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Protective effects of angiotensin AT_1 receptor blockade in malignant hypertension in the rat

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ABSTRACT

We investigated the role of angiotensin II and endothelin-1 using the angiotensin AT_1 receptor antagonist losartan and the endothelin ET_A receptor antagonist atrasentan, in malignant hypertension and renal failure and damage induced by nitric oxide (NO) synthase inhibition in Harlan Sprague–Dawley (SD) rats. We also evaluated whether the protective effects of losartan go beyond the blood pressure reduction. Within only 3 weeks of treatment with the NO synthase inhibitor N^{ω}-nitro-L-arginine methyl ester (L-NAME), Harlan SD rats developed malignant hypertension with renal failure and injuries. The latter were comprised of fibrinoid necrosis of small arteries and glomerular and tubular necrosis. Although both losartan and atrasentan attenuated the development of hypertension and renal failure, losartan only prevented the renal damage. In contrast to antrasentan, the vasodilator hydralazine reduced blood pressure and prevented the renal injuries similar to losartan. However, when these treatments were prolonged to 5 weeks, losartan, but not hydralazine, was still effective in reducing renal failure and damage, despite a marked increase in blood pressure. Our results indicate that angiotensin II and endothelin-1 play a differential role in the pathogenesis of malignant hypertension and in vascular and renal damage induced by L-NAME in Harlan SD rats. Although the protective effects of atrasentan may depend on the reduction of blood pressure, which was shown to retard the development of renal injury using hydralazine, those of losartan go beyond the blood pressure reduction. Hence, tissue protective effects of angiotensin AT_1 receptor blockade may be pivotal for long-term vascular and renal protection.

1. Introduction

Impaired nitric oxide (NO) release has been documented in most diseases with cardiovascular components such as chronic kidney disease (Morris et al., 2001). In patients with chronic renal failure, the reduction in NO release has been associated, at least in part, to the accumulation in plasma of asymmetric dimethyl-arginine (ADMA), an endogenous inhibitor of NO synthase (NOS) (Vallance et al., 1992). Using the rat remnant kidney model of chronic renal failure, Matsuguma et al. (2006) reported that the increase in plasma ADMA correlated positively with the degree of renal failure and hypertension. Accordingly, improvement of NO release with L-Arginine supplementation in uremic rats slowed down the aggravation of hypertension and the progression of renal failure and damage (Dumont et al., 2001b). Similarly, NOS inhibition in normal animals with the L-Arginine analogue N^{ω}-nitro-L-arginine methyl ester (L-NAME) can induce hypertension associated with vascular and renal injury (Baylis et al., 1992; Ribeiro et al., 1992). However, the latter changes may depend upon the rat strain because of different genetic susceptibility to hypertension and renal damage (van Dokkum et al., 1998). For example, treatment of intact and uninephrectomized Sprague–Dawley CD rats with L-NAME, at a dose of 100 mg/kg/day, for up to 6 weeks, induced hypertension without vascular and renal injury (D'Amours et al., 1999). The hypertensive and renal hemodynamic response to L-NAME has been reported to be higher in Harlan Sprague–Dawley SD rats as compared to the Sprague–Dawley CD rats (Pollock and Rekito, 1998; Qiu et al., 1998), suggesting a greater genetic susceptibility in the former rat strain that remains to be shown.

Increasing evidence suggests that hypertension and vascular and renal abnormalities induced by chronic NOS inhibition are mediated, in part, by angiotensin II due either to unopposed vascular and renal effects and enhanced local tissue renin–angiotensin system activity, but without changes in plasma renin activity (Graciano et al., 2004; Kashiwagi et al., 2000; Michel et al., 1996). In the latter case, increased renal angiotensin II levels has been reported in L-NAME-treated rats and was associated with a reduction in the angiotensin AT_1 receptor levels, both of which were normalized by angiotensin-converting enzyme inhibition (Vandermeersch et al., 2003). Depending upon the experimental condition, renin–angiotensin system blockade can attenuate or prevent the development of hypertension along with the vascular and renal alterations induced by L-NAME treatment (Boffa et al., 1999; Kashiwagi et al., 2000; Pollock et al., 1993; Qiu and Baylis, 1999; Ying et al., 2003). Treatment with angiotensin-converting enzyme inhibitors and angiotensin AT_1 receptor antagonists were shown to reduce the rate

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of progression of renal insufficiency and injury through hemodynamic effects which may include a reduction in systemic blood pressure, renal blood flow and glomerular capillary pressure in addition to other non-hemodynamic mechanisms (Anderson et al., 1986; Lafayette et al., 1992). The blood pressure-independent and tissue protective effects have been attributed to a reduction of the direct effect of angiotensin II on vascular and mesangial cell hypertrophy and extracellular matrix expression, renal glomerular sclerosis and interstitial fibrosis (Dumont et al., 2001a; Kakinuma et al., 1992; Larivière and Lebel, 2003; Lavoie et al., 2005). These effects may also be related to a reduction in vascular and renal endothelin-1 production, which have been shown to mediate, at least in part, the pathological effects of angiotensin II (Dumont et al., 2001a; Larivière et al., 1998). However, the involvement of endothelin-1 in the pathogenesis of hypertension and renal injury induced by NO synthase inhibition is controversial and the protective effect of endothelin receptor antagonism may also depend on the experimental settings (Boffa et al., 2001, 1999; Fujihara et al., 1995; Verhagen et al., 1998).

In the present study, we document that inhibition of NO synthase with L-NAME in Harlan Sprague–Dawley SD rats quickly induces, within 3 weeks, malignant hypertension with marked vascular and renal injury. Using this experimental model of malignant hypertension, we investigated the antihypertensive and tissue protective effect of the angiotensin AT_1 receptor blocker losartan and the endothelin ET_A receptor blocker atrasentan. In addition, to ascertain that the tissue protective effects of losartan go beyond the blood pressure reduction activity, we compared the effects of losartan with the vasodilator hydralazine. We show that at week 3 losartan and hydralazine similarly reduced blood pressure and renal function and damage. However, prolonged treatment in L-NAME-treated Harlan SD rats up to 5 weeks with losartan, but not with hydralazine, led to vascular and renal protection.

2. Materials and methods

2.1. Animal experiments

All animal experiments were approved by the Animal Care Committee of Laval University and were performed on 300 g male Harlan Sprague–Dawley (SD) rats (Harlan, Indianapolis, IN, USA). The animals were allowed free access to standard laboratory rat chow and tap water and were housed under controlled humidity and temperature conditions with a 12-h light–dark cycle. In a preliminary study (not shown), Harlan SD rats were divided into 2 groups; one group of controls ($n=10$) received tap water and the second group ($n=12$) received the NOS inhibitor L-NAME 0.1% (Sigma Chemical, St. Louis, MO, USA) in drinking water, which corresponds to a daily intake of about 100 mg/kg (Ribeiro et al., 1992). Unexpectedly, L-NAME-treated Harlan SD rats developed malignant hypertension within only 3 weeks, beyond which period all animals died or underwent hind-limb paralysis due to cerebrovascular lesions and had to be sacrificed. In fact, the survival rate at week 3 was about 75%. Thus, in the following studies, the animals were studied at week 3, unless specified (see below).

In a first series of experiments designed to evaluate the role of angiotensin II and endothelin-1, Harlan SD rats were divided into 4 groups and received; 1) tap water (control; $n=8$); 2) L-NAME (100 mg/kg/day; $n=12$); 3) L-NAME + losartan 20 mg/kg/day ($n=12$; Merck & Co., Rahway, NJ, USA); 4) L-NAME + atrasentan 10 mg/kg/day ($n=12$; Abbott Laboratories, Abbott Park, IL, USA). All drugs were given in the drinking water. The concentration of L-NAME and the drugs was adjusted every two days according to body weight and drinking behavior to ensure similar dosage in the different groups of rats. The dose of losartan was selected according to our study in L-NAME-treated Sprague–Dawley CD rats where losartan was shown to attenuate the development of hypertension (D'Amours et al., 1999), and in uremic rats where losartan was shown to normalize blood pressure, proteinuria and endothelin-1 production (Dumont et al., 2001a). The dose of atrasentan was selected according to our study in uremic rats where it was shown to reduce blood pressure (Rodrigue et al., 2003).

In a second series of experiments designed to determine potential blood pressure independent and tissue protective effects of the angiotensin AT_1 receptor blocker losartan, Harlan SD rats were divided into 4 groups and received; 1) tap water (control; $n=8$); 2) L-NAME (100 mg/kg/day; $n=12$); 3) L-NAME + losartan 20 mg/kg/day ($n=12$); 4) L-NAME + hydralazine 12.5 mg/kg/day ($n=12$; Sigma Chemical Co.,). As above, the drugs were given in the drinking water and adjusted every two days to assure proper dosage in the different groups of rats. The dose of hydralazine was selected according to a study conducted by Takemoto et al. (1997) who reported that hydralazine prevented the increase in blood pressure in L-NAME-treated Wistar Kyoto rats. Since both losartan and hydralazine caused similar blood pressure and renal protective effects after the 3 week period of treatment (see Results), another series of experiments was conducted to assess, in L-NAME rats, the effect of a longer period of treatment with losartan ($n=12$) and hydralazine ($n=12$) at the same doses as above. This protocol was stopped at week 5 since, beyond this period, the health status of L-NAME rats treated with hydralazine markedly declined similar as Harlan SD rats treated with L-NAME alone beyond the 3 week period as indicated above.

In all the experimental protocols, systolic blood pressure was measured by the tail-cuff method as described elsewhere (D'Amours et al., 1999; Dumont et al., 2001a). At the end of each treatment period, the rats were placed in metabolic cages, acclimatized for 24 h and then 24-h urine samples were collected and stored at −20 °C. The animals were then anesthetized with pentobarbital (50 mg/kg, *i.p.*; MTC Pharmaceuticals, Cambridge, ON, Canada) and exsanguinated by an abdominal aortic puncture. The kidneys were harvested and cut in halves longitudinally. One half was fixed in buffered formalin solution for histological analysis (Lebel et al., 2006). The papilla and medulla were removed from the other half and the renal cortex was then quickly frozen, cut into pieces and stored at −80 °C for measurement of endothelin-1 expression and tissue levels.

2.2. Biochemical analyses

Serum creatinine and urinary protein and creatinine were measured with an auto-analyzer system (Ilab 1800; Instrumentation Laboratory, Lexington, MA, USA). Plasma renin activity was measured using a specific RIA kit for angiotensin I purchased from DuPont NEN (Boston, MA, USA).

2.3. Assessment of angiotensin II and endothelin-1

The frozen renal cortex were each weighed and then homogenized twice with a Tissue-Tearor (Biospec Products, Bartlesville, OK, USA) in 2 ml ice-cold extraction solution containing 1 N HCl, 1% acetic acid, 1% trifluoroacetic acid (TFA) and 1% NaCl, and centrifuged at 3000 *g* for 30 min at 4 °C (D'Amours et al., 1999; Dumont et al., 2001a). The supernatant was then extracted on a C_{18} Sep-Pak column (Waters, Milford, MA, USA). Similarly, urine samples (5 ml) were acidified with TFA and extracted on a C_{18} Sep-Pak column. Endothelin-1 in the sample extracts was measured by a radioimmunoassay using a specific rabbit endothelin-1 antiserum that had been previously prepared in our laboratory (Lebel et al., 1994). The concentrations were corrected for loss in the extraction and purification steps using small amounts of [^{125}I]-endothelin-1 (~1000 cpm; DuPont NEN). Renal cortex angiotensin II was measured using the same extraction and RIA methods as for endothelin-1. The rabbit angiotensin II antiserum, standard angiotensin II and [^{125}I]-angiotensin II were purchased from Peninsula Laboratories (Belmont, CA, USA) and DuPont NEN, respectively.

2.4. Urine NO_2/NO_3 (NO_x)

Urine samples were diluted (1:1) in potassium phosphate buffer pH 7.4, deproteinized by ultrafiltration in Centrisart tubes with a molecular