Falsely High Urinary Catecholamines Induced by Labetalol

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Kobayashi, K., Miura, Y., Tomioka, H., Sakuma, H., Adachi, M., Abe, K. and Yoshinaga, K. Falsely High Urinary Catecholamines Induced by Labetalol. Tohoku J. exp. Med., 1979, 127 (1), 63-69 — A preoperative patient with pheochromocytoma was satisfactorily treated with oral labetalol, while a conspicuous increase in urinary output of catecholamines (CA) and of vanillylmandelic acid (VMA) was observed after labetalol therapy. Exaggerated increases in urinary CA and in VMA were also confirmed in normal volunteers immediately after oral labetalol. These effects of labetalol on urinary CA and VMA, however, were proved to be largely due to the interference with the usual photometries rather than due to its stimulation of CA release. Therefore, it should be emphasized that clinical evaluations of CA and its metabolites must be performed before labetalol therapy to avoid an erroneous diagnosis of pheochromocytoma.

labetalol; catecholamine; vanillylmandelic acid; pheochromocytoma

A newly synthesized adrenergic blocking agent, labetalol, is unique in being a competitive antagonist at both α- and β-adrenoceptor sites in the experimental animals and in man (Farmer et al. 1972; Richards et al. 1974). This new profile of action would be expected to provide a potent antihypertensive agent in the clinical medicine. Extensive clinical trials have proved its usefulness in the treatment of various types of hypertension (Frick and Försti 1976; Rosei et al. 1976).

The present report concerns our experiences with labetalol in the treatment of pheochromocytoma. A preoperative patient had been satisfactorily managed with labetalol, while urinary output of catecholamines (CA) and of vanillylmandelic acid (VMA), measured by our usual methods, was remarkably elevated after labetalol treatment. An elevation of CA was also confirmed after labetalol in normal persons. This effect of labetalol on urinary CA output, however, was proved to be due to the interference with the photometries used widely for CA and VMA assays.

Subjects and Methods

A patient with pheochromocytoma and five volunteers were the subjects in this study. The case of pheochromocytoma was a 26-year-old woman who was admitted to Tohoku...
University Hospital because of hypertension with episodic palpitation and perspiration. The blood pressure was 194/124 mmHg and the pulse rate was 60 beats/min on admission. Laboratory studies revealed marked elevation of 24-hr urine norepinephrine (NE, 973–1730 μg/day; normal, up to 65 μg/day) and of VMA (9–23 mg/day; normal, 3.0–7.0 mg/day). Advanced hypertensive retinopathy (grade III of Keith, Wagner and Barker’s classification) and proteinuria were observed, but the levels of creatinine clearance, blood urea nitrogen, serum electrolytes, serum cholesterol and fasting blood sugar remained normal. Abdominal aortography depicted a walnut-sized tumor located in the right adrenal region. The tumor, weighing 21 g, extra-adrenal in origin, was successfully removed by surgery and its histologic or biochemical examination confirmed the benign nature of pheochromocytoma. Effects of oral labetalol on both clinical symptoms and urinary CA were serially studied during the preoperative period. An influence of labetalol on CA and VMA assays was also examined in five healthy volunteers aged from 28 to 34 years.

Urine specimens for CA and VMA assays were collected in a glass jar containing a small amount of 6 N HCl (2 ml for timed urine and 20 ml for 24-hr urine) at room temperature and stored at 4°C until the assay. Every assay was performed within a fortnight. Urinary NE and epinephrine (E) were determined separately with our modification of the trihydroxyindole (THI) method which was primarily devised for plasma CA assay (Miura et al. 1977). Instead of plasma specimens, 1.0–10 ml of urine specimens were used for the assay and an aliquot (usually 0.3 ml) of alumina-column eluate was directly oxidized in 1.0 ml of 0.1 M sodium tetraborate-HCl buffer (pH 7.0) according to the THI procedures. External standards of NE and E (25 ng of each amine) and a reagent blank without amines were prepared in the medium containing 0.3 ml of 0.05 M perchloric acid solution passed through alumina column and 1.0 ml of 0.1 M sodium tetraborate-HCl buffer (pH 7.0), and oxidized as sample eluates. A faded blank for every urine specimen was prepared by the method of Renzini et al. (1970). The oxidized mixture was left at room temperature (20–25°C) for 50–60 min after oxidation. The fluorescence of the reaction mixture was then measured on a fluorescence spectrophotometer (Hitachi, MPF type IV) in quartz cuvettes (7×7×40 mm) at two sets of excitation/emission wavelengths (410/490 and 452/490 nm uncorrected) with the selection of an adequate slit system and sensitivity. Urinary VMA was determined with the method reported by Pisano et al. (1962) and the absorbance of the reaction mixture was measured on a spectrophotometer (Beckman, model 24) in quartz cuvettes (10×10×40 mm) at the wavelength of 335 nm.

Catecholamine content of the tumor was measured by the same method as described in the assay of urinary CA using an aliquot of the supernatant which was obtained by centrifuging 0.3 M sucrose homogenate of the fresh tumor tissue at 800 × g at 4°C. The activity of phenylethanolamine-N-methyltransferase (PNMT) in the same preparation was determined by the procedure developed by Axelrod (1962).

**RESULTS**

Before the treatment, the patient showed remarkably fluctuating hypertension and complained of a variety of episodic symptoms including headache, palpitation, substernal discomfort, excessive sweating and so forth. Fig. 1 illustrates the circulatory effects in the patient following oral administration of several adrenergic antagonists. The patient was initially treated with a combination of phenoxybenzamine (POB, 20–60 mg daily) plus a small dose of propranolol (PRP, 20 mg daily). During these medications, neither clinical symptom nor hypertension had been ameliorated. Then, oral labetalol was tried at an initial dose of 300 mg daily in addition to the above medications. These combinations rendered her blood pressure significantly lowered and the fluctuation reduced, relieving the patient from any kind of episodic symptoms. A dose related reduction
Fig. 1. Circulatory effects of various combinations of adrenoceptor antagonists in a patient with pheochromocytoma. Daily oral dosage of each agent is indicated in the upper frame. POB, phenoxybenzamine; PRP, propranolol; LAB, labetalol.

TABLE 1. Effect of various adrenoceptor antagonists on urinary catecholamines and vanillylmandelic acid (VMA) in a patient with pheochromocytoma

<table>
<thead>
<tr>
<th></th>
<th>Number of determinations</th>
<th>Epinephrine (µg/day)</th>
<th>Norepinephrine (µg/day)</th>
<th>VMA (mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretreatment period</td>
<td>3</td>
<td>Trace</td>
<td>1080±170</td>
<td>14.3±1.8</td>
</tr>
<tr>
<td>POB 60 mg/day</td>
<td>4</td>
<td>Trace</td>
<td>1070±96</td>
<td>14.7±1.2</td>
</tr>
<tr>
<td>PRP 20 mg/day</td>
<td></td>
<td></td>
<td>794±41</td>
<td>2350±90</td>
</tr>
<tr>
<td>POB 60 mg/day</td>
<td></td>
<td></td>
<td>2350±90</td>
<td>13.5±4.8</td>
</tr>
<tr>
<td>PRP 20 mg/day</td>
<td></td>
<td></td>
<td>LAB 300 mg/day</td>
<td></td>
</tr>
<tr>
<td>POB 30 mg/day</td>
<td>3</td>
<td>1320±50</td>
<td>3420±160</td>
<td>19.0±4.6</td>
</tr>
<tr>
<td>PRP 20 mg/day</td>
<td></td>
<td></td>
<td>LAB 450 mg/day</td>
<td></td>
</tr>
<tr>
<td>Postoperative period</td>
<td>3</td>
<td>9.7±2.5</td>
<td>52.5±2.6</td>
<td>3.3±0.8</td>
</tr>
</tbody>
</table>

Values indicate mean±S.E.M. POB, phenoxybenzamine; PRP, propranolol; LAB, labetalol.

of blood pressure was also obtained by increment of labetalol 450 mg daily. The patient was satisfactorily managed by repeated intravenous labetalol during the surgical removal of pheochromocytoma. The tumor contained predominantly NE (9.02 mg/g wet tissue) rather than E (0.49 mg/g). No significant activity of PNMT was detected in the tumor.

Changes of mean levels of urinary CA and VMA induced by various therapy were summarized in Table 1. Twenty-four hour urinary NE, E and VMA during the pre-treatment period were 1080±170 µg (mean±S.E.M.), trace and 14.3±1.8
mg, respectively. These values did not vary significantly after treatment with POB and PRP. But a marked elevation of urinary CA was observed after oral labetalol. Particularly, urinary E fraction was increased from trace to extremely high levels over 500 µg/day. Urinary VMA tended to increase after 450 mg of labetalol, although the rate of increase was much less than that of CA.

Labetalol showed native fluorescence with a peak located at excitation and emission wavelength of 315/420 nm as previously reported (Richards et al. 1977). This fluorescence was much intensified in the ammonium alkaline medium rather than in HCl acidic medium. This fluorescence, however, did not interfere with the CA assay, when the fluorimetry was carried out at excitation/emission wavelength of 410/490 and 452/490 nm, respectively. On the other hand, labetalol has another small but significant fluorescence with a peak of 420/490 nm which is similar to the main peak of CA fluorescence (Fig. 3). When 10 mg of labetalol was treated through the whole course of assay procedure of CA, a considerable fluorescence was measured, which was calculated as 24 ng of NE and 56 ng of E. Further, labetalol

Fig. 2. Changes of urinary epinephrine (E), norepinephrine (NE) and vanillylmandelic acid (VMA) before (●) and after (○) 50 mg of oral labetalol in normal persons. Stippled area indicates normal range.

When urinary CA and VMA were determined in five normal volunteers at supine position, urinary NE, E and VMA were 0.924±0.175 µg/hr (mean±s.e.m.), 0.567±0.067 µg/hr and 73±19 µg/hr, respectively. Those respective values increased to 4.52±2.58 µg/hr, 9.66±4.15 µg/hr and 73±19 µg/hr immediately after 50 mg of oral labetalol. Urinary E was distinctly higher than the upper limit of normal range (Fig. 2).
Labetalol and Catecholamine

Fig. 3. Excitation spectra of (a) 10 ng of norepinephrine, (b) 10 ng of epinephrine, (c) 0.5 mg of labetalol and (d) its faded blank. These spectra were obtained after processing each agent in 1.0 ml of 1.0 M sodium tetraborate-HCl buffer (pH 7.0) through a THI oxidation procedure (Miura et al. 1977). The fluorimetries were carried out at the following conditions: Energy mode, Ex. slit 2 nm, Em. slit 14 nm, Em. W.L. 490 nm, Fluorescence filter 35, Sample sensitivity × 10 and Measuring voltage of the recorder 10 mV.

Fig. 4. Absorption spectra of (a) labetalol 15 mg plus vanillylmandelic acid 10 µg, (b) labetalol 15 mg only and (c) nonoxidized blank. These spectra were obtained after processing each agent through a complete procedure of Pisano et al. (1962) and its oxidized compounds via procedure of Pisano et al. (1962) showed an absorption spectrum indistinguishable from that of vanillin (Fig. 4). Fifteen mg of albetalol passed through the whole procedure of VMA assay were calculated as 0.16 mg of VMA.

DISCUSSION

The present study confirmed the previous report (Rosei et al. 1976) that labetalol is useful in the treatment of pheochromocytoma. Although a combination therapy of α- and β-antagonists has been established in the management of pheochromocytoma, Ross et al. (1967) emphasized that blockade of α-adrenoceptor must be achieved prior to the β-adrenoceptor blockade, otherwise hypertension might
be aggravated. Viewed from their opinion, labetalol may not be suitable in the beginning of the treatment. However, administration of \( \alpha \)-antagonist elicits severe tachycardia in a majority of patients so that simultaneous prescription of \( \beta \)-antagonist is often required. These clinical experiences support the opinion that labetalol is not inconvenient, but adequate from the beginning of the management of pheochromocytoma. The minimal effect of labetalol on the central nervous function (Martin et al. 1976) is an additional advantage, since larger doses are required quite often in these patients.

A conspicuous increase in urinary CA and VMA was observed after the administration of labetalol in our patient with pheochromocytoma. These increments might be due to an enhancement of CA release from the chromaffin tissues or due to the interference by a number of noncatecholamine fluorescence in the assay system. Blakeley and Summers (1977) reported that labetalol induces an enhancement of CA release from cat spleen by blocking neuronal uptake of NE. This mechanism may partly account for elevation of urinary CA and VMA. However, this explanation seems incompatible with the fact that a remarkable increase in both NE and E was observed after oral labetalol, while administration of both POB and PRP did not influence on urinary CA output in this patient. Exaggerated increase in E fraction after labetalol is also quite contradictory with the fact that NE fraction was exclusively detected in the tumor, and both E fraction and PNMT were negligible. These findings indicate that an elevation of values calculated as CA and VMA might be due largely to the biochemical properties of labetalol rather than its stimulation of CA release.

This study disclosed that labetalol, when processed through a complete course of CA or VMA assay, emits a considerable fluorescence and has an absorbance in the similar areas of the spectrum as those of CA and VMA, respectively. It is beyond doubt that these biochemical natures of labetalol cause falsely higher values of CA and/or VMA. However, the interference with the assay by labetalol seems too weak to explain the great increase in values of CA, when the rate of interference is calculated on the basis of the data that 10 mg of labetalol are miscalculated as 24 ng of NE and 56 ng of E. Besides, unmetabolized labetalol in urine has been shown to account for 10% or less of the oral dose (Martin et al. 1976). From these reasons, it is most likely that a major portion of the interference is caused by urinary metabolites of labetalol rather than labetalol itself, although these fluorescent metabolites remain speculative.

Marked increase in urinary CA was also observed in normal subjects immediately after labetalol (Fig. 2). This finding is in line with recent correspondence by Harris and Richards (1977), suggesting that the hypertensive patients under labetalol therapy may be erroneously diagnosed as pheochromocytoma. Therefore, it should be emphasized that clinical evaluations of CA and its metabolites have to be done prior to the treatment with labetalol.
Acknowledgments

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References