Permanent alterations of the dendritic tree of cerebellar Purkinje neurons in the rat following postnatal exposure to cis-dichlorodiammineplatinum

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Summary. The aim of this study was to characterize further the effect of cis-dichlorodiammineplatinum (cisDDP) on cerebellar Purkinje neurons of the immature rat. Ten-day-old rats were treated with cisDDP subcutaneously and killed after 1, 7, 20 or 65 days. The cerebellar vermis was impregnated by the Golgi-Cox method to evaluate the extent of morphological maturation of the Purkinje cell dendritic tree. One day after treatment, the dendritic network of Purkinje cells of treated animals was poorly developed and the cell somata still showed numerous perisomatic processes. This indicates that cisDDP interferes with the organization of microtubules and microfilaments by the cell. Later, several abnormal shapes of the Purkinje cell dendritic tree were observed. These included: (1) elongated primary dendrites; (2) asymmetrical dendrites; (3) sprouting of secondary and spiny branches in two planes of the molecular layer; and (4) damming of spiny branchlets at the pial surface. Moreover, all the dendritic networks of Purkinje cells in treated animals were of a lower Strahler order than in controls. All these data suggest that the late anomalies of the dendritic trees are secondary to the general cisDDP-induced damage of the cerebellar cortex, rather than being a primary effect of the drug on the dendritic tree growth.

Key words: Purkinje cells - Histogenesis - Cis-dichlorodiammineplatinum - Dendritic tree

It has been reported that a single subcutaneous injection of cis-dichlorodiammineplatinum (cisDDP), an inhibitor of DNA synthesis used for therapy of tumours [14], into immature rats caused cell death in the germinative compartment of the external granular layer (EGL) of the cerebellum [13]. As a consequence, some abnormalities, consisting of reduction of the cerebellar size in the neopallial part of the vermis, ectopia of granule cells at the pial surface and scanty cellularity in the internal granular layer (IGL), occurred [13]. Postmitotic neurons, such as Purkinje and Golgi cells, were also affected and, after initial cytoplasmic damage revealed by dilatation of the endoplasmic reticulum and ribosome segregation, some cells degenerated [15]. At late post-treatment intervals (towards 15 and 20 days, i.e., at the end of cerebellar histogenesis), although the cellular deficit persisted, the total size of the cerebellum was restored by reactive glial cell fibers, which hypertrophied after initial edema [15]. Moreover, the animals apparently did not show sign of impaired locomotion, thus suggesting that there was compensation for the cerebellar hypocellularity.

To characterize the effect of cisDDP treatment on postmitotic neurons further, we have examined the development of the Purkinje cell dendritic tree by a Golgi method. Our aim was a better definition of what was the
Fig. 2. a Control rats 17 day old. The dendritic network of Purkinje cells is well-developed and reaches the 6th Strahler order. b–f cisDDP-treated animals. b A Purkinje cell with severe asymmetry of the dendritic tree. c In some cells the dendritic tree is stunted and there is a long unbranched stem dendrite. d A Purkinje cell with differently developed dendrites. Secondary branches sprout in two different planes of the molecular layer. e A Purkinje cell with a dendrite with damming of spiny branchlets at the pial surface. f Microphotograph of the cell in e. a–e Camera lucida drawings, f planachromat; a–f x 40. Scale bar = 50 μm

Material and methods

CisDDP dissolved in saline (5 μg/g of body weight, a therapeutic dose) was injected subcutaneously into 10-day-old Wistar rats. Control animals were given saline only. After 1, 7, 20 or 65 days the animals (three treated specimens and three controls per stage) were killed by decapitation, the cerebella immediately excised and immersed in Golgi-Cox solution (solution A: 16 ml of 5% potassium chromate and 40 ml of distilled water; solution B: 20 ml of 5% potassium dichromate and 20 ml of 5% mercuric chloride. Solutions A and B are combined immediately prior to removal of the cerebellum). The entire cerebella were hardened in the solution in the dark for 24 h. The next day, the Golgi-Cox solution was changed and the cerebellar hemispheres were removed by parasagittal cuts on either side of the vermis, and the vermis left undisturbed in the final change of impregnating solution for 3 to 6 months in the dark. The blocks were then dehydrated in ethanol, briefly exposed to oil of turpentine ethereal and enclosed in paraffin at 45°C. Microtome sagittal sections (100 μm thick) were rehydrated in 70% ethanol and water, developed in 3% NaOH for 5 min, fixed in 5% sodium sulfite for 15 min and routinely dehydrated in ethanol and passed into xylol for coverslipping.

Purkinje cells with well-impregnated, entire dendritic trees were drawn by camera lucida at objective magnification 25 or 40 X. Since it is known that there are differences in the maturation of different cerebellar lobules [2] and between Purkinje cells in the depths of fissures, along fissures and at the surface of folia, only cells along the fissures of the neopallial part of the vermis (lobules VI to VII according to Larsell [12]) were considered. At least 30 Purkinje cells in 15 sections per animal were observed.

Results

Purkinje cells of the neopallial part of the vermis of 11-day-old control animals had irregularly shaped...