Spontaneous Tumors of the Central Nervous System of Fischer 344/DuCrj Rats

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ABSTRACT. Thirty-four spontaneous tumors of the central nervous system were found in 1705 male and 1705 female Fischer 344/DuCrj rats. The tumors developed in 24 (1.4%) males and 10 (0.6%) females ranging in age from 38 to 109 weeks. There were 11 astrocytomas, 5 oligodendrogliomas, 5 mixed gliomas, 5 meningiomas, 4 granular cell tumors, 2 ependymomas, one medulloblastoma, and one reticulosarcoma. The astrocytomas were divided into 4 fibrillary, 3 protoplasmic, 3 pilocytic, and one malignant types. The meningiomas were divided into 2 meningotheliomatous, 2 fibromatous, and one malignant types. One of the ependymomas originated in the tentorium cerebelli and was diagnosed as extraventricular ependymoma. Seven of 34 tumor-bearing rats revealed neurological signs and these signs were related with anatomical locations of tumors.—**KEY WORDS:** central nervous system, F344 rat, spontaneous tumor.

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Information on the natural incidence of tumors in laboratory animals is essential for the assessment of carcinogenic bioassays. In spite of the wide use of Fischer 344 (F344) rats for chronic or carcinogenicity tests, only a few groups of workers [4, 10, 12, 13] have dealt with a relatively large number of spontaneous tumors of the central nervous system (CNS) of rats of this strain. In these studies, it has been shown that types of tumors detected were astrocytoma, oligodendroglioma, mixed glioma, ependymoma, medulloblastoma, ganglioma, ganglioneuroma, neurofibroma, meningioma, granular cell tumor, malignant reticulosis, choroid plexus papilloma, and sarcoma, and that the incidences of the tumors varied from 0.7% to 1.3% with a tendency to occur more frequently among aged rats and males.

We detected 34 spontaneous CNS tumors in 1705 male and 1705 female F344/DuCrj rats, which had been used for toxicity studies in our laboratory. The present paper describes pathologic features of these tumors and neurological signs of rats related with the tumors.

MATERIALS AND METHODS

The rats were obtained from the Charles River Japan Co. Ltd. at the age of 5 weeks and used as untreated or treated animals in 6 separate 104-week toxicity studies of 6 different test substances. Out of 34 CNS tumors detected, 14 tumors were found in 866 untreated control rats and the remainder in 2544 rats treated with the test substances. The data obtained in these studies indicated that the test substances were non-carcinogenic for rats and that the occurrence of CNS tumors was not related to the test substance in each study. The rats were housed one or two animals per stainless steel cage under barrier conditions in rooms controlled at $23 \pm 2^\circ\text{C}$, $50 \pm 20\%$ relative humidity, and a 12-hr light-dark cycle. Standard commercial laboratory diet for rats (CRF-1, Charles River Japan Co. Ltd.) and chlorinated tap water were available *ad libitum*.

Complete necropsies were performed on all rats found dead, killed when moribund, or killed at the end of the studies. Tissues

Table 1. Spontaneous central nervous system tumors in Fischer 344/DuCrj rats^{a)}

Tumors	No. of rats with tumors out of		Age range (average) in weeks
	1705 males	1705 females	
Astrocytoma	6	5	94-109(103)
Oligodendroglioma	3	2	38-109(72)
Mixed glioma	3	2	53-109(77)
Ependymoma	2	0	89-105(97)
Meningioma	4	1	57-109(86)
Granular cell tumor	4	0	84-109(103)
Medulloblastoma	1	0	83 (83)
Reticulosarcoma	1	0	84 (84)
Total (%)	24(1.4)	10(0.6)	

a) Surviving rats were killed at 109 weeks of age.

Table 2. Neurological signs observed in Fischer 344/DuCrj rats with central nervous system tumors

Sign	Anatomical location of tumor	No. of rats showing sign (tumor type)
Rolling movement	Cerebellum, corpus medullare	2 (2 astrocytomas)
Torticollis	Cerebellum, corpus medullare	1 (medulloblastoma)
Lethargy	Thalamencephalon	3 (2 oligodendrogliomas, 1 mixed glioma)
Lumber paralysis	Spinal cord, intumescentia lumbalis	1 (ependymoma)

from these rats were fixed in 10% formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H. & E.). Selected sections from some tumors were also stained with periodic acid-Schiff (PAS) with or without diastase digestion, phosphotungstic acid hematoxylin (PTAH), Watanabe's silver for reticulin, azan-Mallory, elastica van Gieson, Holzer, Luxol fast blue, and cresyl violet. Some formalin-fixed specimens were examined by electron microscopy after being post-fixed with osmium tetroxide and processed by the conventional method.

RESULTS

The types and numbers of tumors detected, and ages of tumor-bearing rats are

shown in Table 1. Out of 34 tumors, 24 (1.4%) tumors were found in males and 10 (0.6%) tumors in females. Ages of rats with tumors ranged from 38 to 109 weeks, and tumors tended to occur more frequently in older rats. These tumors were 11 astrocytomas, 5 oligodendrogliomas, 5 mixed gliomas, 5 meningiomas, 4 granular cell tumors, 2 ependymomas, one medulloblastoma, and one reticulosarcoma. Their sizes varied from a microscopic lesion to a grayish mass 10×10×5 mm. No distinct difference was seen among gross findings of the tumors. Each tumor occurred as a solitary lesion, and no metastases were detected in any of the tumors.

As shown in Table 2, 7 rats developed neurological signs 1-7 weeks before death. Out of these animals, 2 with astrocytoma

Table 3. Anatomical distribution of astrocytomas

Type	Anatomical location	No. of rats with tumors
Fibrillary	Peri-3rd ventricular area	1
	Peri-lateral ventricular area	1
	Cerebellum, corpus medullare	1
	Cerebral hemisphere	1
Protoplasmic	Cerebral hemisphere	2
	Cerebellum, corpus medullare	1
Pilocytic	Hippocampus	3
Malignant	Cerebral hemisphere	1

Fig. 1. Male rat, 105-week-old. Protoplasmic astrocytoma arising predominantly in perivascular areas. H. & E. $\times 180$.

Fig. 2. Male rat, 109-week-old. Pilocytic astrocytoma consisting of spindle-shaped or undulating cells with ovoid to elongated nuclei. H. & E. $\times 250$.

and one with medulloblastoma in their cerebellar medullary corpus showed such involuntary movement as rolling and torticollis; 2 with oligodendroglioma and one with mixed glioma in their thalamencephalon exhibited lethargy; and one with ependymoma in the lumbar enlargement of the spinal cord developed lumbar paralysis. The remaining rats with tumors died during the course of the studies or survived up to 109

weeks without showing any neurological signs.

Neuroglial tumors: Astrocytomas were the most common tumor, accounting for 32.4% of all tumors detected. They were classified into 4 fibrillary, 3 protoplasmic, 3 pilocytic, and one malignant types. Anatomical distribution of these types is shown in Table 3. All astrocytomas were poorly circumscribed and extended into the adja-

Fig. 3. Female rat, 98-week-old. Malignant astrocytoma showing pseudorosette-formation. Mitotic figures are frequent. H. & E. $\times 600$.

cent tissue, and neither necrosis nor hemorrhage was seen except the malignant type. Cells that formed fibrillary and pilocytic astrocytomas stained positively with PTAH and Holzer stain for glial fibers, whereas those of protoplasmic and malignant types were faintly positive or negative by both stainings. Electron microscopy demonstrated sparsely arranged glial filaments in their cytoplasm. Tumor cells in fibrillary and protoplasmic astrocytomas proliferated mainly in perivascular area and had round or elongated cytoplasm and a small, dense nucleus (Fig. 1). Pilocytic types consisted of spindle-shaped or undulating cells with ovoid to fusiform nuclei (Fig. 2). All pilocytic astrocytomas arose in the hippocampus. Malignant astrocytoma was composed of pleomorphic cells with a pale nucleus and abundant cytoplasm, and occasional multinucleated giant cells. There were pseudorosettes formed by tumor cells (Fig. 3).

Fig. 4. Female rat, 109-week-old. Oligodendroglioma showing a honeycomb pattern and capillary-endothelial cell proliferation. H. & E. $\times 220$.

Mitotic figures were frequent.

Three oligodendrogliomas occurred in the cerebral cortex and the other two in the thalamencephalon. The tumors developed as a well-circumscribed mass with hemorrhagic foci and capillary-endothelial cell proliferation. The mass was made up of small round cells with hyperchromatic nuclei surrounded by clear intercellular spaces, giving an appearance of honeycomb (Fig. 4). Mixed gliomas arose in the white matter around the lateral ventricle of 3 rats and in the thalamencephalon of 2 rats. They were composed of neoplastic oligodendrocytes and a number of clusters of degenerative astrocytes, seemingly gemistocytes. Their histologic features were similar in part to oligodendrogliomas showing well-demarcated border, hemorrhage, capillary-endothelial cell proliferation, and a honeycomb pattern.

One of ependymomas developed in the

Fig. 5. Male rat, 105-week-old. ependymoma in the tentorium cerebelli showing pseudorosette-formation in perivascular area. H. & E. $\times 260$.

tentorium cerebelli bearing no relation to the ventricular system. This case was, therefore, diagnosed as extraventricular ependymoma. The other ependymoma occurred in the lumbar enlargement of the spinal cord. Tumor cells were round to ovoid in shape and had round nuclei with evenly dispersed chromatin. The cells formed a uniform structure of sheet, and some parts of the tumors contained areas in which cells were arranged in pseudorosettes around canal-like spaces or in perivascular spaces (Fig. 5). Mitotic figures were frequent.

Meningiomas: Meningiomas developed in the ventral cerebral meninges of 4 males and one female. They were divided into following 3 types: 2 meningotheiomatous, 2 fibromatous, and one malignant meningiomas. The meningotheiomatous type consisted of small whorls formed by elongated cells with pale nuclei (Fig. 6). The fibromatous type was composed of well-differentiated fibro-

Fig. 6. Male rat, 109-week-old. Meningotheiomatous meningioma characterized by small whorls consisting of elongated cells. H. & E. $\times 280$.

blast-like cells accompanied by abundant collagen fibers (Fig. 7). The malignant meningioma infiltrated into the brain tissue. It was composed of ovoid and fusiform cells with round nuclei (Fig. 8). Giant cells and mitoses were often seen. A small amount of collagen fibers lying among tumor cells was demonstrated by the azan-Mallory method.

Granular cell tumors: Three of 4 granular cell tumors arose from the cerebellar leptomeninges and the remaining one from the perivascular area of the cerebral cortex. They were small in size and could be detected only by light microscopy. The tumor mass was sharply demarcated. The neoplastic cells contained eosinophilic granules in their cytoplasm (Fig. 9) which were positive for PAS reaction with or without diastase digestion. No mitosis was seen.

Medulloblastoma and reticulosarcoma: One medulloblastoma was found in the cerebellar medullary corpus of a male rat.

Fig. 7. Male rat, 72-week-old. Fibromatous meningioma consisting of fibroblast-like cells and abundant collagen fibers. H. & E. $\times 260$.

Fig. 8. Female rat, 57-week-old. Malignant meningioma consisting of ovoid to fusiform cells and occasional giant cells. H. & E. $\times 280$.

Fig. 9. Male rat, 109-week-old. Granular cell tumor arising from the cerebellar leptomeninx. H. & E. $\times 90$.

Fig. 10. Male rat, 83-week-old. Medulloblastoma consisting of round to fusiform cells with scanty cytoplasm. H. & E. $\times 260$.

Fig. 11. Male rat, 84-week-old. Reticulosarcoma showing indistinct cytoplasmic borders. H. & E. $\times 450$. Inset shows dense, pericellular reticulin network. Watanabe's silver impregnation, $\times 450$.

The tumor was poorly demarcated, and cellular mass showed an interlocking arrangement of cells. Neoplastic cells were fusiform to round in shape and had scanty cytoplasm (Fig. 10). Mitotic figures were frequently seen.

One reticulosarcoma was detected in the meninx near the nervus oculomotorius and slightly compressed the brain base. Tumor cells proliferated into the lacrimal fossa, auris interna, and intracranial space. The cells had round nuclei and indistinct border. A dense, pericellular reticulin network was demonstrated by Watanabe's silver impregnation. Mitosis was occasionally seen (Fig. 11).

DISCUSSION

The results obtained in the present study are generally consistent with previous re-

ports on spontaneous CNS tumors of rats of the same strain [4, 10, 12, 13], although Maekawa *et al.* [10] described only 6 gliomas and one granular cell tumor in 692 male and female rats. As experienced in our study, astrocytoma was the most common tumor, and oligodendroglioma, mixed glioma, meningioma, granular cell tumor, and ependymoma were frequently occurring tumors in these studies.

In other strains of rats, it has been reported that the incidences of CNS tumors ranged from 0.09 to 1.90% [2, 3, 5, 8, 11], and that the most frequent tumor was meningioma [11], astrocytoma [2, 3, 5], or granular cell tumor [6, 8]. Furthermore, Krinke *et al.* [8] listed neoplastic reticulosis as the second most common tumor, which has not been detected in F344 rat by any workers. Such heterogeneities might result from, in addition to strain-difference, genetic background of rats examined, length of experiments, diets, environmental conditions, and variations in procedures and criteria of pathologic examinations. In this regard, the incidence of CNS tumors was higher in F344/N rats of life-span studies than in those of 2-year studies, suggesting the age-related occurrence of the tumors [12].

The histologic features of neuroglial tumors and meningiomas described here were similar generally to those of counterpart tumors of humans [9], domestic [1] and laboratory animals [7]. Malignant astrocytoma found in our study appeared to correspond to glioblastoma multiform in humans [9] and dogs [1], but differed from them in that our case did not have distinct pseudopalisading cells around necrotic areas. Our mixed gliomas were composed predominantly of neoplastic oligodendrocytes and reactive gemistocytes. This type of tumors has been classified as oligodendroglial tumor by the new World Health Organization classification of brain tumors [14].

Extraventricular ependymomas have been reported in man [9, 14] but not in rats. Accordingly, extraventricular ependymoma described here may be the first case in rats.

Granular cell tumors have been included in meningeal tumors, because of their exclusive location in the leptomeninges [5, 8, 13]. Three granular cell tumors described here were also located in the leptomeninges. The histogenesis of this tumor type remains to be determined. Neoplastic reticulosis characterized by multiple masses consisting of perivascular proliferation of reticulo-histiocytic and microglial cells has occasionally been observed in rats [2, 8]. One reticulosarcoma documented here exhibited some degree of malignancy but differed from neoplastic reticulosis in its location and morphologic feature. This tumor resembled those 2 cases of reticulosarcoma described by Mawdesley-Thomas and Newman [11] in Sprague-Dawley rats. The cerebellum was regarded as a predilection site for medulloblastoma in humans [9]. Our medulloblastoma also originated from the cerebellum as reported by Ward and Rice [13] in 3 cases in F344 rats.

In coincidence with a previous finding that only a small proportion of CNS tumor-bearing rats revealed evidence of associated clinical abnormalities [5], 7 of our 34 tumor-bearing rats manifested neurological signs during the course of diseases. It is of interest to note that irrespective of tumor types, involuntary movements were associated with tumors in the cerebellar medullary corpus and lethargies had relation to the presence of tumors in the thalamencephalon.

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要 約

Fischer 344/DuCrj ラットの中枢神経系における自然発生腫瘍：山手丈至，田島正典，布谷鉄夫，工藤 悟（日本生物科学研究所）——Fischer 344/DuCrj ラット雌雄各1,705匹中34匹の中枢神経系に自然発生腫瘍が認められた。腫瘍は38-109週例の雄24匹（1.4%）及び雌10匹（0.6%）に発生し，星状膠細胞腫11例，希突起膠細胞腫 5 例，混合膠腫 5 例，髄膜腫 5 例，顆粒細胞腫 4 例，上衣細胞腫 2 例，細網肉腫及び髄芽細胞腫各 1 例であった。星状膠細胞腫は線維型 4 例，原形質型 3 例，毛様型 3 例及び悪性型 1 例に分けられた。髄膜腫は髄膜内皮腫型 2 例，線維腫型 2 例及び悪性型 1 例に分類された。上衣細胞腫の 1 例は小脳天幕から発生し，脳室外上衣細胞腫と診断された。担腫瘍ラット34匹中 7 匹が神経症状を示し，それら症状と腫瘍の解剖学的局在とが関係づけられた。