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Biochemical link between chromogranin A and cyclooxygenase -2 in pheochromocytoma pathology

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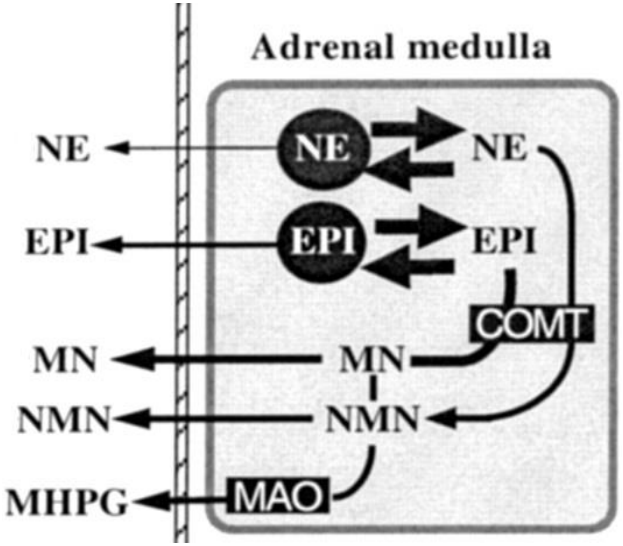
Graphical Abstract



Cox-2 ➡ CgA ➡ Pheochromocytoma Dense-core chromaffin granules NMNp/MNp/CgA

Catecholamine metabolism Contemporary view
Eisenhofer et al.Pharmacol Rev 56:331-349,2004

Abstract: The precise biological function of elevated chromogranin A (CgA) in neuroendocrine and nonneuroendocrine neoplasms remains unclear. In a neuroendocrine tumor (NET)-derived cell line study it was demonstrated that cyclooxygenase-2(Cox-2) up-regulates both CgA expression and bioactivity with implications of this polypeptide in neuroendocrine cancer. In our study, we indirectly tested the link between Cox-2 and CgA in 15 patients clinically suspected of pheochromocytoma by comparison with a 15 matched controls without endocrine dysfunction. Biochemical diagnosis of pheochromocytoma was realized by differentially assay of plasma free normetanephrines (NMNp) /free metanephrines (MNp) and by plasma assay of CgA. Cox-2 was tested as a new parameter. All four parameters were assayed both in tumoral and normal subjects. We established statistically significant differences between all parameters assayed. Multiple regression showed important correlation coefficients between: NMNp/CgA; CgA/Cox-2;NMNp/MNp. Practically, we proved the traffic control of the noradrenergic metabolite NMNp by CgA and Cox-2. Using Relative Operating Curve Analysis (ROC) we could compare sensitivity and specificity of all four assayed parameters. Cox-2, CgA, NMNp proved the best sensitivity and a great specificity. We can conclude that Cox-2 could be used as a prediction marker in pheochromocytoma pathology together with CgA/NMNp/MNp.

Keywords: *Pheochromocytoma; Chromogranin A; Cyclooxygenase-2; Free Metanephrines; Relative Operating Curve Analysis*

Introduction

- The precise biological function of elevated chromogranin A (CgA) in neuroendocrine and nonneuroendocrine neoplasms remains unclear
- Limited studies in cell and animal models have provided contradictory evidence as to whether CgA promotes or inhibits tumorigenesis
- In a neuroendocrine tumor (NET) - derived cell line study it was demonstrated that cyclooxygenase-2(Cox-2) up-regulates both CgA expression and bioactivity with implications of this polypeptide in neuroendocrine cancer
- It has been reported that Cox-2 was expressed in pheochromocytoma tissue but it was not present in normal adrenal medulla tissue
- The aim of the current biochemical study was to prove the link between Cox-2 and CgA based on a clinically suspected pheochromocytoma patients group by comparison with a control group subjects

Results and discussion

- In our retrospective study (2013-2015), we investigated from biochemical point of view 15 patients: 13 women aged 33-72 years and 2 men aged 43-77 years (clinically suspected of pheochromocytoma) by comparison with a lot of 15 matched controls without endocrine dysfunction
- One plasma (EDTA vacutainer) and one total blood vacutainer were sampled before 9am from all subjects (after a night fasting without no drugs)
- Free plasma normetanephrines (NMNp) and free plasma metanephrines (MN) by Elisa Research differentially procedures
- Serum Chromogranin A (CgA) by an Elisa method for in-vitro diagnostic use
- Plasma Cyclooxygenase-2(Cox-2) by an Elisa research procedure
- Statistical analysis was performed using Med Calc Software version 14.12.0 Windows 98/NT/Me/2000/XP
- Sensitivity and specificity for all parameters were tested by Receiver Operating Curves (ROC analysis)

Results and discussion

- Biochemical diagnosis of pheochromocytoma was based on plasma differential assay of ***NMNp*** and ***MNp*** and serum assay of ***Cg A*** both in tumoral cases and normal subjects
- We tested also plasma ***Cox-2*** by an adapted research Elisa assay specific for cell lysates
- In **Table 1 mean values** for all 4 parameters: ***NMNp, MNp, CgA*** and ***Cox-2*** were overincreased in tumoral cases vs normal subjects
- In **pheochromocytoma group** vs **controls**: ***NMNp*** was **30-times higher**; ***MNp*** was **84-times higher; CgA** was ***12-times higher*** and ***Cox-2*** was ***6- times higher***
- All 4 parameters were statistically analyzed in both groups of subjects and in tumoral group they were significantly increased by comparison with the same parameters in control group ($P < 0.05$)
- We calculated a high **Spearman correlation coefficient** between **NMNp and CgA (R=0.86**), **NMNp and MNp (R=0.70**) and a good correlation between **CgA and Cox-2 (R=0.56)**

Results and discussion

- In **Table 2 ,threshold values** calculated were as it follows: ***NMNp***: 101pg/mL**;** ***MNp***: 68pg/mL**;*CgA*** : 90ng/mL; ***Cox-2*** : 0.3ng/mL
- The best **sensitivities** calculated were equal for: ***NMNp***; ***CgA***; ***Cox-2***
- The best **specificities** were in decreased order: ***MNp, CgA>NMNp>Cox-2.*** Percentage of **Positive predictive values** was in decreased order: ***MNp, CgA > NMNp > Cox-2***
- Percentage of **Negative predictive values** was in decreased order: ***NMNp, CgA***, ***Cox-2 >MNp***
- In **Table 3, ROC** comparison between **areas of different parameters pairs**: ***Cox-2/CgA***;***Cox-2/NMNp***; ***Cox-2/MNp*** and ***CgA/NMNp*** showed no statistically significant differences

Results and discussion

Table 1 - Mean values comparison in pheochromocytoma cases vs normal subjects

LOT/number of cases	NMNp pg/mL mean± SE	MNp pg/mL mean± SE	CgA ng/mL mean± SE	Cox-2 ng/mL mean± SE
TUMORAL/15	2056.13 ± 510.96	3365 ± 236.12	773.86 ± 170.59	4,96 ± 1.39
NORMAL/15	67.20 ± 7.99	40.20 ± 4.54	65,8 ± 3,91	0,78 ± 0.34
t-Test	P < 0.0001	P = 0.001	P = 0.0041	P < 0.0001
Spearman's coefficient R	NMNp/CgA 0.86	NMNp/MNp 0.70	CgA/Cox-2 0.56	

Results and discussion

Table 2 - Cut-off values,sensitivity,specificity, positive and negative prediction values for all 4 parameters

Parameter	Threshold value	% Sensitivity	% Specificity	+ PPV*	- PPV**
NMNp	>101	100	93.3	93.7	100
MNp	>68	66.7	100	100	75
CgA	>90	100	100	100	100
Cox-2	>0.3	100	80	83.3	100

*Positive predictive value
**Negative predictive value

Results and discussion

Table 3- Areas comparison by ROC analysis

Parameters/Pairs	Areas comparison	Statistical significance
Cox-2/CgA	0,098 ± 0,059	p=0.098
Cox-2/NMNp	0,093 ± 0,057	p=0.103
Cox-2/MNp	0,104 ± 0,085	p=0.221
CgA/NMNp	0,004 ± 0,013	p=0.724

Results and discussion

- Although pheochromocytoma is a rare cause of hypertension it may be removed surgically in more than 90% of patients but in untreated cases it could be lethal
- Early diagnosis is important to avoid hypertensive complications but also because of the approximately 10% incidence of malignancy
- Independent studies showed that initial screening of pheochromocytoma should always include plasma or both plasma/urine measurements of free metanephrines as degradation products of intratumoral metabolism of catecholamines/2/
- CgA is an acidic protein costored and coreleased by exocytosis, along with catecholamines from chromaffin granules of normal adrenal medulla and pheochromocytoma
- In patients with pheochromocytoma plasma CgA is markedly elevated and parallels tumor mass/5/
- In our study, we diagnosed all 15 cases clinically suspected of pheochromocytoma by differentially plasma metabolites NMNp/MNp assays and by CgA plasma assay

Results and discussion

- Cyclooxygenase is the key enzyme in the conversion of arachidonic acid to prostaglandin and thromboxane eicosainoids
- Cox-2 is the inducible form of the enzyme and was associated with carcinogenesis
- Cox-2 is overexpressed in many human malignancies/1,3,4,6/
- Our plasma Cox-2 data were significantly increased in all tumoral cases
- By courtesy, we assayed a tissue lysate from a pheochromocytoma operated in another medical unit not in our institute and belonging to one patient from our investigated group
- So, we could verify the presence of tissue Cox-2 in pheocromocytoma 6-times higher than Cox-2 value in normal tissue
- With respect to neuroendocrine tumors, it has been reported that although absent from normal adrenal medulla,Cox-2 is expressed in pheochromocytoma

Results and discussion

- Cox-2 up-regulates both CgA expression and bioactivity in a neuroendocrine cell line
- Cox-2 dependent CgA up-regulation was associated with significantly increased chromaffin granules number
- Cox-2 dependent CgA up-regulation was associated with significantly increased chromaffin granules number/1,3,4,5,7/
- Multiple regression proved a high multiple correlation coefficient(R= 0.77) with dependent variable NMNp and independent variables:Cox2,CgA
- Cox-2 plasma values proved a good positive prediction value and the best negative prediction value
- Cox-2,CgA,NMNp proved the best sensitivity and a great specificity
- Our results pointed out plasma Cox-2 could be used as a pheochromocytoma prediction marker besides NMNp/MNp and CgA
- Some authors suggested a relationship between CgA-mediated chromaffin granule biogenesis necessary for catecholamine storage

Results and discussion

- The ability to regulate the number of granules formed in neuroendocrine cells is unique to CgA/1,3,5,7,8/
- We could speculate the presence of a great number of chromaffin granules in our pheochromocytoma cases regulated by CgA under Cox-2 stimulation
- This argument could explain the presence of an intense synthesis of catecholamines and an excessive intratumoral metabolism to free metanephrines illustrated by increased plasma values of plasma NMNp/MNp
- Practically, we proved the traffic control of the adrenergic metabolites NMNp/Mp by CgA and Cox-2

Conclusions

- In our study we could not precise the nature of pheochromocytoma because all cases biochemically diagnosed by us were operated in other medical units
- Our study underlined indirectly the link between Cox-2 and CgA in pheochromocytoma cases using plasma data
- In our opinion, we have not found such an approach in the literature consulted
- Practically, we proved the traffic control of the adrenergic metabolites NMNp/MNp by CgA and Cox-2
- Cox-2, CgA, NMNp proved the best sensitivity and a great specificity
- We can conclude that Cox-2 could be used as a prediction marker in pheochromocytoma pathology together with CgA/NMNp/MNp.

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Acknowledgments

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