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Original Article

Sulforaphane in Experimental Hypertension

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Abstract

Background: Hypertension is defined as a failure to achieve a blood pressure (BP) target – smaller than 140/90 mmHg. The worldwide burden of hypertension has been associated with globally increased rates of death and disability. There is increasing evidence of strong relation between hypertension and oxidative stress, where either increased oxidative stress or depressed antioxidant level may lead to hypertension. Using stroke-prone spontaneously hypertensive rats (SHRSP) rats, previous studies in our laboratory have shown that broccoli sprouts (high in sulforaphane, a phase-2 protein inducer) attenuate BP and inflammation. **Objectives:** The question this study addressed was whether sulforaphane (a potent phase-2 protein inducer) can attenuate hypertension in the experimental model using the stroke-prone spontaneously hypertensive rats (SHRsp). **Materials and Methods:** Sulforaphane (*LKT* Laboratories) or vehicle was orally gavaged to SHRsp or Sprague–Dawley rats (SD) daily for 15 weeks. The body weight and BP were determined weekly, using a standard tail-cuff BP measurement. Tissues such as hearts and kidneys were collected, weighed, and stored under −80°C for further analysis. **Results:** When compared to BP in SHRsp control rats (179.9 ± 4.32), sulforaphane significantly reduced BP to 157 ± 5.21 (10 μmol/kg body weight), 136.57 ± 1.96 (20 μmol/kg body weight), and 129.33 ± 6.10 (5 μmol/kg body weight), respectively, in SHRsp rats. **Conclusion:** Administration of sulforaphane, a potent phase-2 enzyme inducer, daily for more than 3 months, significantly improves BP in SHRsp rats, but it did not have any effects on normotensive rats – SD.

Keywords: Blood pressure, hypertension, SHRsp, Sprague–Dawley, sulforaphane

Introduction

Persistent hypertension is linked to an increased risk of morbidity and mortality. The escalating health problem, hypertension, increases at an alarming rate. Worldwide, approximately 1 billion individuals are hypertensive; by 2025, this number is projected to increase to 29%, 1.56 billion, and more than 50% of Canadians aged from 55 to 74 years old are hypertensive.[1] A new therapeutic approach, such as the dietary intervention, could increase the percentage (5%–23%) of patients with normal blood pressure (BP) (<140/90 mmHg), in so doing, it will increase the successful rate of the pharmacotherapy and decrease the number and severity of adverse effects associated with the use of pharmacotherapy.[2] Various data support the therapeutic effects of the cruciferous vegetables (mustard family) of the genus brassica including cauliflower, broccoli, cabbage, and Brussels sprouts in several diseases.[3] In young (<mid-20s) healthy male cigarette smokers (10 cigarettes/day), 10 day-broccoli consumption lowers lymphocyte DNA oxidative damage when compared with nonsmokers.[4] Small quantities of young crucifer sprouts (e.g., 3-day-old broccoli sprouts) protect against chemical carcinogens.[3] Previously demonstrated in our lab that in SHRsp rats, diet-containing broccoli sprouts decreases inflammation, oxidative stress, hypertension and ageing-related degenerative changes in aging population.[5] Also, feeding hypertensive rats 200 mg/day of dried broccoli sprouts for 14 weeks, ameliorates hypertension and atherosclerotic changes.[6] Most of the available raw plant extracts contain a mixture of compounds, for instance, broccoli extracts contain multiple bioactive components such as indole glucosinolates, a precursor of indole-3-carbinol (enhance tumorigenesis), flavonoids (i.e., quercetin), selenium, and sulforaphane.[5,6] Consequently, the health beneficial effects seen with broccoli consumption in our previous studies could be due to the synergistic or inhibitory

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effects in complex mixtures. Therefore, the aim of this study is to investigate whether the potent inducer of the cytoprotective phase-2 protein, sulforaphane (independent of other ingredients in the broccoli sprouts), attenuates hypertension in female SHRsp.[7]

Materials and Methods

Animal model

A total of Fourty two 5-week-old female rats, including 21 SHRsp rats and 21 age-matched Sprague–Dawley (SD) rats (end of the 4th week postnatal), were purchased from Charles River Laboratories (St. Constant, Quebec Canada). The rats were treated in accordance with the guidelines of the Canadian Council on Animal Care, and the experimental protocols were approved by the Animal Care Committee at the University of Saskatchewan. With free access to water and regular food, the animals were kept under standard 12 h light/12 h dark cycle and humidity condition.

Animal groups

After 1 week of adaptation, the 5-week old female SHRsp and SD rats were divided into four groups and administered daily (8–10 a. m.) by gavage: (i) corn oil (vehicle) alone (control, $n = 5$); (ii) sulforaphane (5 μmol/kg body weight, $n = 5$) in corn oil; (iii) sulforaphane (10 μmol/kg body weight, $n = 5$) in corn oil; and (iv) sulforaphane (20 μmol/kg body weight, $n = 6$) in corn oil. Systolic BP was determined weekly (8–10 p. m.) using a standard tail-cuff noninvasive BP measurement system (model 29-SSP; Harvard Apparatus, St. Laurent, QC, Canada). The gavage treatment lasted for 15 weeks (20 weeks old). At the end of the treatment period, the animals were euthanized and perfused with normal saline, and tissues, such as hearts and kidneys, were collected and stored under −80°C for further analysis.

Statistical methods

All data are expressed as means ± standard error of the mean. Statistical significance was tested using Student's *t*-test or one–way analysis of variance followed by a *post hoc* analysis. Check test of homogeneity of variances was performed. If equal variances were determined, the Bonferroni test was used, whereas if equal variances were not determined, Tamhane's T2 test was used using SPSS software version 14.0 for windows (SPSS Inc. Chicago, IL, USA). The significance level was set at $P < 0.05$.

Results

Sulforaphane administration daily for 15 weeks attenuates hypertension in hypertensive SHRsp rats. Sulforaphane could neither affect rats' body weights nor influence organ weights.

Organs of Sprague–Dawley and SHRsp female rats

At the end of the study, we collected and weighed the hearts and kidneys of SD and SHRsp rats (data not shown). Within the strains, SHRSP and SD rats did not have any significant difference in weights when compared with the controls.

Body weights of Sprague–Dawley and SHRsp female rats

To investigate any effects of sulforaphane on body weights (g), we weekly measured the body weights of the animals. For 15 weeks, despite the initial (184.34 ± 3.94 vs. 120.14 ± 3.84) and the final body weights of SD rats being significantly higher (320.08 ± 11.3 vs. 216.2 ± 1.84) than SHRsp rats (strain difference), 15 weeks of daily sulforaphane administration did not have any effect on body weights [Figure 1].

Blood pressure in SD and SHRsp female rats.

During the 15-weeks study, we measured BP weekly in the morning (8–10 a. m.) using tail-cuff machine. Sulforaphane administration attenuated BP in SHRsp rats, but it did not have any effect in normotensive SD rats. Comparing with the SD control, sulforaphane did not have any effect in SD rats (83.98 ± 4.3 mmHg). However, when compared with the SHRsp rats (179.9 ± 4.3 mm Hg), 15 weeks of sulforaphane administration significantly attenuate BP to 157 ± 5.21 (10 μmol/kg body weight), 136.57 ± 1.96 (20 μmol/kg body weight), and 129.33 ± 6.10 (5 μmol/kg body weight), respectively, in SHRsp rats [Figure 2]. Previous studies in our laboratory have shown that higher doses than 5.5 μmol sulforaphane may have more profound antihypertensive effects.

Discussion

SHRsp is an adequate experimental model with elevated BP, together with structural and functional abnormalities.[8] As an animal model of hypertension, our laboratory has been using this model to study the therapeutic effects of broccoli sprouts (containing sulforaphane precursor) on hypertension. In this study, using an animal model of hypertension such as SHRsp, we evaluated the antihypertensive effects of pure sulforaphane. Our studies have shown that chronic oral administration of sulforaphane, a phase-2 protein

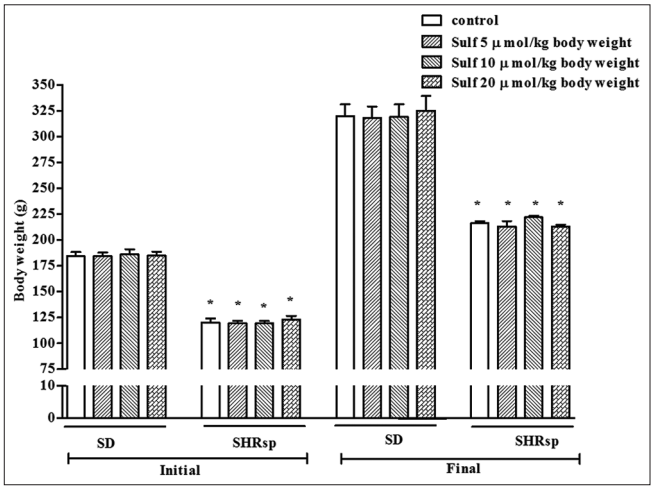

Figure 1: Effect of sulforaphane on initial and final body weight of SHRsp and Sprague–Dawley rats, *$P < 0.05$ versus the Sprague–Dawley control at the two respective time points, $n = 5$–6 rats per group, means ± standard error of the mean

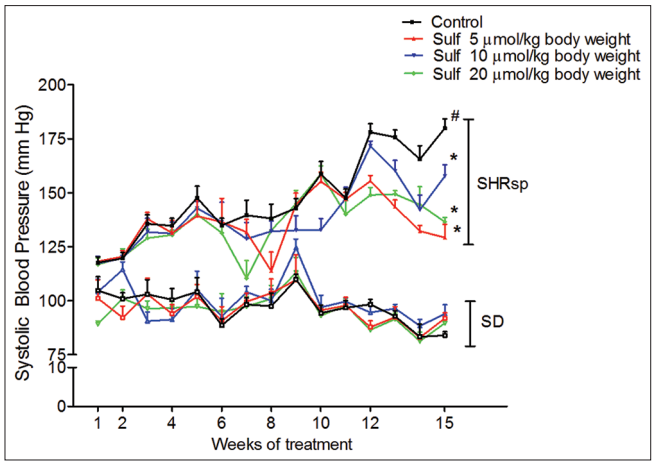

Figure 2: Effect of sulforaphane on systolic blood pressure (measured by tail-cuff) in SHRsp and Sprague–Dawley rats. The systolic blood pressure in SHRsp and Sprague–Dawley rats is significantly different (#$P < 0.05$). The blood pressure measured in sulforaphane-treated SHRsp rats is significantly lower than that in corn oil-treated SHRsp rats, *$P < 0.05$ versus the control of the same age and strain, $n = 5$–6 rats per group, mean ± standard error of the mean

inducer, (a) had comparable effects in body weights among all sulforaphane-treated groups throughout the experiment [Figure 1], (b) significantly reduced BP at the end of the 15-week treatment [Figure 2], and (c) it did not have any effects on animals of normal redox physiology such as SD.

The comparable body and organ weights (i.e., hearts and kidneys) among the sulforaphane-treated and untreated groups suggest that there were no toxicities associated with the chronic oral administration of sulforaphane. One could easily understand this since sulforaphane is of the many ingredients in broccoli sprouts, little or no adverse effects would be expected from sulforaphane administration. These findings are in agreement with those of Conaway.[9] In A/J mice (lung cancer animal model), sulforaphane, phenyl isothiocyanate, and their N-acetylcysteine conjugate-treated mice had comparable body weights as compared to untreated groups.

In our study, we have shown that sulforaphane reduced hypertension in SHRsp rats. Unlike SHRsp rats, the normotensive SD rats which their BP remain constant during the sulforaphane administration. This means that sulforaphane needs time to produce therapeutic effects when given daily by gavage to rats. In normal physiology rats (SD), sulforaphane did not have any effects; this needs more investigations to find an answer for this outcome. Similarly, sulforaphane reduces hypertension in SHRsp rats but did not affect SD rats.[10] Moreover, glucoraphanin, a sulforaphane precursor, is high in broccoli sprouts. In SHRsp rats, diet-containing broccoli sprouts high in glucoraphanin reduce oxidative stress, inflammation, and hypertension.[11]

Growing body of evidence supports the protective effects of broccoli (which contains sulforaphane precursor) in several chronic diseases, which have the oxidative stress and inflammation components. For example, both *in vitro* and in a diabetic rat model, broccoli offers a protective effect by reducing the oxidative stress load.[12] Moreover, in the hypertensive rat model, 14-week administration of rats with 200 mg/day of dried broccoli sprouts that contained glucoraphanin (0.5 and 5.5 µmol sulforaphane equivalents) attenuates blood pressure.[6] Cruciferous vegetables (i.e., broccoli) possess similar chemistry, metabolism, and protective effects as sulforaphane (e.g., glucobrassicin, gluconasturtiin–phenethyl isothiocyanate, glucoerucin [sulfide analog of sulforaphane], and glucoiberin-iberin).[13] Not only sulforaphane but also broccoli contains multiple bioactive components such as indole-3-carbinol and flavonoids (i.e., quercetin).

Our study shows that independent of these bioactive components, long term oral administration of sulforaphane alone reduces BP in hypertensive rats. How does long-term administration of sulforaphane decrease BP in hypertensive in hypertensive rats? Further experiments are needed to understand the mechanism(s) by which sulforaphane reduces BP in SHRsp rats or why sulforaphane did not have any effects in SD rats.

Conclusion

According to this study, (i) a minimal changes in our diet (adding broccoli sprouts that contain sulforaphane) may have a major impact in our health, (ii) the beneficial health effects previously seen with consumption of broccoli sprout are due to conversion of the sulforaphane precursor – glucoraphanin to sulforaphane – a potent phase-2 protein inducer, and (iii) the health-promoting effects of sulforaphane can be seen after long-term administration (~3 months).

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Nil.

Conflicts of interest

There are no conflicts of interest.

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ملخص المقال باللغة العربية

السلفورافان في ارتفاع ضغط الدم التجريبي في فئران التجارب

المؤلفون:

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الخلفية: يُعرَّف ارتفاع ضغط الدم على أنه الفشل في تحقيق هدف ضغط الدم أقل من 140/90 مم زئبق. ارتبط العبء العالمي لارتفاع ضغط الدم بزيادة معدلات الوفاة والعجز. هناك أدلة متزايدة على وجود علاقة قوية بين ارتفاع ضغط الدم والإجهاد التأكسدي، حيث قد يؤدي زيادة الإجهاد التأكسدي أو انخفاض مستوى مضادات الأكسدة إلى ارتفاع ضغط الدم. باستخدام الفئران المعرضة للسكتة الدماغية الذاتية، أظهرت الدراسات السابقة في مختبرنا أن براعم البروكلي (عالية في السلفورافان، محفز بروتين المرحلة 2) يخفف من ضغط الدم ويمنع الالتهاب.

الأهداف: كان السؤال الذي تناولته هذه الدراسة هو ما إذا كان للسلفورافان إمكانية تخفيف ارتفاع ضغط الدم في النموذج التجريبي باستخدام الفئران المعرضة للسكتة الدماغية ذات ارتفاع ضغط الدم التلقائي.

المواد والطرق: تم حقن سلفورافان (مختبرات LKT) أو حمّال السلفورافان فموياً إما إلى مجموعة من الفئران المعرضة للسكتة الدماغية ذات ارتفاع ضغط الدم التلقائي أو لمجموعة مماثلة من فئران سبراغ-دولي يوميًا لمدة 15 أسبوعًا. تم تحديد وزن الجسم وضغط الدم لكل فأر أسبوعياً باستخدام قيد الذيل. ثم جمع ووزن وتخزين أنسجة مثل القلوب والكلى في درجة حرارة -80 درجة مئوية.

النتائج: بالمقارنة مع ضغط الدم في الفئران غير المعالجة والمعرضة للسكتة الدماغية ذات ارتفاع ضغط الدم التلقائي (179.9± 4.32 مم زئبق)، قلل السلفورافان بشكل كبير من ضغط الدم إلى 157±5.21 مم زئبق عند جرع تساوي 10 ميكرومول/كجم من وزن الجسم، وإلى 136.57±1.96 مم زئبق عند جرع 20 ميكرومول/كجم من وزن الجسم، و 129.33± 6.10 مم زئبق عند جرع تساوي 5 ميكرومول/كجم من وزن الجسم، في فئران المعالجة والمعرضة للسكتة الدماغية ذات ارتفاع ضغط الدم التلقائي. ولكن لم يكن له أي تأثير على الفئران فئران سبراغ-دولي العادية.

الخلاصة: إن إعطاء السلفورافان، وهو محفز إنزيم قوي في المرحلة - 2، يوميًا لأكثر من 3 أشهر، يحسن بشكل كبير من ضغط الدم في فئران المعرضة للسكتة الدماغية ذات ارتفاع ضغط الدم التلقائي.

الكلمات المفتاحية: ضغط الدم، ارتفاع ضغط الدم، الفئران المعرضة للسكتة الدماغية ذات ارتفاع ضغط الدم التلقائي، سبراغ-داولي، سلفورافان.