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Effects of Citrulline Combined with Tadalafil on Monocrotaline-Induced Pulmonary Hypertension in Rats Compared with Arginine

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[1]

housed in a temperature-controlled environment and free access to food and water.

All rats were induced pulmonary hypertension by 5 time's subcutaneous injection of MCT (10 mg/kg/day) within 2 weeks. Next, we orally administrated tadalafil (0.4 mg/kg/day) or saline (vehicle group) for 6 times every other day. Citrulline and arginine (KYOWA HAKKO BIO CO., LTD., Tokyo, Japan) were administered from water supply (10 g/L) (Figure 1).

This study was approved by the Osaka University Medical School Animal Care and Use Committee and was in compliance with the Osaka University Medical School Guidelines for the Care and Use of Laboratory Animals. All institutional and national guidelines for the care and use of laboratory animals were followed.

Estimation of cardiac function by echocardiography

Echocardiography was performed at 6-weeks and 8-weeks. Rats were anesthetized with sodium pentobarbital (40 mg/kg, ip). Right and left ventricular end-diastolic area were measured at the papillary muscle level using SONOS5500 (Philips Medical Systems, USA) with an s12 probe (frequency: 5-12 MHz, frame rate ≥120 Hz) (Figure 2). At the aortic valve level in the short axis view, Pulmonary artery (PA) flow

using log-rank analyses of Kaplan-Meier curves. Statistical analysis was performed with Statcel software for Windows (Version 2, OMS Publishing, Inc., Saitama, Japan) and the ystat 2000 Statistical Program File (Igaku Tosho Shuppan, Tokyo, Japan).

Results

Survival rate

The survival rate of a tadalafil and citrulline group is much higher than other 4 groups (Figure 4). However, the survival rate of a tadalafil and arginine group is almost same as that of a tadalafil group.

Two-dimensional echocardiographic parameters

At 6 weeks, the ratio of right to left ventricular end-diastolic area in all groups was almost same. At 8 weeks, the ratio of right to left ventricular end-diastolic area in 4 groups (tadalafil, citrulline, tadalafil and citrulline, tadalafil and arginine) was lower than that in a vehicle group (Figure 5).

Pulmonary artery flow

Maximum flow velocity of the pulmonary artery at 8 weeks in 4 groups except for a tadalafil+arginine group is lower than that at 6

Citation:

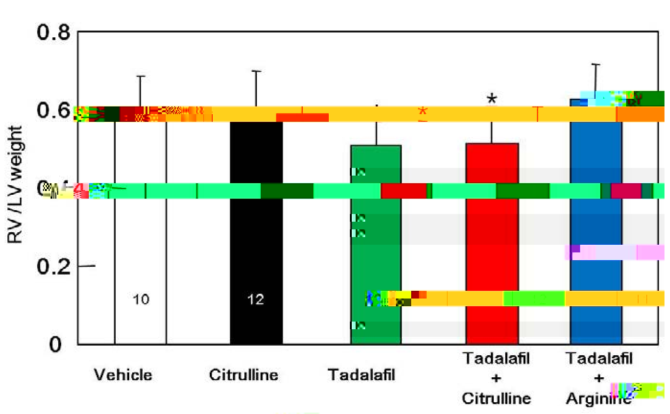

Figure 8:

Expression of PDE5 was found to be greater in lung tissues from patients with PH compared with controls [22]. Highly expression of PDE5 in lung tissues causes excess contraction of the pulmonary artery and leads to PH. Now PDE5 inhibitors are the first line to treat patients with PH. PDE5 is the major negative regulator of cGMP. Its expression has been observed in corpus cavernosum, vascular smooth muscle cells, and platelets. cGMP is one of the secondary messengers involved in the regulation of vascular homeostasis. Up-regulation of cGMP is a well-established strategy for vasodilatation and increased blood flow.

It is known that NO is effective to improve PH [23]. NO increased the intercellular cGMP level by activating soluble guanylate cyclase (sGC), but cGMP is quickly broken down by PDE5. Thus, it is possible that combination of the PDE5 inhibitor and NO donor is very effective to treat PH.

However, a direct NO donor, such as nitroglycerin is contraindication of concomitant use with PDE5 inhibitors. Arginine is amino acid and physiological substrate for NOS, so arginine could be concomitant use with PDE5 inhibitors. Citrulline, one of the amino acid which is used as a safe food ingredient, [15,24] is not a direct NO donor, but citrulline is converted to arginine by argininosuccinate synthetase. The survival rate of a tadalafil and arginine group is almost same as that of a tadalafil group. The survival rate of a tadalafil and citrulline group is much higher than other 4 groups involving in tadalafil and arginine group. This is because citrulline is a more effective extracellular supplement to enhance NO production. Diminished global arginine bioavailability ratio is associated with higher systolic pulmonary artery pressure and higher central venous pressure [25]. In a study of orally ingested arginine, arginine is extensively metabolized by arginase in the intestine and liver [26,27]. This may limit its bioavailability as a substrate for NOS and subsequent effects on vascular function. In contrast, citrulline is not metabolized in the gut wall or liver, and is more effective in maintaining plasma arginine concentrations than arginine itself [11]. So, oral intake of citrulline increased plasma arginine and produced NO by NOS. NO up-regulated cGMP by sGC, and tadalafil inhibited the PDE5 that breaks down the cGMP. Therefore combination of citrulline and tadalafil increased cGMP efficiently. Activation of NO-cGMP pathway leads to vasodilatation and increased blood flow. That is why the survival rate of a citrulline and tadalafil group is much higher than arginine group. To our knowledge, this is the first study to demonstrate the therapeutic effect of the combination of tadalafil and citrulline on PH.

Pulmonary hypertension is a disorder characterized by an increase in mean pulmonary arterial pressure, which is responsible for the transport of blood from the heart to the lungs. Increased pulmonary vascular tone causes stricture of pulmonary artery and then leads to right ventricular failure [28]. RV to LV weight in a tadalafil group and a tadalafil+citrulline group were lower than other 3 groups. In addition, estimated pulmonary artery pressure in a citrulline and tadalafil+citrulline group seem to be lower than those in other groups. This is because citrulline reduced right ventricular failure by inducing vasodilation. Low RV to LV weight and estimated pulmonary artery pressure might result in improving survival rate in tadalafil+citrulline group. The survival rate in 4 groups except for a tadalafil+citrulline group is low. So, we could not perform echocardiography for all rats, as well as Vmax of PA flow and RVEDA/LVEDA ratio. Pulmonary hypertension in survival rats might not be severe, so echocardiographic parameters were almost same in 4groups. The survival rate and the ratio of RV to LV weight could be the most reliable data. In this study, we didn't have blood pressure and histopathological data. It has been reported that oral administration of citrulline suppressed the increase of blood pressure and lung histopathological changes in pulmonary hypertensive rats [8,10]. Therefore, the efficacy of tadalafil + citrulline in preventing deterioration of pulmonary hypertension is likely to be partially mediated by these effects.

We tried to establish a rat model of pulmonary hypertension with sufficient tricuspid regurgitation by 15 times injections of 5 mg/kg/day of MCT. In this model, we obtained sufficient tricuspid regurgitation in 78% of rats. Other parameters, such as right ventricle area and right ventricle or lung weight, in the PH group were greater than those in the control group, and AT/ET and VTI in the PH group were smaller than those in the control group. The findings were very almost similar to those in other reports [6,7,21].

It is more effective to administrate tadalafil every day, but to be performed forced oral dosage is very stressful for rats and affects survival rate. Therefore we orally administrated tadalafil for 6 times every other day in this study. In this condition, the combination of tadalafil and citrulline demonstrated survival benefit. It is possible that administrating tadalafil with citrulline every day is more efficient therapeutic effect on PH.

Conclusion

Combination therapy of tadalafil and citrulline might be useful to prevent deterioration of pulmonary hypertension and improve survival rate compared with concomitant use of arginine.

Conflict of interest

Fuminobu Ishikura, Mai Egawa, Yuri Takano, Kota Kumagai, Takashi Suzuki and Masahiko Morita declare that have no conflict of interest.

References

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