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Research Article

Detection of the Mixed Disulfide Coenzyme A-S-S Glutathione: A Hypertensive Factor in Human Plasma of Young and Adult Individuals With or Without Hypertension

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Abstract

Background: We have previously demonstrated the presence of a nucleotide peptide from rat liver which was identified as a mixed disulfide formed by Coenzyme A-SS-Glutathione linked by a disulfide bridge. Also informed about the presence in rat liver and other tissues of the NADPH-dependent Coenzyme A-SS-G reductase. Now we communicate about the presence of Coenzyme A-SS- Glutathione as a hypertensive agent in human plasma of 22 young and adult individuals with or without hypertension.

Methods: In the present work the isolation of Coenzyme A-SS-Glutathione from rat liver to be used as standard and the human blood samples were extracted with Perchloric acid (HCLO4) neutralized with 2 N KOH; the supernatant was lyophilized and dissolved in a minimal amount of water in order to be separated by High Pressure Liquid Chromatography (HPLC) system with UV detector capable of measurement at 254 nm.

Results: We have demonstrated for the first time the presence in human blood of young and adult individuals of the hypertensive compound CoASSG. Two subgroups could be observed among the young individuals according to the amount of CoASSG in plasma: in subgroup number 1 an average amount of CoASSG/ul of plasma of 1.895 and in group number 2 with an average amount of 7.92.

In the group of adults also two subgroups could be detected: subgroup 3 with an average of 2.756 and subgroup 4 with an average of 12.95.

Conclusion: According to our results it can be appreciated that the mixed disulfide compound (CoASSG) is present in the blood of young individuals and in higher amounts in older people.

Introduction

In this paper we report the presence of the mixed disulfide Coenzyme A-SS- Glutathione in human plasma of 22 individuals.

This compound was originally demonstrated and characterized in our laboratory from rat liver as well as the corresponding enzyme NADPH-dependent CoASSG reductase [1-9] and lately identified by other authors in bovine adrenal [10] and human parathyroid glands as a potent vasoconstrictor [11] also as a potent modulator of angiotensin II inducing vasoconstriction in the isolated perfused rat kidney [12]. CoenzymeA glutathione disulfide (CoASSG) has recently been isolated from bovine adrenal glands and is assumed to play an important role in Blood Pressure (BP) control. It has

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been concluded that CoASSG is able to potentiate the vasoactive properties of AngII, and that CoASSG might play an important role in BP regulation via modulating effects of AngII. Am J Hypertens 2001;14:164–168 [13].

According to the World Health Organization (WHO), hypertension is a serious medical condition that significantly increases the risks of other diseases. An estimated 1.13 billion people worldwide have hypertension, most living in low and middle-income countries.

Based on the above findings we decided to study the presence of this hypertensive factor (CoA-SS-G) in human blood plasma of young and adult individuals in order to see if there is a correlation between the presence and amount of this compound in normal and hypertensive people.

Methods

1. Isolation of Coenzyme A-SS-Glutathione from rat liver to be used as standard

Perchloric acid ($HClO_4$) extracts following neutralization with 2N KOH were made from rat liver Wistar rats fed on stock laboratory diet: one extract from two young rats (one liver with a weight of 9.69 g and another of 14.54 g) and from two adult rats (rat liver weight of 26.58 grs and another of 20.74 grs).

The supernatant was lyophilized and dissolved in a minimal amount of water in order to be separated by High-Performance Liquid Chromatography (HPLC) system with UV detector capable of measurement at 254 nm (Figure 1).

2. Separation of CoASSG from human blood plasma samples by High-performance liquid chromatography (HPLC)

From each of the 22 individuals approximately 5 mL of blood were obtained in the presence of anticoagulant and N-ethyl maleimide (NEM) in order to block the thiol compounds such as CoASH. After centrifugation the cell fraction was eliminated and the plasma precipitated with 4% perchloric acid ($HClO_4$) and neutralized with KOH. The precipitate was eliminated by centrifugation and the extract submitted to HPLC chromatography with UV detector capable of measurement at 254 nm. The area corresponding to CoASSG was submitted to mass spectrometry and gave a molecular weight of 1072.7

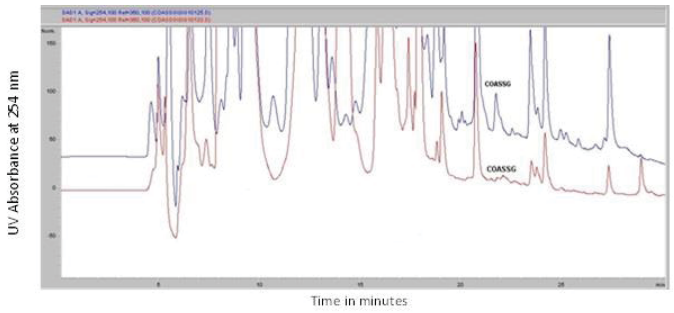

Figure 1: Comparison of two HPLC elution diagrams: one obtained from adult Rats showing the presence of CoASSG compared with the extract from young rats which did not showed enough amount (blue line represents adult rats and red line the young animals).

The present project was approved by the Research Division of the Faculty of Medicine UNAM, through the Research and Ethical Commissions on May 6 of 2014. (No FMED/SPLR/149/2014) To obtain blood samples and related information the donors provided informed consent, confirming that their data would remain confidential and be used solely for research purposes.

Note: The obtaining of the human blood samples was done under the direction of Malaquías López Cervantes, by Ricardo Antonio Escamilla, and Julia B. Ramirez Gonzalez. The acid extracts were prepared by Eva Hernández, the HPLC separation and Mass spectrometry analysis of the mixed disulfide CoASSG by Gerardo Hurtado.

Results

In order to have the CoASSG used as standard and be able to identify it in the human blood samples we made extracts from rat liver of young and old animals as it can be appreciated in Figure 1.

Then we proceeded to study 22 human blood plasma samples corresponding to two groups:

A - 10 young people between 23 to 40 years old;

B - 12 adults from 41 to 60 years old;

Table 1 presents the sex, age, and blood pressure of the 22 individuals. It can be appreciated that the first 10 young subjects have normal blood pressure. The following 12 adults also have normal blood pressure except the female M4 S325 of 54 years old with blood pressure of 138/100 mmHg and 2 old adults with high blood pressure (A11 and A12).

In Table 2, it can be appreciated the quantitative results of CoASSG (nanomoles/ul) in plasma of 22 normal and hypertensive individuals. Two adults (A11 and A12) had high blood pressure but were not receiving antihypertensive treatment are shown below.

1. In the above Table 2 it can be observed in the group corresponding to young individuals two subgroups according to the amount of CoASSG in plasma:(J1=2.25; J2=1.93; J3=2.8; J6=1.7; J7=0.76; J8=1.93) with an average amount of CoASSG/ul of plasma of 1.895 and

2. (J4 =7.62; J5 =7.61; J9 =9.15; J10 =7.33) with the average amount of 7.92

In the group of adults also two subgroups can be detected:

3. (M1= 0.00; M3 = 3.09; M4 = 1.33; M5 = 3.68; M8 = 6.27; M9 =2.17) with an average of 2.756

4. (M2 =11.22; M6 = 11.51; M7 =14.14; M10 =10.42; A11= 20.3 and A12= 10.12) the average was 12.95.

In the next two elution HPLC diagrams appear as examples the results corresponding to:

A young female (J1) 26 years old) with 2.25 nanomoles of CoASSG/ul of plasma (Figure 2),

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Table 1: Data of 22 individuals showing their sex, age and with normal or high blood pressure.

ID SAMPLE	SEX	AGE	HAS	SYS	DIA
J1	F	26	NORMAL	120	80
J2	M	28	NORMAL	104	78
J3	F	25	NORMAL	102	64
J4	F	29	NORMAL	100	62
J5	M	23	NORMAL	114	68
J6	F	36	NORMAL	110	80
J7	F	25	NORMAL	100	64
J8	M	34	NORMAL	110	68
J9	F	40	NORMAL	100	70
J10	F	38	NORMAL	120	80
M1	F	48	NORMAL	108	78
M2	F	55	NORMAL	120	80
M3	F	53	NORMAL	120	76
M4	F	54	HIGH	138	100
M5	F	43	NORMAL	126	80
M6	F	41	NORMAL	112	70
M7	M	59	NORMAL	110	80
M8	F	55	NORMAL	126	70
M9	F	41	NORMAL	112	76
M10	F	52	NORMAL	127	80
A11	F	60	HIGH	165	113
A12	M	60	HIGH	157	110

Table 2: Quantitative data of CoASSG from 22 plasma of normal and Hypertensive Human individuals.

ID SAMPLE	SEX	AGE	HAS	nanoM/ul	
J1	F	26	NORMAL	2.25	Young with Normal blood pressure
J2	M	28	NORMAL	1.93	
J3	F	25	NORMAL	2.8	
J4	F	29	NORMAL	7.52	
J5	M	23	NORMAL	7.61	
J6	F	36	NORMAL	1.7	
J7	F	25	NORMAL	0.76	
J8	M	34	NORMAL	1.93	
J9	F	40	NORMAL	9.15	
J10	F	38	NORMAL	7.33	
M1	F	48	NORMAL	0	Adult individuals
M2	F	55	NORMAL	11.22	
M3	F	53	NORMAL	3.09	
M4	F	54	HIGH	1.33	
M5	F	43	NORMAL	3.68	
M6	F	41	NORMAL	11.51	
M7	M	59	NORMAL	14.14	
M8	F	55	NORMAL	6.27	
M9	F	41	NORMAL	2.17	
M10	F	52	NORMAL	10.42	
A11	F	60	HIGH	20.30	
A12	M	60	HIGH	10.12	

An adult female (M2, 55 years old) with 11.22 nmol/µL of plasma CoASSG (Figure 3),

It can be appreciated that there is a great difference in the amount of CoASSG in plasma of the young female of 26 years old with 2.25 nanomoles/ul of plasma and the adult female 55 years old with 11.22 nanomoles/ul of plasma.

Additionally, we could obtain two results from older individuals with high blood pressure that were not receiving recently any antihypertensive treatment, one is an adult female 60 years old with 20.30 nanomoles of CoASSG / ul of plasma (Table 2) and the other is an adult man 60 years old with 10.12 nanomoles of CoASSG / ul of plasma.

Discussion

We are aware that the hypertension problem in the human population has multiple causes and represents one of the most significant health conditions [14]. One of these factors is Arginine Vasopressin (AVP) since when it is released in high concentrations may also raise blood pressure by inducing moderate vasoconstriction, although it has a very short half-life, between 16–24 minutes. Arginine vasopressin was first described by du Vigneaud in 1954 [15] as a cyclic nonapeptide with a molecular weight of 1084 Da and showing a disulphide bridge.

This fact of the presence of a disulfide bridge in both compounds is very important since there is a chemical reaction known as a thiol / disulfide exchange which permits the dissociation of the compound either by chemical or enzymatic reaction [16,17].

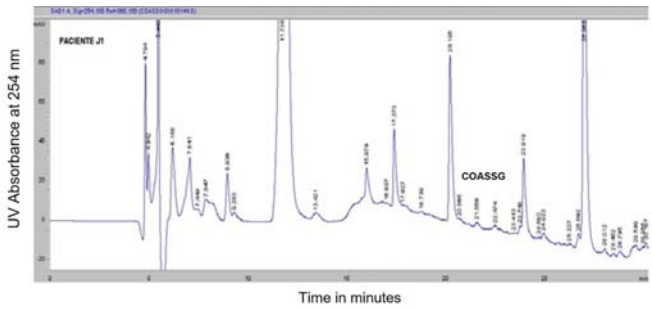

Figure 2: HPLC elution diagram of a young female (J1) 26 years old with 2.25 nanomoles of CoASSG/ul of plasma.

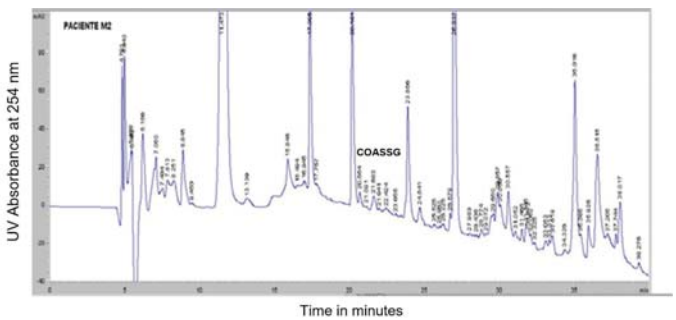

Figure 3: HPLC elution diagram of an adult female (M2) 55 years old with 11.22 nanomoles of CoASSG/ul of plasma.

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According to our results it can be appreciated that the mixed disulfide compound (CoASSG) is present in the blood of young individuals and in higher amounts in older people but we don't know what is the reason of this increase, possibly due to reduced NADPH-dependent CoASSG reductase activity with ageing the NADPH-dependent CoASSG reductase loses activity and then the substrate accumulates, contributing to hypertension.

Originally, our hypothesis was based on the idea that young people with a high concentration of CoenzymeA-SS-Glutathione in their blood would help to diagnose a major predisposition to have hypertension.

With the results presented here this hypothesis seems plausible but it will be necessary to run a large number of analyses with blood samples from young and old people in order to establish the normal and abnormal parameters of CoASSG and its enzyme NADPH- dependent CoASSG reductase.

Acknowledgement

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