Nitric Oxide Mediates the Blood-Pressure Lowering Effect of Garlic in the Rat Two-Kidney, One-Clip Model of Hypertension\textsuperscript{1,2}

Khaled K. Al-Qattan,\textsuperscript{3} Martha Thomson, Sausan Al-Mutawa’a, Dalal Al-Hajeri, Hana Drobiova, and Muslim Ali

Department of Biological Sciences, Faculty of Science, Kuwait University, Safat 13060, Kuwait

ABSTRACT Garlic reduces blood pressure (BP) in two-kidney, one-clip (2K-1C) rats, and enhances nitric oxide (NO) synthesis in in vivo and in vitro experiments. NO is an important modulator of BP in the 2K-1C model. This study investigated the role of NO in the BP-lowering effect of garlic in the 2K-1C model. BP readings (mm Hg) were obtained from 2K-1C rats in 4 groups treated intraperitoneally for 2 wk with either normal saline (NS), garlic, \textsuperscript{-}NAME, or \textsuperscript{-}NAME + garlic (n = 4 × 5). BP was determined using the tail-cuff method and compared with data of 4 similarly treated groups of normal (unclipped) rats (NRs). The BP readings of NR groups were 120 ± 3 mm Hg for the NS-treated group, 120 ± 2 mm Hg for the garlic-treated group, 167 ± 3 mm Hg for the \textsuperscript{-}NAME treated group (higher than NS or garlic, \textit{P} < 0.001) and 128 ± 5 mm Hg for the garlic + \textsuperscript{-}NAME-treated group (higher than \textsuperscript{-}NAME, \textit{P} < 0.001). The BP readings of 2K-1C rat groups were: for the NS group, 165 ± 6 mm Hg (higher than NRs, \textit{P} < 0.001); for the garlic group, 116 ± 7 mm Hg (lower than NRs, \textit{P} < 0.001); for the \textsuperscript{-}NAME group: 184 ± 8 mm Hg (higher than garlic, \textit{P} < 0.001), and for the \textsuperscript{-}NAME + garlic group: 130 ± 6 mm Hg (lower than garlic or NS, \textit{P} < 0.001). The data show that \textsuperscript{-}NAME increases the BP of both NRs and 2K-1C rats, with the rise more evident in the NRs (39 vs. 9%, respectively). Garlic counteracts the hypertensive effect of \textsuperscript{-}NAME in NRs as well as 2K-1C rats. We conclude that the BP-lowering effect of garlic in the rat 2K-1C model may be partly mediated through the NO pathway. J. Nutr. 136: 774S–776S, 2006.

KEY WORDS: • nitric oxide • garlic • 2K-1C • hypertension

The two-kidney one-clip (2K-1C)\textsuperscript{4} model exhibits a transient increase in the activity of the renin-angiotensin system and a sustained rise in blood pressure (BP) (1). Hypertension in this model is primarily the result of augmented total peripheral resistance and, in mild cases of renal artery stenosis, bilateral reduction in renal-clearance function (2). These physiological abnormalities are principally the result of a considerable increase in tissue and circulating levels, and direct actions of angiotensin II (AngII) (3). Evidence shows that as the condition advances, the role of AngII in maintaining hypertension subsides, and other mediators become more effective in determining the level of BP (4,5).

Nitric oxide (NO) is an important mediator of BP homeostasis. It has been reported that pharmacologically reducing the bioavailability of NO can lead to hypertension in NRs (6). Furthermore, it is strongly suggested that loss of the vasodilatory action of NO is a main cause to the development of hypertension in some forms of the disease (7).

For the 2K-1C model the literature suggests that NO is vital to restraining hypertension, preserving normal blood circulation, and thus preserving organ functionality. However, what remains unclear is the nature of the shift in the concentration of NO and the level of influence this radical exerts on the BP-regulation mechanism (8). This ambiguity could be due to the fact that NO and AngII reciprocally influence each other’s rate of synthesis and potency (9). In addition, the dynamic interaction between these vasoactive agents seems to be inconsistent and varies as the 2K-1C condition progresses (10).

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\textsuperscript{3} To whom correspondence should be addressed. E-mail: qattan@kuco1.kuniv.edu.kw.

\textsuperscript{4} Abbreviations used: AngII, angiotensin II; BP, blood pressure; \textsuperscript{-}NAME, \textsuperscript{-}NAME + garlic; \textsuperscript{-}NAME, \textsuperscript{-}NAME + garlic; NS, normal saline; 2K-1C, two-kidney, one-clip.

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Garlic was reported to lower BP in the 2K-1C model (11). Garlic appears to exert this effect by modulating the activity of several mechanisms that are vital in BP homeostasis in favor of hypotension. These include the prostaglandin system (5), renin-angiotensin system (12), and renal tubular transport mechanisms (13). Another possible mechanism by which garlic might induce its hypotensive effect could be through the direct and indirect vasodilatory actions of NO (14,15). Garlic was reported to contain arginine and enhance the synthesis of NO (16,17).

In this preliminary study, the role of NO in the hypotensive effect of garlic in the 2K-1C model rat was investigated. The BP of 2K-1C rats was compared with the BP of NRs subjected to the same experimental protocol.

METHODS

**Animals.** The animals used in this study were 40 healthy male Sprague-Dawley rats (birth wt 50 ± 4 g, Laboratory Animals Inc.). The rats were provided with a free supply of standard rodent diet and filtered tap water. They were housed under moderated ambient climatic conditions with a normal light/dark cycle in the animal care facility at the Department of Biological Science, Kuwait University. At the end of the experiments each rat received an overdose of sodium thiopentone and was kept under supervision until all vital signs ceased. The animal treatment is in accordance with the Guide for the Care and Use of Laboratory Animals.

**Two-kidney, one-clip model.** A 2K-1C model was induced in 20 rats according to a previously described method (2). The remaining 20 rats were used as a control group.

**Animal treatment.** The rats were almost 4 wk old when the model was induced, and treatment started 4 wk after. They received different treatments as a single daily intraperitoneal injection for 2 wk as described here: 1) control treatment: each of 5 NRs and 5 2K-1C rats received 0.5 mL of NS; 2) garlic treatment: each of 5 NRs and 5 2K-1C rats received 500 mg/kg 0.5mL of aqueous extract of fresh garlic; 3) L-NAME treatment: each of 5 NRs and 5 2K-1C rats received 10 mg/kg 0.5 mL of L-NAME (Sigma), and 4) L-NAME + garlic treatment: each of 5 NRs and 5 2K-1C rats received L-NAME + garlic. Treatments were administered at midnight from aliquots of preprepared L-NAME solution and extract of fresh garlic that were stored at −70°C.

**Blood pressure measurements.** The BP of all rats was recorded 2 h following the last injection on d 14. The rat’s systolic BP was determined using the tail-cuff method (Harvard apparatus) after several test runs. The BP reading for each rat was the mean of 3–5 comparable measurements.

**Data presentation and statistical analysis.** Bar graphs represent mean ± SEM of BP readings. BP values after different treatments in the NRs as well as in the 2K-1C rats were compared using a one-way ANOVA test, whereas BP readings of NRs and 2K-1C rats receiving the same treatment were compared using the independent samples t test (SPSS program, version 12). Differences were considered significant if P < 0.05.

RESULTS

**Normal rats.** As shown in Figure 1, the BP of NRs that received NS was normal at 120 ± 3.4 mm Hg. NRs that received garlic showed no change in their BP compared with the NS-treated group. In contrast, NRs that received L-NAME had a higher (P < 0.0001) BP by 39% compared with the readings of NRs treated with either NS or garlic. The BP of NRs that received L-NAME plus garlic was 30% lower (P < 0.0001) than the BP of NRs treated with only L-NAME and not different from the BP of NRs treated with either NS or garlic.

**2K-1C rats.** The BP of 2K-1C rats treated with NS was of a hypertensive level at 169 ± 5.5 mm Hg and was 40% higher than the BP of NRs that were treated similarly (P < 0.0001) (Fig. 2). The 2K-1C rats that received garlic had a normal BP. The BP of this group was (not different from the BP of NRs treated with NS, garlic or L-NAME + garlic) and was 47% lower (P < 0.0001) than the BP of the 2K-1C group that received NS. The 2K-1C rats treated with L-NAME had a slightly higher but not significantly different BP compared with the NS treated group. The BP of the 2K-1C group treated with L-NAME was different from the BP of the NRs group receiving the same treatment (P < 0.05). As with NRs, the 2K-1C rats that received garlic plus L-NAME had a normal BP that was 42% lower (P < 0.001) than the BP of the 2K-1C group that was treated with either NS or L-NAME and not different from the BP of clipped rats that received only garlic.
DISCUSSION

The results support the concept that NO availability is imperative for maintaining normotension and restraining the magnitude of pathological increases in BP. Here and previously reported, treating normotensive rats with L-NAME for an extended time made them hypertensive. The BP increase in these animals might have resulted mainly from diminishing the vasodilatory effect of NO. Nonetheless, since NO under normal conditions imposes a suppressor influence on the level of AngII (18,19), and probably other agents with similar effect on the vasculature (8), then an increase in the level of such vasoconstrictors, or their ability to express their action unopposed in the absence of NO, could have contributed to the observed rise in BP.

Treating 2K-1C rats with L-NAME did not cause a further increase in their BP; an observation inconsistent with the findings of other studies (18,19). This discrepancy might be due to 2 main reasons: 1) a difference in the activity of the NO pathway (10,20). It is possible that in the present study the NO activity was low and therefore its attenuation with L-NAME did not have a pronounced effect on the BP; or 2) the effect of pathologically elevated levels of several potent vasoconstrictors in this model, such as AngII, 8-iso-prostaglandin F2α (PGF2α), and Thromboxane-A2. It is possible that this masked the response to a decline in the availability of NO. Irrespective of the present finding, it is highly probable that NO has an important role in restraining the development of hypertension. This view is supported by the observation that the BP readings of the L-NAME-treated NRs and 2K-1C rats were different (P < 0.05) indicating that the difference that existed between these groups when receiving NS remained during the L-NAME protocol.

The response of the 2K-1C rats to L-NAME plus garlic might suggest that NO does not play a role in the BP-lowering effect of garlic in this model. We do not believe that this is the case. It must be taken into consideration that the 2K-1C model represents a condition of drastic and compounded modulation in the biochemistry and physiology of substantial number of vasoactive agents. Furthermore, garlic treatment in general increases the concentration and activity of vasodilators and decreases those of vasoconstrictors. Since garlic caused a fall in BP, the effect of the reduction in NO concentration due to L-NAME treatment was not apparent in the BP of the 2K-1C group treated with both L-NAME and garlic. It is also possible that because of the overwhelming competitive nature of garlic, the herb enhanced the formation of NO even in the presence of L-NAME (21). The response of the normotensive rats supports this assumption. In these animals garlic did not have an effect on BP. However, when garlic was given in addition to L-NAME, it prevented a rise in the BP of NRs similar to that observed when only L-NAME was administered. Under this uncomplicated circumstance, such as hypertension induced only by diminishing NO actions, the role of NO in the BP lowering effect of garlic is clear. Thus, its is most highly likely that garlic lowers the BP in the 2K-1C model by enhancing the concentration and activity of an array of vasodilatory agents including NO.

The findings suggest the following: 1) garlic prevents the rise in BP induced by chronic inhibition of NO production; 2) NO availability is important in restraining hypertension in the 2K-1C model; and 3) the BP-lowering effect of garlic in the 2K-1C model may be partly mediated through the NO pathway.

LITERATURE CITED