Implementation of the technique in isolated organ vascular as tool for the validation of medicinal plants: Study of the vasodilator effect of the *S. scutellarioides**

JORGE HERNÁN RAMÍREZ, M.D., M.SC.[1], MAURICIO PALACIOS, M.D., M.SC.[1], OSCAR GUTIÉRREZ, M.D., M.SC.[2]

SUMMARY

***Introduction*:** *Salvia scutellarioides* is a plant with antihypertensive and diuretic properties. In this study, its vasodilator effect is evaluated using the isolated organ technique.

***Objectives*:** To standardize the isolated vascular organ, and to study the effect of *S. scutellarioides* in vasoconstriction by $\alpha 1$ receptors stimulation.

***Methodology*:** Rat aortic rings were placed in an organ bath equipment (BIOPAC®), at 37°C temperature and constant carbogen gas bubbling. Tension changes were registered with an isometric tension transducer and data acquisition equipment. During the standardization, six curves of concentration-tension with phenylephrine at 1, 3 and 5 hours were registered, in order to determine maximum tension (T_M), and the effective concentration 50 (EC_{50}). Finally, three concentration-tension curves were obtained for phenylephrine with *S. scutellarioides* at a concentration of 10 mg/dl and 20 mg/dl.

***Results*:** During the technique standardization, no statistically significant differences were found in T_M for phenylephrine at 1, 3 and 5 hours. The administration of *S. scutellarioides* produced a T_M dose dependent reduction with no EC_{50} changes.

***Discussion*:** The implemented isolated organ technique is viable up to 5 hours, and does not produce $\alpha 1$ receptors desensibilization. The T_M diminution by *S. scutellarioides* is explained by a non competitive $\alpha 1$ antagonism effect. Studies to isolate active principles for allowing the initiation of phase I human clinical trials are required.

Keywords: *Salvia; Hypertension; Mastranto; Ethnopharmacology; Vascular isolated organ; Vasoconstriction.*

Implementación de la técnica en órgano aislado vascular como herramienta para la validación de plantas medicinales: Estudio del efecto vasodilatador de la Salvia scutellarioides

RESUMEN

Introducción: La *Salvia scutellarioides* es una planta con efecto antihipertensivo y diurético. En este estudio se evalúa su efecto vasodilatador con la técnica de órgano aislado vascular.

Objetivos: Estandarizar la técnica de órgano aislado vascular y estudiar el efecto de *S. scutellarioides* en la vasoconstricción por estimulación de receptores $\alpha 1$.

Metodología: Se colocaron anillos de aorta de rata en un equipo de baño de órganos (BIOPAC®) a una temperatura de 37°C y burbujeo constante de gas carbógeno. Los cambios en la tensión se registraron con un transductor de tensión isométrica y un equipo de adquisición de datos. En la estandarización, se realizaron seis curvas de concentración-tensión con fenilefrina a las horas 1, 3 y 5, para determinar la tensión máxima (T_M) y la concentración efectiva 50 (EC_{50}). Finalmente, se obtuvieron curvas de concentración-tensión para fenilefrina con *S. scutellarioides* a una concentración de 10 mg/dl y 20 mg/dl.

Resultados: En la estandarización de la técnica no se encontraron diferencias estadísticamente significativas en la T_M y la EC_{50} para fenilefrina en la primera, tercera y quinta horas. La administración de *S. scutellariodes* ocasionó una disminución concentración dependiente de la T_M sin cambios en la EC_{50}.

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1. Assistant Professor, Department of Physiological Sciences, Universidad del Valle, Cali, Colombia.
e-mail: jorgehramirez31@yahoo.com e-mail: maopalacios@yahoo.com
2. Associate Professor, Department of Physiological Sciences, Universidad del Valle, Cali, Colombia.
e-mail: ogutie@telesat.com.co

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Discusión: La técnica de órgano aislado implementada es viable hasta por cinco horas y no ocasiona desensibilización de receptores α1. La disminución de la T_M por la *S. scutellarioides* se explica por un efecto de tipo α1 antagonista no competitivo. Se requieren estudios para aislar principios activos que permitan iniciar ensayos clínicos fase I en los seres humanos.

Palabras clave: *Salvia; Hipertensión; Mastranto; Etnofarmacología; Órgano aislado vascular; Vasoconstricción.*

Studies in isolated organ are a useful tool to evaluate the pharmacological activity of a drug in the receptors, channels and enzymes of a tissue[1,2]. Recently, the use *in vitro* techniques has increased in the area of ethnopharmacology to evaluate the effects of plant derived extracts and molecules. The growing use of this technique by the international scientific community is due to the fact that it is cheap, requires fewer animals in comparison with *in vivo* models, and permits the evaluation of the pharmacological activity of a great variety of extracts and molecules of vegetal origin in a short period of time[3].

Colombia has the greatest density of vegetal biodiversity of the planet, and occupies the second place in number of species after Brazil. Nevertheless, more than 99% of the vegetal species have not been studied as potential source of medicines, despite that an extensive proportion of the Colombian population depends on the use of medicinal plants. Accordingly, it is necessary to apply cost-effective methods that use a reduced number of animals and permit to evaluate in the shortest possible time the pharmacological activity of the Colombian biodiversity. The investigation in Colombian ethnopharmacology is a priority need for the country to permit the social appropriation of the knowledge, important to avoid biopiracy in the era of globalization and free commerce[4-6].

In this study the implementation of the technique of isolated vascular, the effects of time in the contraction of smooth muscle and the action of repeated dose of phenylephrine in the sensibility of α1 receptors is presented. Finally, the vasodilator effect of *Salvia scutellarioides* (Lamiaceae) was evaluated since is a plant used extensively by the Colombian population for the treatment of arterial hypertension and recently reported by our group as antihypertensive and diuretic[7,8].

The project was approved by the Committee of Animal Ethics of the Universidad del Valle, Minute N° 16 July 18 of 2002.

MATERIAL AND METHODS

Tissue preparation. Five male Sprague-Dawley rats with weight from 200 to 250 g were anesthetized with pentobarbital (100 mg/kg) for thoracotomy and extraction of the toracic aorta. The artery was placed in a petri dish with Krebs-Henseleit solution (119 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$ and 5.6 mM glucose) at 4ºC plus a continuous supplement of carbogen (CO_2: 5% - O_2: 95%). The aorta was carefully dissected with a scalpel inside the solution to obtain three concentric rings of approximately 5 mm each; besides, the endothelium was removed with a PE-100 polyethylene tube. For the assembly, each vascular ring was fixed by its endings with two silver wires and placed in an organ bath [Tissue Bath Station, BIOPAC™] with Krebs-Henseleit solution (20 ml) at 37ºC plus a continuous supplement of carbogen (Photo 1). One of the wires was fixed to the organ bath and the other to a 50 g isometric transducer. [TSD125, BIOPAC™] connected to a data acquisition equipment [MP-100, BIOPAC™] and this to the computer. The basal tension applied to the vascular rings was 1 g. The results of the vascular smooth muscle tension and the effects of drugs were observed, in real time, with the use of Acknowledge software v. 3.07 for Windows XP® (Graphic 1). All the results were stored in electronic format for subsequent analysis.

Determination of the time dependent effects on vascular smooth muscle contraction. Once the vascular ring was adapted in the organ bath, we proceeded to stimulate the tissue response during the first hour of experimentation adding KCl (25 mM) every 20 minutes, and continuously changing the Krebs-Henseleit solution. After the first hour had elapsed, and once the stabilization of the tissue was reached, a concentration curve vs. tension with phenylephrine (SIGMA®) was carried out added to the organ bath in a cumulative way (10 μM to 100). After obtaining the maximal tension (MT) with phenylephrine, acethylcoline was added (10 μM) to verify the non viability of the endothelium. The curve with phenylephrine was repeated at the third, fifth and seventh hour in six vascular rings to determine *in vitro* the temporary changes of the vascular smooth muscle maximal tension and the desensitization of α1 receptors to phenylephrine.

Effect of Salvia scutellarioides in vascular smooth

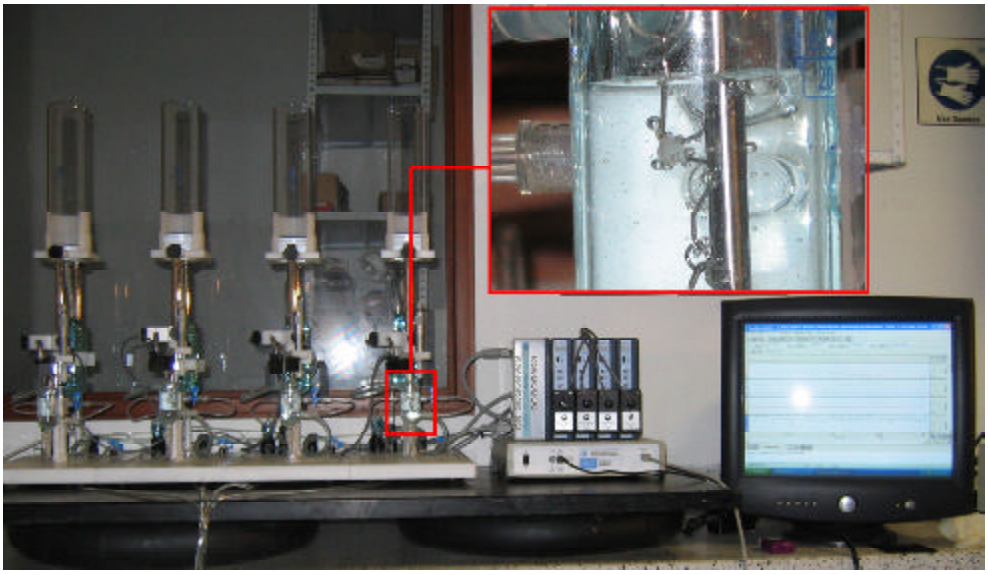
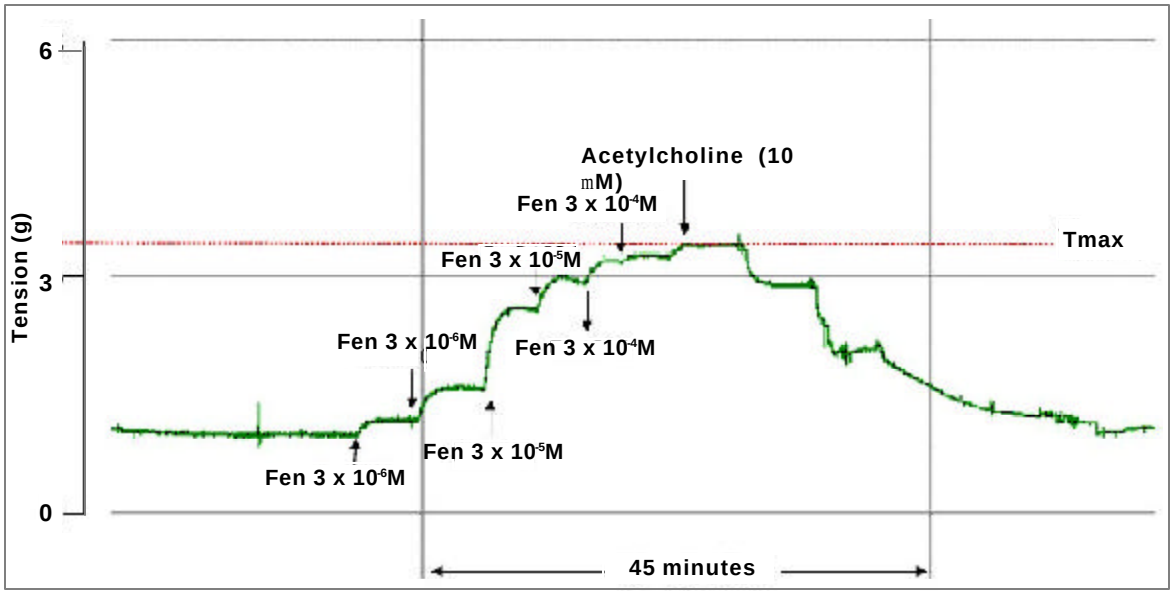


Photo 1. ***Setting of the vascular isolated organ.*** **The organ bath with the data acquisition equipment is shown. In the insert, an aorta suspended by two silver hooks in Krebs-Henseleit solution, with continuous carbogen gas supplement.**



Graphic 1. ***Acknowledge Software 3.07 data Acquisition.*** **The graphic shows the acquisition of data and the vascular smooth muscle contraction induced by phenylephrine (fen). The acetylcholine did not cause a decrease of the maximal tension (MT) proving the vascular rings endothelial destruction.**

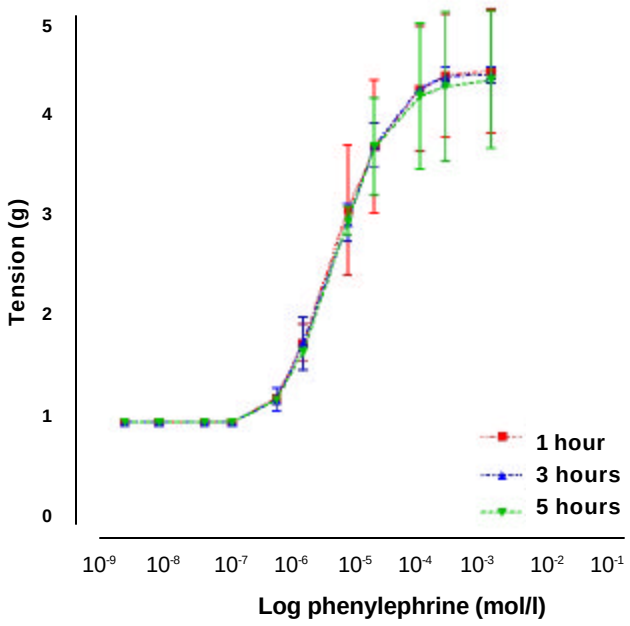
muscle. The place of plant collection, the certification by botanic biology and the processes for the preparation of the extract are described with detail in a previous publication[7]. The effect of the *S. scutellarioides* acuous extract was determined with the use of three vascular rings. During the first hour, a concentration - tension control curve with phenylephrine was carried out with each vascular ring. At the third and fifth hour, a new curve was obtained with phenylephrine plus the aqueous extract of *S. scutellarioides* at concentrations of 10 mg/dl and 20

mg/dl respectively.

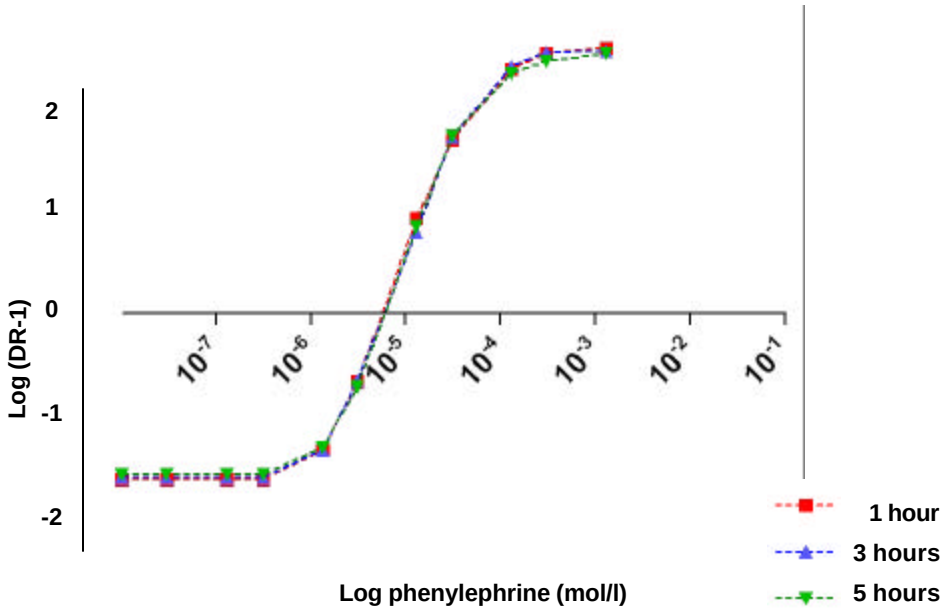
Statistical analysis. The data of the obtained tension with the phenylephrine concentrations were analyzed and graphicated with the GraphPad 4.02 for Windows program. To calculate the concentration of the drug that cause a 50% of the maximum tension (EC_{50}) the analysis of Hill[9] was used. The data were analyzed by means of two way ANOVA (concentration vs. tension) a $p<0.05$ being accepted *a priori* as indicator of statistically significant differences. The results are shown as the average of the group ± standard error of the mean (SEM).

RESULTS

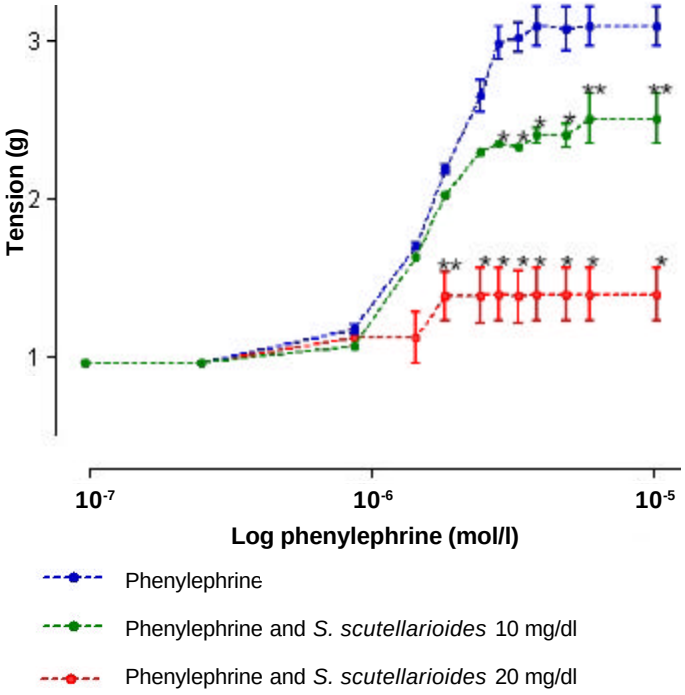
Effect of time in the contraction of vascular smooth muscle and EC_{50} for phenylephrine. The maximum tension for phenylephrine at the third and fifth hours did not show statistically significant differences in comparison with the curve control of the first hour (Graphic 2). At seventh hour of experimentation, no contractile response was obtained with phenylephrine in none of the vascular rings studied. The analysis of Hill (Graphic 3) gave as a result an EC_{50} of 5.4 x 10-6 mol/l of phenylephrine for the curves obtained at the first, third and fifth hour of experimentation.



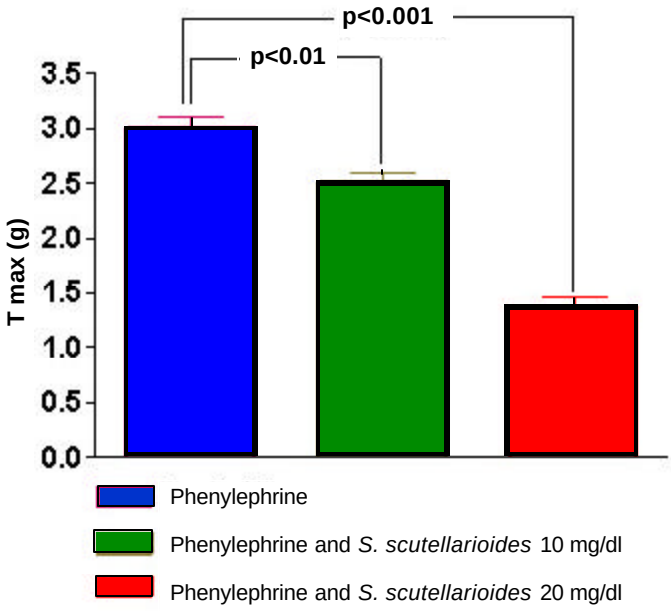
Graphic 2. ***Log Curve phenylephrine [mol/l] vs. Tension (g).*** **It shows the tension curve obtained with cumulative doses of phenylephrine at the first, third and fifth hours. No statistically significant differences between the curves were observed. The data are expressed as the average of the group ± SEM.**



Graphic 3. ***Hill plot.*** **The graphic shows the necessary phenylephrine concentration to reach 50% of the maximum tension (EC_{50}). The EC_{50} for phenylephrine did not show differences at the first, third and fifth hours of experimentation.**



Graphic 4. *Effect of S. scutellarioides in vascular isolated organ.* The graphic shows the effect of *S. scutellarioides* in the decrease of the vascular smooth muscle MT with phenylephrine (n=3). The data are expressed as the average of the group ± SEM. **p<0.01; *p<0.001



Graphic 5. Effect of the *S. scutellarioides* in phenylephrine MT. The graphic shows the effects of the administration of the aqueous extract of *S. scutellarioides* in the MT for phenylephrine.

Effect of the S. scutellarioides in the contraction of the vascular smooth muscle. The maximum tension for the phenylephrine in combination with the aqueous extract of *S. scutellarioides* at a concentration of 10 mg/dl was 2.44 g ± 0.13, equivalent to a decrease of the maximum tension of 17% in comparison with the control curve for phenylephrine ($p<0.01$). With the aqueous extract of *S. scutellarioides* at a concentration of 20 mg/dl a maximum tension obtained for phenylephrine was 1.4g ± 0.15, equal to a decrease of the maximum tension of 54% in comparison with the control curve for phenylephrine ($p<0.001$) (Graphics 4 and 5). The aqueous extract of *S. scutellarioides* at the concentrations studied did not produce differences in the EC_{50} for phenylephrine.

DISCUSSION

The studies in isolated organ are considered as a useful technique for the evaluation of the effect of medicines and plants derived extracts in smooth muscle. Nevertheless, the technique of vascular isolated organ presents as disadvantage the difficulty to maintain the tissue viable for the execution of the pharmacological assays. This itself is due to the excessive surgical manipulation of the tissue, tissue hypoxia by changing carbogen gas bubbling and inappropriate concentrations of electrolytes in the Krebs solution[1]. Moreover, with the to elapse of minutes, the vascular muscle smooth could present fatigue and decrease of the maximum tension, producing a confusion factor when the agonist first curve is taken as control. Another factor that causes difficulties for the implementation of the technique, is the desensitization of the receptors caused by the administration of agonistas, phenomenon that occurs due to the phosphorilation of the receptor or its recapture to the cytoplasm in a process mediated by chlatrine[10].

In the reproduction of the model of vascular isolated organ, no statistically significant differences in the maximum tension were found for phenylephrine at the first, third and fifth hours. Nevertheless, at seventh hour no contractile response was obtained with phenylephrine, therefore the experiments in vascular isolated organ should only be carried out for a maximum of five hours. The EC_{50} for the phenylephrine at the first, third and fifth hours did not show

statistically significant differences, indicating that the sensibility of the adrenergic receptors was maintained without changes during the five hours of experimentation. The implementation of this technique will permit the evaluation of Colombian plants with effects in vascular smooth muscle, as a screening method in the search of active principles with cardiovascular action. The *S. scutellarioides* (10 mg/dl and 20 mg/dl) caused a decrease in the maximum tension with phenylephrine, without altering its EC_{50}. This type of effect is due to the fact that at least one of the molecules that compose the aqueous extract of the *S. scutellarioides* has a non competitive antagonist type effect to some medicines as phenoxibenzamine[11]. Other plants of the Lamiaceae family as *Satureja obovata* and *Orthosiphon aristatus* present a similar vasodilator effect as *S. Scutellarioides*[12,13]. Besides, protection against the endothelial cells oxidative stress and the inhibition of the proliferation of vascular smooth muscle by *Salvia miltiorrhiza* was recently described[14,15].

The vasodilator effect of the *S. scutellarioides* can explain the decrease of the diastolic arterial tension in the rats L-NAME hypertensive model[7]. Nevertheless, it should be clarified that the pharmacological action of a plant can result of the additive and/or synergic action of various active principles[16], so that other mechanisms such as the increase of diuresis and natriuresis can also explain the antihypertensive effect of the *S. Scutellarioides*[8]. Recently, it was determined that *S. scutellarioides* did not present subacute toxicity when administered for 28 days to Wistar rats[17]. The continuity of the studies with the *S. scutellarioides* is justified due to the relative safety of the plant in humans, the extensive use in Colombian traditional medicine and the positive results obtained with animal models and *in vitro*. Specifically, the main active principles of the plant should be isolated and its pharmacodynamic characteristics defined, so the clinical trials phase in humans could start.

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