shwartzman Regime

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Summary

Ageing rats are known to have an increased incidence of myocardial fibrosis and dyspnoea caused by pulmonary intravascular coagulation. In order to determine whether endotoxin can be responsible for such responses in ageing rats we have exposed rats of differing ages (2 months, 16 months and 24 months) to single or repeated (two doses 24 h apart; generalized Shwartzman regime) intravenous doses of endotoxin (*E. coli* 0111 B4). Only the 2-year-old rats reacted adversely. Two doses of endotoxin produced death, with focal myocardial necrosis, haemorrhage and pulmonary and hepatic intravascular coagulation. The increased susceptibility of aged rats to the toxic effects of endotoxin explains some of the changes found in the tissues of old rats. The sporadic nature of both cardiac failure and dyspnoea as a cause of morbidity and mortality in ageing rats may be related to the need for two endotoxin episodes in a period of 24 h to provoke a generalized Shwartzman reaction, an occurrence likely to be relatively uncommon under natural conditions.

Introduction

Chronic glomerulonephropathy, polyarteritis nodosa, chronic myocarditis, radiculoneuropathy and skeletal muscle degeneration are the major non-neoplastic lesions found in ageing rats (Anver and Cohen, 1979; Anver, Cohen and Lattuada, 1982; Berg, 1967), but dyspnoea as a result of disseminated intravascular coagulation (DIC) has recently been found to be a major cause of morbidity (Carthew, Aldred, Hill, Riley and Edwards, 1989). The DIC was attributed to the systemic procoagulant effect of endotoxin lipopolysaccharide (LPS) released into the blood stream from pathogenic bacilli invading the epithelium of the caecum. That endotoxin LPS, rather than septicaemia, was responsible for the DIC seemed reasonable because of the failure to culture or to identify histologically any bacteria in the blood or organs. In an effort to reproduce this syndrome and determine whether endotoxin LPS is responsible, we exposed rats of differing ages to intravenous endotoxin whilst blocking the fibrinolytic pathway with AMCA (trans-4-amino-ethylcyclohexane carboxylic acid) (Gerdin, Diffang and Saldeen, 1981). This allowed any fibrin formed to persist for histological demonstration at necropsy and made possible a qualita-

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tive assessment of the relative amounts of fibrin deposited in various organs, revealing whether there was an increase in the procoagulating response of rats to endotoxin with age. It also had the effect of removing from our experimental system the variable of decreased fibrinolytic activity occurring naturally in older animals.

It is possible that the generalized Shwartzman phenomenon (Shwartzman, 1928) contributes to systemic intravascular coagulation with ageing. The generalized Schwartzman phenomenon is an experimentally induced increased sensitivity of animals to the toxicity of endotoxins, owing to the intravenous administration of two doses of endotoxin LPS 24 h apart (Goto, Baez and Orkin, 1981). This causes systemic procoagulation and haemorrhage, because the first dose of endotoxin saturates the reticuloendothelial system, which is responsible for the detoxification of the vascular system in animals following endotoxin exposure (Beeson, 1947). The saturation or subsequent reduced efficiency of the systemic phagocytic cells in absorbing and deotoxifying endotoxin LPS, results in the second dose administered 24 h later being less effectively dealt with and, therefore, more toxic (Wiznitzer, Better, Rachlin, Atkins, Frank and Fine, 1960). This results in an enhancement of the procoagulant effect of endotoxin LPS, resulting in occlusive thrombi in the glomerular capillaries of the kidneys in rabbits (Thomas and Good, 1952) causing subsequent necrosis (bilateral renal cortical necrosis). To determine whether there might be a naturally occurring form of the Shwartzman phenomenon involved in the myocardial and pulmonary lesions found commonly in ageing animals, we have used a generalized Shwartzman regime in rats of various ages in an attempt to reproduce similar lesions experimentally.

Materials and Methods

Source of Rats

Male LACP rats aged 2, 16 and 24 months were bred in the MRC Toxicology Unit's own breeding establishment. Twenty-four rats were used; they were serologically negative for antibodies to Sendai virus, pneumonia virus of mice, Kilham rat virus, the rat coronaviruses and were also examined for the presence of *Mycoplasma pulmonis* by culture of the nasopharynx.

Treatment of Rats with Endotoxin and an Inhibitor of Fibrinolysis

Prior to injection of endotoxin, all rats were treated with the fibrinolysis inhibitor AMCA (trans-4-aminoethyl-cyclohexane carboxylic acid; Sigma Chemical Co., Poole, Dorset, U.K.) as described previously (Gerdin *et al.*, 1981). Rats that were given two injections of endotoxin were not treated with AMCA before the first injection. Groups of rats 2, 16 and 24 months of age (four per group) were injected in the tail vein, under ether anaesthesia, with either a single dose of *Escherichia coli* (*E. coli*) endotoxin lipopolysaccharide (LPS) 0111 B5 (Sigma Chemical Co.) at a dose of 16 mg per kg body weight in phosphate buffered saline (PBS) or two doses of endotoxin 24 h apart. The injected solution was administered continuously over a period of 2 mins. Two and a half h after the administration of the single, or the second of the two doses of endotoxin, the rats were killed with carbon dioxide gas and the lungs were removed and distended with 10 per cent formol saline. Kidneys, livers, spleens, hearts and intestines were removed and immersion-fixed in 10 per cent formol saline. The tissues were paraffin-wax embedded and 5 μm sections were cut and stained with

haematoxylin and eosin (HE) and Martius Scarlet Blue for fibrin. Serial unstained sections were also cut to demonstrate fibrin by the immunoperoxidase technique as described previously (Carthew *et al.*, 1989). Groups of control rats (four per group) were treated by the same protocol, except that equivalent volumes of PBS were administered intravenously instead of endotoxin LPS.

Results

Clinical and Post-mortem Findings

All rats in groups (irrespective of age) given a single dose of endotoxin LPS after AMCA survived the exposures without any obvious clinical effects other than a temporary increase in respiratory rate after endotoxin injection. At necropsy the group of oldest rats (24 months) showed some lung congestion and one had petechiae on the intestine. The oldest rats in the group given a second intravenous exposure to LPS reacted more dramatically. One was found dead from the initial dose 24 h after exposure, but the three remaining animals, 24-months-old, given a second dose of intravenous LPS, became moribund with loss of motor function of the hind limbs and died between 1 and 2 h after the second dose. At necropsy all cases showed widespread petechiae with severe lung congestion. The rats in the other two groups of younger animals survived the second injections and had no gross abnormalities at necropsy.

Histopathological Examination of Treated Rats

Histological examination of the groups of oldest rats given two doses of endotoxin showed a consistent pattern of focal liver necrosis (Fig. 1) and focal myocardial necrosis (Fig. 2), especially of the papillary muscles of the left ventricles and ventricular septa, with subendocardial haemorrhage (Fig. 3). There was also a pronounced detachment of the vascular endothelium of the aorta in the old rats that died, with oedema in the subendothelial space (Fig. 4). These lesions were greater both in extent and severity than those in the other groups of rats. The 24-month old rat that died from a single injection of LPS was found to have pre-existing myocardial fibrosis indicative of impaired cardiac function, which increased its susceptibility to the experimental dose of LPS. The liver sinusoids in all of the 24-month-old rats given two doses of endotoxin were also congested as a result of fibrin deposition and infiltrating polymorphonuclear cells and there were also extensive fibrin deposits in the central veins of the livers (Fig. 5) as well as the capillaries in the glomeruli of the kidneys and the veins (Fig. 6) and capillaries throughout the lungs. The lungs also showed extensive congestion and haemorrhage (Fig. 7) with polymorphonuclear cells marginating both in the blood vessels and in the capillaries in the alveoli. Only the other group of 24-month-old rats given a single injection of endotoxin LPS had any other pathological reaction, with a less severe myocardial necrosis (mostly single cells) and no haemorrhage, but some fibrin deposits in the sinusoids of the liver and the pulmonary vasculature. The results are summarized in Table 1.

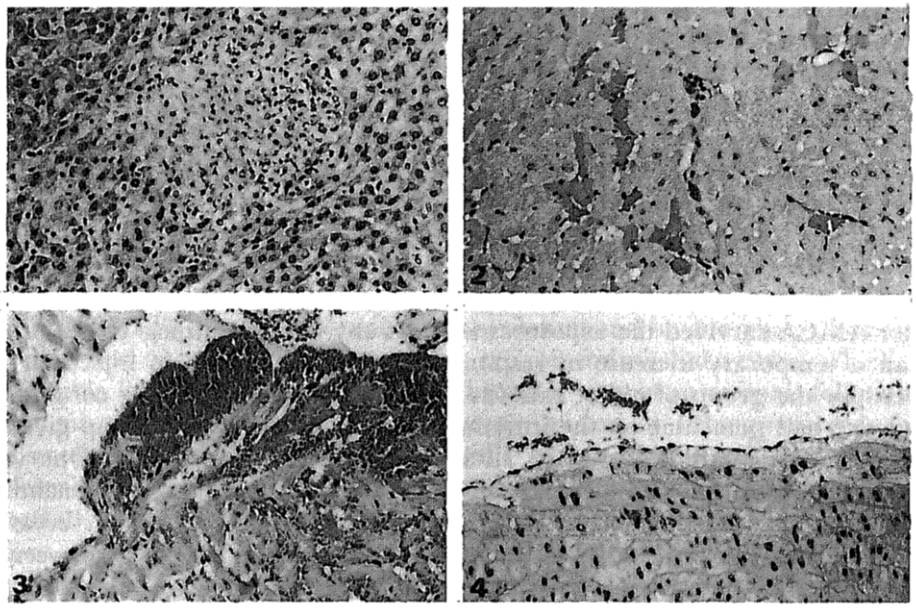

Fig. 1. Liver; focal hepatic necrosis caused by endotoxin. HE × 53.

Fig. 2. Heart; several foci of myocardial necrosis. HE × 53.

Fig. 3. Heart; subendocardial haemorrhage due to endotoxin. HE × 53.

Fig. 4. Aorta; endotoxin induced oedema of the subendothelial space. HE × 105.

Table 1
Pathological effects of single and repeat doses of intravenously administered endotoxin (*E. coli* 0111 B4) on rats 24 months old*

	Number of animals with evidence of haemorrhage		*Number of animals with evidence of necrotic lesions*		*Number of animals with evidence of intravascular coagulation*	
Age and treatment of rats	*Papillary muscle and ventricular septum*	*Lungs*	*Liver focal necrosis*	*Heart myocardial necrosis*	*Lungs*	*Liver*
24 month old, single dose of endotoxin	0/4	0/4	0/4	4/4	4/4	4/4
24 month old, two doses of endotoxin	4/4	4/4	4/4	4/4	4/4	4/4

* No lesions were seen in groups of four rats aged 2 or 16 months, regardless of whether they received 1 or 2 doses of endotoxin.

Discussion

The results obtained by intravenous administration of endotoxin LPS to old rats showed clearly that endotoxin can precipitate hypercoagulation, especially after a second dose. This resolves the problem of a causative agent responsible,

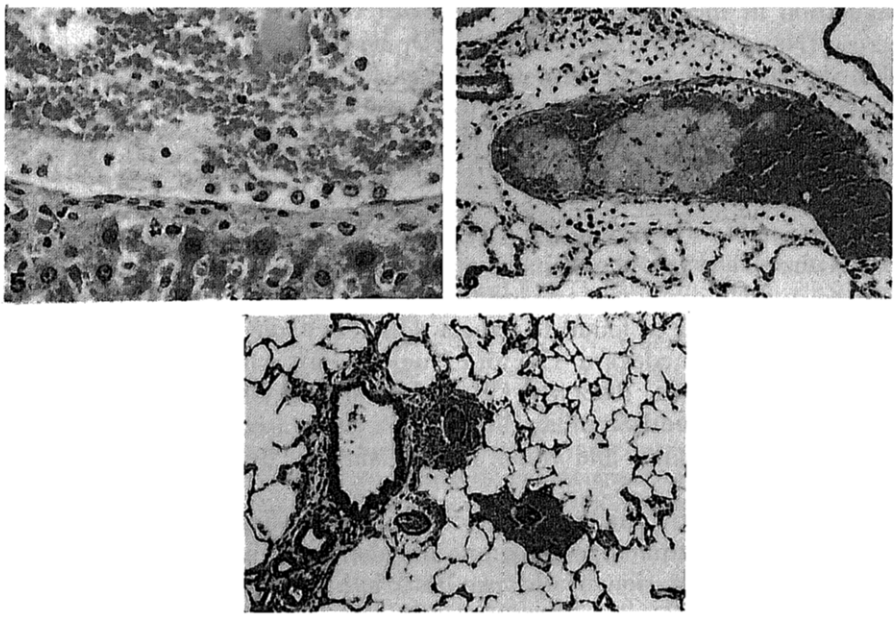

Fig. 5. Liver; margination of inflammatory cells with fibrin visible in a vein after endotoxin treatment. HE ×210.

Fig. 6. Lung; intravascular fibrin aggregates after repeated endotoxin treatment. HE ×53.

Fig. 7. Lung; perivascular haemorrhages induced by repeated endotoxin administration. HE ×23.

under natural conditions, for DIC in old rats (Carthew *et al.*, 1989) and is consistent with the enhanced sensitivity of old rats to endotoxin-induced pulmonary injury previously described (Durham, Horan, Brouwer, Barfelds and Knook, 1989) and the increased aggregation of platelets in aged rats (Giani, Masi and Galli, 1985). The co-existing haemorrhage associated with our experimental administration of endotoxin to old rats, is also consistent with previous findings at necropsy in rats affected by this syndrome (Carthew *et al.*, 1989). Whether overt haemorrhage occurs is probably a function of the dose of endotoxin experienced by the rat, as well as whether it has had a recent episode of endotoxin exposure, which would increase the damage, especially to the myocardium. During the life-time of such affected animals, myocardial fibrosis will increase with age and repeated natural endotoxin exposures. The original cases of DIC were discussed in terms of whether ageing was associated with a decrease in natural fibrinolytic activity, or perhaps an increased susceptibility to hypercoagulation caused by LPS with age (or both). By removing the fibrinolytic variable (blocking fibrinolysis by pretreatment with AMCA prior to LPS) in these animals, we succeeded in demonstrating that older rats have a greater hypercoagulative response to endotoxin LPS, both as a single or repeat dose. Hypercoagulation is thought to be at least partly mediated by tumour necrosis factor (TNF) (Bevilacqua, Pober, Majeau, Fiers, Cotran and Gimbrone, 1986; Nawroth and Stern, 1986) and this pathology can play an important part in the mechanism of tumour necrosis, owing to

coagulation in the neovascularized areas of tumours, leading to ischaemic necrosis (Nawroth, Handley, Matsueda, De Waal, Gerlach, Blohm and Stern, 1988; Shimomura, Manda, Mukumoto, Kobayashi, Nakano and Mori, 1988). TNF has been shown experimentally to cause fibrin deposition in the glomeruli of animals given exogenous TNF (Bertani, Abbate, Zoja, Corna, Perico, Ghezzi and Remuzzi, 1989). Whether the hypercoagulation of older rats exposed to LPS is mediated through this mechanism remains to be clarified. It seems reasonable to suggest that LPS may elicit increased TNF production from macrophages in older animals and that this could be one of the factors involved in the induction of systemic hypercoagulation. Complement component C5a has also been found to have a significant role in endotoxin-induced shock in the rat (Smedegard, Cui and Hugli, 1989), as has platelet activating factor (Doebber, Wu, Robbins, Choy, Chang and Shen, 1985; Terashita, Imura, Nishikawa and Sumida, 1985) which is released by TNF stimulation of vascular endothelial and other cells (Camussi, Bussolino, Salvidio and Baglioni, 1987).

The demonstration that repeated doses of LPS administered to induce a generalized Shwartzman phenomenon can enhance both the haemorrhage and intravascular coagulation in old rats to a lethal extent, could explain the sporadic nature of death or morbidities owing to endotoxin exposure. The likelihood of episodic endotoxin exposures in old animals occurring sufficiently frequently to satisfy the requirements of the generalized Shwartzman reaction (exposures must be 24 h apart to induce exacerbated hypercoagulation) is probably not great. Hence it is also possible to understand why episodes of dyspnoea or myocardial damage occur irregularly, but with greater frequency with age where the relative resistance is reduced (especially for cardiac damage) owing to the cumulative weakening of the heart as a result of previous natural exposures to LPS.

Recently a rat model of DIC has been described using dexamethazone-induced immunosuppression, and the intraperitoneal injection of *Escherichia coli* (Galera-Davidson, Lopez-Garrido, Ortega-Medina, Gonzalez-Campora and Rubi-Uria, 1989; Lopez-Garrido, Galera-Davidson, Ortega-Medina, Martinez-Navarro, Leal-Bermudez and Rodriguez-Fernandez, 1987). Given that endotoxin response may differ in elderly compared with young individuals (Horan and Fox, 1984; Norman, Grahn and Yoshikawa, 1985), a model of DIC involving the enhanced sensitivity of older animals to the Shwartzman reaction may clarify the role of endotoxin in the development of DIC. Robbins and Angell (1976) stated that "disseminated intravascular coagulation is the human equivalent of the experimentally produced generalized Shwartzman reaction". DIC is also implicated in adult respiratory distress syndrome (Putterman, 1988), where abnormalities of local coagulation and fibrinolytic systems are thought to occur before the development of the clinical syndrome (Idell, James, Levin, Schwartz, Manchanda, Maunder, Martin, McLarty and Fair, 1989).

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