Effect of Quercetin on the Short-term Impairment of Learning Induced by X-rays in Wistar Rats. Nonlinear Regression Analysis of Morris Water Maze Latencies

R. Marina[1], P. González[2], J.P. Barrio[3]

Departamento de Ciencias Biomédicas e Instituto de Biomedicina, Universidad de León, León, Spain.
[1]raquelina_soria@hotmail.com [2]mfgons@unileon.es [3]jpbarl@unileon.es

Introduction

Neurocognitive impairment is a serious adverse effect of radiation-based therapy [1]. It is well known that ionizing radiations induce oxidative stress on target tissues, mainly through the generation of reactive oxygen species (ROS) [2,3]. Spatial learning and memory are compromised after ionizing irradiation in rats using the Morris water maze test, and antioxidant-based diets are able to improve these radiation-induced behavioural deficits [4]. Quercetin is one of the most commonly found flavonoids in the diet with reportedly antioxidant and anti-inflammatory properties, and has been shown to have radioprotective actions in mice under gamma-radiation [5].

The analysis of learning curves obtained from Morris water maze tests has been traditionally carried out using repeated-measures analysis of variance [6], analysis of linear regression slopes [7], and analysis of non-linear exponential regression coefficients [8]. However, when the learning curve is altered by applying a disruptive agent (such as X-irradiation), these approaches may not be appropriate to best explain the behavioural changes induced. Piecewise (segmented) linear regression could be useful when a change in experimental conditions can be presumed to produce a sharp transition point in the response curve.

Objectives

The present study aimed to test the hypothesis that intragastric (IG) administration of quercetin shows a positive radiomodifier effect over spatial learning and memory dysfunction induced by X-irradiation in male Wistar rats, when the behavioural tests started either before or after X-irradiation.

Materials and methods

Experiments were designed to test the response of rats to a single sub-lethal X-irradiation (6 Gy, 0.4 Gy/min) on a Maxishot 200 X-ray machine, with a source-skin distance (SSD) of 50 cm. According Spanish legislation, irradiation procedures were performed by qualified technical staff. Male Wistar rats, two-months old (Harlan, Barcelona, Spain), were divided into four groups (CQ, CV, RQ, RV, n=8 each) according IG administration of quercetin (50 mg/kg body weight) in vehicle (propyleneglycol) (Q), vehicle IG-administered (V), whole-body irradiated with 6 Gy X rays after anaesthesia with pentobarbital (R), and sham-irradiated (C). Quercetin or vehicle IG administration started five days before behavior was first measured and lasted along all experimental period.

Learning was assessed using the Morris water maze test over the six days following X-irradiation (Experiment 1). We used a pool (120 cm in diameter and 60 cm height) made on glass fiber and painted on acrylic black, filled with water at 23 °C. In Experiment 2, with a new batch of similar, naïve rats, Morris tests started as previously four days before X-irradiation, to be resumed by the three days immediately after radiation exposure at the same conditions as above. All the tests were conducted during the morning, and the order in which animals were tested was randomized in sucessive days. Each rat was allowed to swim by 60 seconds in each of four sessions starting sequentially from each pool quadrant edge with 30 seconds resting time between trials. The black, circular escape platform was only visible to the rat on the first testing day.

Proceedings of Measuring Behavior 2012 (Utrecht, The Netherlands, August 28-31, 2012)
Eds. A.J. Spink, F. Grieco, O.E. Krips, L.W.S. Loijens, L.P.J.J. Noldus, and P.H. Zimmerman

One B/W camera (512x512 pixels) was set up 2 m high to record the trials. We used a Dell Optiplex GX280 computer running Noldus Ethovision 3.0 (Noldus Information Technology, Wageningen, The Netherlands). The behavioral parameter studied was the latency to reaching the escape platform when the animal was placed in the pool from any of the four quadrants.

Learning data were analyzed using nonlinear regression fitting to exponential and piecewise segmented linear equations. Segmented regression fits one line to all latency (L) points with time (t) less than some value t0, and a second line to all points with t greater than t0, ensuring that the two lines intersect at t0 [9]. The model was built according the following statements:

L1 = intercept1 + slope1*t
L at t0 = slope1*t0 + intercept1
L2 = L at t0 + slope2*(t – t0)
L = IF(t<t0, L1, L2)

There was also an imposed constraint so that X0 was greater than 4 days, that is, the first line should include the data from the four first days without irradiation, and the second line the data from the next Morris tests after irradiation.

Nonlinear fitting procedures were carried out using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego CA, USA, www.graphpad.com).

The experimental protocol used was approved by the University of León Ethical Committee, and adhered to the European Community Guiding Principles for the Care and Use of Animals.

Results

When learning started after experimental X-irradiation, the relationship latency (L) vs. time (t) fitted an exponential decay curve given by the equation L=(L0 - Plateau)*exp(-K*t) + Plateau [9]. RV rats showed the highest latency plateau level following six test days after X-irradiation, which was indicative of a learning slowdown. However, learning was improved in X-irradiated animals administered quercetin (Figure 1). When the fitting model was a straight line, the slope was taken as a measure of the learning speed. RV rats showed the slowest learning speed, which was improved by quercetin treatment in a way similar to non-irradiated animals.

In Experiment 2, learning was interrupted by X-irradiation after four days, and behavioural tests were resumed on the next three post-irradiation days. All latency data were fitted to segmented linear regression.

Upon resuming the tests, the slope of the corresponding segment showed that learning speed was depressed in all irradiated animals, with a non-significant improvement by quercetin treatment (Figure 2).

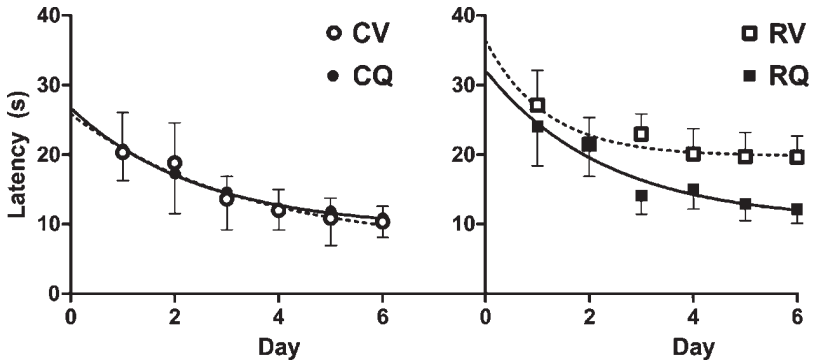

Figure 1. Exponential decay fitting of the latency to reach the escape platform vs. day for successive Morris water maze tests on rats after X-irradiation applied on day 0.

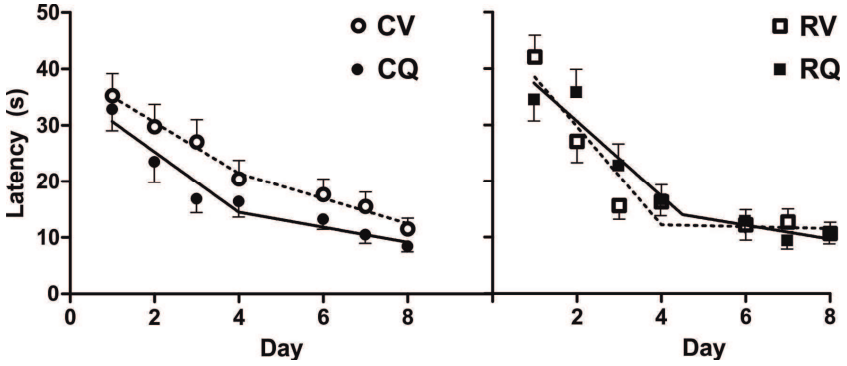

Figure 2. Segmented linear regression fitting of the latency to reach the escape platform vs. day for successive Morris water maze tests on rats before (days 1-4) and after X-irradiation (applied on day 5).

Discussion

Learning curves from Morris water maze experiments can be fitted to multiple equations, with the best results usually obtained using exponential decay equations [8]. However, when the experimental setup is such that disruption can be presumed to alter significantly the behavioral response, segmented linear regression may be a useful tool to quantify the difference in the time course of the observed process. In our work, the disruption was mainly due to X-irradiation taking place at the 5th day, but also there is an effect of the anaesthesia in CV and CQ animals (Figure 2, left). We are not aware of this fitting procedure being applied to Morris water maze data analysis in the literature.

The results of the present work show that quercetin administration before and after sub-lethal X-irradiation is able to counteract the learning slowdown induced by X rays only when the learning process starts once irradiation has been applied. These findings could be helpful to design clinical interventions aimed to reduce the impact of radiotherapy on cognitive function.

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