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Brain cyclooxygenase-2 gene induction by interleukin-1β following chemotherapy

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RESUMEN

Antecedentes: algunos estímulos inflamatorios elevan levemente la expresión de la COX-1, pero son bien conocidos por conducir un rápido aumento de ARNm de COX-2, lo que sugiere que la COX-2 desempeña un papel en el proceso de inflamación. COX-2 también puede jugar un papel importante en la neurodegeneración y en particular en la muerte neuronal citotóxica.
Objetivo: analizar en tejido celular de autopsias de 13 pacientes con cáncer –cinco de ellos tratados con quimioterapia y los otros ocho pacientes, sin quimiuoterpaia– la expresión de la ciclooxigenasa-2 (COX-2), ARNm e interleucina-1β.
Material y métodos: se analizó en tejido cerebral de autopsias la interleucina-1β y la expresión de ciclooxigenasa-2 de 13 pacientes con diferentes tipos de tumores malignos primarios, de los cuales cinco fueron tratados con quimioterapia y ocho sin quimioterapia. Se realizó RT-PCR en tiempo real de ARN total de tejido de la corteza cerebral y materia blanca preservados a -80°C, se utilizó fluoróforo marcado con sondas TaqMan específicas para la COX-2 e IL-1β.
Resultados: en el tejido cerebral de los pacientes con quimioterapia se observó significativamente mayor expresión de la ciclooxigenasa-2 e interleucina-1β en la corteza frontal y en el hipocampo.
Conclusión: estos resultados sugieren que la exposición a los fármacos citotóxicos se asocia con la inducción de los genes de COX-2 y de la interleucina-1β, lo que genera la disfunción neuronal que podría preceder a las complicaciones neurológicas de la quimioterapia.
Palabras clave: cerebro, quimioterapia, interleucina-1β, ciclooxigenasa 2.

ABSTRACT

Backgrond: Inflammatory stimuli have been found to exert little effect on COX-1 expression, but are well-known for leading to a rapid rise in COX-2 mRNA, suggesting that COX-2 plays a role in the inflammation process. COX-2 may also play an important role in neurodegeneration, and in particular in citotoxic neuron death.
Objective: To analyze in brain tissue from autopsies of 13 patients with cancer –five of them treated with chemotherapy and the other eight without chemotherapy– expression of cyclooxygenase-2 (COX-2), mRNA and interleukin-1β.
Material and methods: In brain tissue of 13 patients with different kinds of primary malignant tumors the interleukin-1β and cyclooxygenase-2 expression was analyzed in autopsy, five patients were treated with chemotherapy and eight without chemotherapy. It was realized by real time RT-PCR of total RNA of cerebral cortex and white materia tissue preserved in -80°C, and it was used, primers and fluorophore-labeled TaqMan probes targeting human COX-2 and IL-1β.
Results: Brain tissue from patients with chemotherapy had significantly higher interleukin-1ß and cyclooxygenase-2 expression in frontal cortex and hippocampus.
Conclusion: These findings suggest that exposure to cytotoxic drugs is associated with COX-2 gene induction by interleukin-1β, neuronal dysfunction that could precede the neurologic complications of chemotherapy treatment.
Key words: brain, chemotherapy, interleukin-1β, cyclooxygenase-2.

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Neurologic complications of cancer therapy are an increasingly important concern in patient management. Increasing use of local treatments to target specific tumor sites, such as brain or leptomeningeal metastases have resulted in increased incidence of treatment toxicity in the central nervous system. Recognition of specific treatment-related toxicities, and more importantly differentiation of treatment toxicity from reversible causes of neurologic dysfunction, is critical.[1] Neurotoxic side effects of chemotherapy occur frequently and comprise a reason for limiting the chemotherapy dose. The central neurotoxi-

city of cytotoxic drugs depends on their ability to cross the blood-brain barrier.[2] Cyclooxygenase (COX) is the rate-limiting enzyme in prostaglandin production and as such is a key target for many anti-inflammatory drugs. There are two known isoforms, COX-1 and -2, which have quite distinct expression patterns and biological activities. COX-1 is a constitutively expressed protein found in the majority of tissues, whereas COX-2 expression can be induced by a variety of mitogens, including cytokines, hormones, and phorbol esters.[3,4] Inflammatory stimuli have been found to exert little effect on COX-1 expression, but are well-known for leading to a rapid rise in COX-2 mRNA, suggesting that COX-2 plays a role in the inflammation process.[5] Recent evidence indicates the role of interleukin-1β in up-regulating COX-2 expression at the mRNA, protein, and enzyme activity levels in human endometrial stromal cells, as a model system of inflammatory disease, and additionally found that COX-2 gene induction by interleukin-1β involved the ERK1/2 signaling pathway.[6] COX-2 may also play an important role in neurodegeneration, and in particular in citotoxic neuron death.[7]. The aim of this study is to analyze brain tissue from autopsies of 13 patients with cancer treated, five with chemotherapy

and eight without chemotherapy, expression of cyclooxygenase-2 (COX-2) mRNA and interleukin-1β.

MATERIAL AND METHODS

We conducted a study using autopsy brain samples from 13 patients with cancer, five with chemotherapy and eight without chemotherapy. Data available for all subjects included age, gender, clinical history, nutritional status, cause of death, and time between death and autopsy. Inclusion criteria included the following: Patients with anything kind of cancer treatment with or without chemotherapy with recent complete clinical information within three months of death through medical records, interviews with private practitioners, and evaluation of job performance. With out evidence of neurological disease or cognitive abnormalities in the medical history and complete neuropathologic examination; body mass index (BMI) between 19 and 25 kg/m^2. Negative family history of dementia, drug addictions, and negative history exposure to potential neurotoxicants or steroidal compounds. Cause of death was considered for all subjects to rule out the possibility that infection, inflammatory events, brain ischemia, or hypoxia might impact gene-expression levels measured in the study. Based on evaluation of clinical medical records and information obtained from relatives and patient co-workers by two physicians, each subject was considered cognitively and neurologically intact.

Autopsy and tissue preparation

Autopsies were performed 4.1 ± 1.3 h after death; the post-mortem period was similar for patients with and without chemotherapy. The skull was opened and selected areas from alternating right- and left-cerebral hemispheres were immediately frozen in liquid nitrogen and maintained at -80ºC. Frozen tissue for reverse transcriptase-polymerase chain reaction (RT-PCR) was taken from brain cortex and white matter, taking care to make a perpendicular cut to the brain surface and maintaining similar amounts of cortex and white matter for each method. Sections adjacent to the frozen material were immersed in 10% neutral formaldehyde, fixed for 48 h, and transferred to 70% alcohol. Sections were taken from superior frontal gyrus and hippocampus. Paraffin sections 6-μm thick were cut and H&E stained for the histopathologycal control.

Real-time RT-PCR

Total RNA was extracted from frozen tissues using Trizol reagent (Invitrogen Corp., Carlsbad, CA, USA) according to manufacturer instructions. Random-primed, first-strand cDNAs were generated as described[8] except that 1 μg of total RNA was used as template. Relative abundances of mRNAs encoding COX-2 and IL-1β were estimated by quantitative fluorogenic 5′ nuclease (TaqMan) assay of first-strand cDNAs as described.[8] Amounts of COX-2 and IL-1β cDNA in each sample was normalized to the amount of 18s ribosomal RNA (rRNA), yielding an index (molecules per femtomol 18s rRNA) proportional to the relative abundance of mRNA in each sample.

Primers and fluorophore-labeled TaqMan probes targeting human COX-2 and IL-1β were designed using Primer Designer software (Scientific and Educational Software, Durham, NC, USA) based on sequence information in GenBank. Probes were labeled at the 5′ end with 6-carboxy-fluorescein (FAM) and at the 3′ end with Black Hole Quencher-1 (BHQ1). To control for unintentional sample to sample variation in *1)* the amount of total RNA reverse transcribed and *2)* reverse

transcription efficiency, the amount of cDNA corresponding to 18s ribosomal RNA in each first-strand cDNA was estimated using TaqMan ribosomal control reagents (Applied Biosystems) and serial dilutions of a recombinant plasmid standard.

Statistical analysis

Statistics were performed using Stata Statistical software (SPSS v.10). We applied the *t*-parametric procedure that considers differences among variances of the variable of interest. COX-2 and IL-1β mRNA abundance in frontal cortex and hippocampus of 13 patients with cancer with and without chemotherapy were analyzed independently. Then, we considered transformation of the response variable to stabilize variances in our model, which considers co-variables such as age and gender. Pearson correlation was also calculated for all model variables, co-variables, and the response variable.

RESULTS

Average age for patient groups with and without chemotherapy groups were 40 ± 13.8 and 48 ± 18.4 years, respectively. Histopathological diagnosis included nine carcinomas, two germ cell tumors, one lymphoma, and one leukemia. Chemotherapy cycles and drugs were variable, and primary causes of death included hypovolemic shock, acute respiratory distress syndrome, and septicemia. Clinical data for patients with and without chemotherapy is presented in Table 1. The gross appearance and routine microscopic brain examinations were unremarkable in all subjects. Genes for cytokines IL-1β and COX-2 were up-regulated in frontal cortex and hippocampus by real-time RT-PCR in the chemotherapy group compared with those of the non-chemotherapy group. Amount of secreted IL-1β was 7.1-fold higher in frontal cortex and hippocampus from the chemotherapy group (16.12×10^2/fentomoles 18s rRNA) compared with the non- chemotherapy group (2.28×10^2/fentomoles 18s rRNA), and the level of COX-2 secreted from frontal cortex and hippocampus from chemotherapy group was 2.6-fold higher (8.60×10^2/fentomoles 18s rRNA) compared with non-chemotherapy group (3.21×10^2/ fentomoles 18s rRNA). The independent samples test and Pearson correlations for patients with and without chemotherapy is presented in Table 2.

DISCUSSION

Chemotherapy-induced neuropathy is clearly related with cumulative dose or dose intensities. Methotrexate, cytarabine (cytosine arabinoside), and ifosfamide are primarily known for their central neurotoxic side effects. Central neurotoxicity ranges from acute toxicity, such as aseptic meningitis, to delayed toxicities comprising cognitive deficits, hemiparesis, aphasia, and progressive dementia. Risk factors include high doses, frequent administration, and radiotherapy preceding methotrexate chemotherapy, which appears more neurotoxic than methotrexate as single modality.[2] In this study the primary tumors were diverse and the chemotherapy regimens too, thus CDDP/VO16, CISCA, CDDP/5FU,CHOP, Doxorrub-Ara-C, and for the same reason, was compared tissue of the patient with and without chemotherapy. Drugs with the highest neurotoxicity are therefore those that cross the blood-brain barrier most easily, including alkylating agents (metabolites of cyclophosphamide and ifosfamide, thiotepa, and high-dose melphalan), busulfan, platinum derivatives, aracytine, and methotrexate. In addition to aracytine-induced cerebellar toxicity, clinical signs suggestive of chemotherapy neurotoxicity are relatively non-specific and include altered level of consciousness, seizures, behavioral disorders, and motor deficits. Nevertheless, good knowledge of the various neurological syndromes likely to occur can allow these to be attributed to a drug-induced cause.[9] In this study, inclusion criteria: patients without evidence of neurological or cognitive disorders in the clinical history, no history of drug addiction, without exposure to potential neurotoxic, no history of non-steroidal anti-inflammatory drugs (NSAIDs) or steroid compounds; in order to avoid bias in the study.

Chemotherapy can exert, nonetheless, significant toxicity on the nervous system. The most common neurologic complications involve acute alterations in consciousness, leukoencephalopathy, seizures, cerebral infarctions, paralysis, neuropathy, and ototoxicity. The majority of information on toxicity derives from prospective reports and adult patient population. Methotrexate, cyclosporin, and platinum compounds are the most frequently cited.[10] Several of the cases in this study were treated with some compounds which have been found toxic.

Up-regulation of COX-2 expression is a common feature of inflammatory responses,[4,5,11] suggesting that increase

Table 1. Clinical data

Autopsy	*Age*	*Gender*	*Histopathological diagnosis*	*Chemotherapy*	*Cycles QT*	*Last QT before death (months)*	*Survival (months)*	*Death cause*
A-02-2	33	M	Testicular mixed germ cell tumor	CDDP-VO1	5	3	4	Hipovolemic shock
A-02-5	52	F	Breast infiltrating duct carcinoma NOS	CISCA	6	2	33	Acute respiratory distress syndrome
A-02-6	34	M	Adrenal primitive neuroectodermal tumor	-	-	-	5	Acute respiratory distress syndrome
A-02-7	76	F	Squamous cell carcinoma of the cervix uteri	-	-	-	302	Hipovolemic shock
A-02-9	42	M	Lung adenocarcinoma	-	-	-	6	Acute respiratory distress syndrome
A-02-10	53	F	Squamous cell carcinoma of the cervix uterine	CDDP y 5FU	4	4	156	Septicemia
A-02-12	54	M	Basal cell carcinoma	-	-	-	45	Respiratory insufficiency secondary to broncoaspiration
A-02-19	37	F	Squamous cell carcinomaof the cervix uterine	-	-	-	4	Septic shock
A-02-20	66	M	Gastric adenocarcinoma	-	-	-	72	Cerebrovascular hemorrhage
A-02-21	20	M	Lymphoblastic limphoma	CHOP	4	1	15	Septicemia
A-02-22	41	M	Acute leukemia	Doxorrub-Ara-C	8	1	5	Septicemia
A-02-23	58	M	Kidney clear cell adenocarcinoma	-	-	-	5	Cerebral edema
A-02.41	20	M	Testicular embryonal carcinoma	-	-	-	1	Pulmonary tromboembolism

Table 2. Independent samples test and Pearson correlation

Equal variances assumed	*With chemotherapy*	*Without chemotherapy*	*Sig. (2-tailed)*	*Pearson correlation*
IL-1β	16.12 ± 1.96	2.28 ± 1.03	.000	.981
COX-2	8.60 ± 1.40	3.21 ± 0.84	.000	.935

of COX-2 in the chemotherapy group is the result of a chronic inflammatory process. Because COX-2 mRNA is highly unstable and interleukin-1β stabilizes COX-2 mRNA in the absence of transcription.[6] In this study we also observed stabilization of COX-2 by Interleukin-1. Suggest that post-transcriptional mRNA stability is an important consequence of interleukin-1β action.

Inflammation is an important contributor to neuronal damage in other neurodegenerative conditions such as Parkinson's disease, Alzheimer's disease, multiple sclerosis, and amyotrophic lateral sclerosis.[12-14] One of the best-known features of inflammation in the central nervous system (CNS) is the production of nitric oxide (NO), which is a product of cytokine-activated macrophages and a microbial compound found in the inflammatory reaction.[15] Lipopolysaccharide, IFN-α, TNF-γ, and IL-1 are responsible for microglial activation and NO production.[16] NO production is associated with the generation of reactive oxygen species of (ROS), including superoxide anions, hydroxyl radicals, lipid hydroperoxides, and hydrogen

peroxide. These compounds are toxic to neurons because they induce lipid peroxidation, DNA fragmentation, and protein oxidation.[17,18]

There are historic suggestions to stimulate the immune system as adjuvant therapy in brain neoplasms, however, the majority of immunotherapy tries have failed in speediness and specificity. One of these suggestions is the interstitial immunotherapy, which consists in the local injection of immune cells or molecules with cytotoxic properties that can destroy neoplastic cells; as an ideal model it will include selective destructiveness of the managed tissues, causing less toxicity, or trying not to cause toxicity.[19,20]

Despite the discussed limitations of the present study, as the sample size and the pursuit of other signaling pathways such as ERK 1, 2 or NFkB that rounds off have been involved in the activity of COX-2, the results of this study show COX-2 gene induction by interleukin-1β following chemotherapy, and this effect may be neurotoxic. Although we have no direct evidence that COX-2 gene induction by interleukin-1β is involved in the neurodegenerative symptoms of chemotherapy toxicity, further investigation of this relationship should clarify the roles of these factors.

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