Chapter from the book *Brain Tumors - Current and Emerging Therapeutic Strategies*
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1. Introduction

The possibility of having experimental models of brain tumors allows for testing therapies applicable to human brain tumors. They can be induced by viruses, chemicals or radiation. Radiation-induced brain tumors have seldom been used, but diverse virus groups have been used to induce brain tumors. Among DNA viruses, both adenoviruses and papovaviruses have been shown to induce brain tumors in animals. The RNA viruses causing experimental brain tumors have consistently belonged to the retrovirus group, and have been generally limited to the murine sarcoma virus, the avian sarcoma virus, and the murine sarcoma virus. In these models, brain tumors are induced in rodents after intracerebral inoculation, with a variable latency, and the induced tumors are generally classified as gliomas, sarcomas, ependymomas or choroid plexus tumors.

On the other hand, the heterotransplantation of human brain tumors into immunodeprived animals gained great interest after the development of the nude mouse model, a thymus-deficient animal that provided the possibility for the xenografting of human brain tumors. It is known that human meningiomas and glioblastomas can grow after subcutaneous transplantation into the nude mouse, maintaining its original morphology. Nevertheless, at present, diverse chemical agents provide the best models of experimental neurocarcinogenesis.

2. Viral neurocarcinogenesis

The role of viruses in human oncology is a question that has interested for many years to researchers and clinicians (Bigner and Pegram, 1976). However, despite the intense research that has been developed over the last decades in this field, we still can not establish a clear etiological association between the presence of certain viruses and tumor development in humans, with some exceptions, such as the case of Epstein-Barr virus associated to Burkitt's lymphoma, although there has never been any conclusive proof that this virus causes the tumor.

From an experimental point of view, one of the models used to trigger the development of neural tumors in experimental animals is inoculation by different routes of virus with oncogenic capacity (Bullard and Bigner, 1980). The potential value of virus-induced gliomas has been questioned, however the information obtained from these experimental models has enabled significant progress in the treatment of human cancers. This experimental model of virus-induced neurocarcinogenesis offers the advantage that some of the viruses used will induce the development of tumors in a short period of time, the tumors are specifically located at the Central Nervous System (CNS) or Peripheral Nervous System (PNS), so that
we can not rule out the possible viral etiology of certain types of brain tumors in humans. However, at present, numerous studies failed to establish any etiological association between viruses and human brain tumors (Minn et al, 2002). We now know different animal viruses that can act as transforming agents in normal cells, since they are capable of causing malignant transformation of a cell through its ability to integrate genetic information. It is well known that, for example, intracerebral inoculation of retroviruses can induce brain tumors in a wide variety of animals. Viral carcinogenesis allows us to induce experimental tumors with a short latency period and with a more specific location that offers radiation carcinogenesis, location depends on the route of administration, animal age and the amount of virus inoculated. However, it is obvious that experimental models of viral neurocarcinogenesis have the inconvenience and risks involved in the handling of virus particles.

Numerous studies have shown that RNA viruses (retroviruses) are able to induce the development of tumors in the CNS of experimental animals. Within this group, we highlight the avian sarcoma virus, murine sarcoma virus and simian sarcoma virus, being the most widely used in experimental neuro-oncology. Avian sarcoma virus (ASV) has been one of the most used in the literature to induce experimental brain tumors. The tumors are usually induced in chickens by intracerebral inoculation, and intracerebral tumors originated showed characteristics of sarcomas. There have been studies of ASV inoculation in the brains of monkeys because of their similarity to man, and tumors induced were fibrosarcomas. Interestingly, no author has reported the existence of glial tumors, but it has shown the ability of this virus to infect and replicate in glial cells when they grow in tissue culture.

Murine sarcoma virus (MSV) with its three strains: Moloney MSV, Kirsten and Harvey, can cause leukemia and sarcomas when inoculated subcutaneously in rodents and also is capable of inducing brain tumors in rats when inoculated intracerebrally. Neoplasms that may result show usually the aspect of glioblastomas, gemistocytic astrocytom as, oligodendrogliomas and hemangioblastomas, depending on the age of the animal and the dose of virus inoculated.

The simian sarcoma virus (SSV), after intracerebral inoculation in marmosets (Sanguinus nigricolli) induces the development of tumors that are morphologically similar to human glioblastoma multiforme, being able to demonstrate the presence of virus particles within tumor cells.

Among the known DNA virus, adenovirus and papovavirus have proved very effective in inducing brain tumors after intracerebral inoculation in animals, preferably in neonatal age. Intracerebral inoculation of polyoma virus induces a high incidence of intracranial sarcomas in experimental animals, increasing their impact in terms of age of the animal (Rabson and Kirschstein, 1960). However, when inoculated cells transformed in vitro with the same virus, tumors of astrocytic aspect can be seen. The SV-40 virus shows no oncogenic effect in monkeys, a species from which it was originally obtained, but it is one of the more capable oncogenic virus in rodents. Intracerebral inoculation in hamsters induces the development of ventricular tumors that were classified as ependymoma, choroid plexus papillomas and meningeal sarcomas. The induction of brain tumors by this type of virus depends very heavily on the dose. The role of SV-40 virus in human tumor development, not only brain tumors but also bone tumors and mesothelioma, has been subject of discussion for decades, but now there is conclusive evidence. Furthermore, human adenoviruses can cause meningeal tumors when inoculated
intracerebrally into experimental animals, with tumor development after latent periods of 35 to 40 days. While most existing data in the literature refer to the oncogenic virus ASV virus and SV-40, other viruses whose first guest is the man also play an important role in viral neurocarcinogenesis, such as Ad12 virus, BKV and JCV, three DNA viruses that have been widely used in experimental studies designed to establish a possible relationship between virus inoculation and the development of brain tumors. The human adenovirus Ad12 is able to induce brain tumors in rats after intracerebral inoculation, with a greater susceptibility of neonatal animals, where the range of incidence may vary between 8 and 100%. Furthermore, induced tumors develop after periods of latency between 31 and 235 days. The human papovavirus BK is capable of inducing brain tumors with different histological features, when inoculated into experimental animals. This virus is specifically used to induce choroid plexus papillomas and ependymomas after being inoculated intracerebrally. The JC virus (JCV), when inoculated subcutaneously or intraperitoneally into young hamsters, induces the development of a variety of tumors, especially mesenchymal neoplasms, and may even induce the development of peripheral neuroblastomas. Moreover, intracerebral inoculation can induce the development of malignant astrocytomas. In human neuro-oncology, this virus has been associated with the development of medulloblastomas and more recently, with recurrence of glioblastomas. On the other hand, it is important to note that in recent years, a new focus on the use of viruses has emerged in experimental neuro-oncology, and currently the use of viruses, or parts of them, are used as therapeutic vectors. Although some success has been reached using oncolytic viruses in experimental treatments for malignant gliomas in humans, the fact is that so far the results with these new techniques do not appear to meet the initial expectations (Zemp et al, 2010).

3. Chemical neurocarcinogenesis

The discovery of chemical carcinogens has stimulated neuro-oncology research because, after systemic application, these compounds induce a high incidence of tumors in the CNS and PNS, such as demonstrated Druckrey et al. in 1965, with the N-methyl-N-nitrosourea (MNU). Subsequently, we have found a greater number of chemical compounds, with equal effectiveness. Some of these compounds only occasionally induce tumors in the CNS of adult animals, but they represent, however, powerful neuro-oncogenic agents when administered transplacentally or during the early stages of postnatal life. Compounds, such as N-propyl-N-nitrosourea, N-butyl-N-nitrosourea, N-dimethyl-N-nitrosourea, and N-Trimethyl-N-nitrosourea, have been used. However, at present, N-ethyl-N-nitrosourea (ENU) is considered the best chemical agent to induce experimental brain tumors, because it is capable of inducing a high incidence of tumors, with known latency and morphology.

3.1 Mechanism of tumor induction in chemical neurocarcinogenesis

Most carcinogenic compounds actually represent precarcinogens which are converted in the host. The final product of this transformation is an electrophilic group that is capable of reacting with various cell constituents. It is clear that neuro-oncogenic compounds exhibit biological effects as alkylating metabolites which are formed during processing in vivo. The molecular basis of malignant transformation is not fully clarified, and at present, the cell
being the target for the initiation of carcinogenesis has not identified. Most investigations
are based on the interaction of carcinogens with nucleic acids and proteins.
Recent studies suggest the possibility that the induction of neural tumors by nitrosourea
compounds may be related to a deficiency in DNA repair mechanisms in the Nervous
System. When applied 14C-ENU in neonatal rats, the loss of O6-ethylguanina in liver DNA
is very rapid. However, it persists for several days in the cerebral DNA (Goth and Ralewsky,
1974). In the non-target organs for carcinogenic action, the O6-alkylation excision is repaired
during replication, or alteration remains in the sequence of DNA bases.
It is assumed that the inability of the neuro-oncogenic agents to induce neuronal tumors
may be because neurons represent a cell population that has no capacity to divide. The
permanent genetic alterations are the result of a mutation (transition) and this requires DNA
replication. On the other hand, no direct evidence exists to affirm that the alkylation of
nucleic acids is the cause that triggers the initiation of malignant tumors development.

3.2 Factors affecting the induction of experimental brain tumors in chemical
neurocarcinogenesis
In chemical neurocarcinogenesis, the incidence, distribution, histology of tumors, and
survival time of animals are influenced by the species and age of animals, in addition to
dose and mode of application of the carcinogen.

3.2.1 Species
The susceptibility of different species to the carcinogenic activity of nitrosoureas has been
investigated by several authors. Thus, Druckrey et al. (1970) observed that strains of rats
such as Sprague-Dawley and Fischer, Long-Evans and Wistar, were susceptible to the
carcinogenic action, producing a high number of tumors in the CNS. However, the response
was not uniform, for example, male Sprague-Dawley rats treated with MNU only developed
brain tumors, while male Fischer rats showed a high incidence of PNS tumors (Swenberg et
al. 1972).

3.2.2 Age of animals
There is evidence that the response to neuro-oncogenic agents in fetuses and newborn rats
differs significantly from the response in adult animals. The main characteristics of the
perinatal induction of tumors in the nervous system by chemical agents can be summarized
in the following points:
1. In adult animals, repeated doses of the carcinogen is needed to obtain a high incidence
   of neurogenic tumors. In the perinatal carcinogenesis, however, a single dose is
   sufficient to induce tumors in the nervous system, approximately in 90-100% of the
   experimental animals. On the other hand, some compounds such as 1,2-
   dimethylhydrazine, only induce tumors in fetuses and newborn rats, but never produce
   neurogenic tumors in adults.
2. Transplacental induction of neurogenic tumors in rats is possible only after day 11 of
gestation. This is not due to lack of penetration of the carcinogen in fetal tissue, because
embryotoxic and teratogenic effects occur after treatment, during the early stages of
development. Nervous system susceptibility to chemical carcinogens increases sharply
after day 11 of gestation and peaks during late intrauterine development period
(Druckrey et al. 1969). After the first month of postnatal development, the response to
neuro-oncogenic agents is broadly similar to that obtained in adult animals.
3. In adult animals, the tumors are located mainly in the brain (Denlinger et al, 1973). However, after perinatal application, tumors typically occur at the level of the spinal cord and the PNS. Trigeminal nerve tumors occur more frequently when the carcinogen is administered at the end of gestation, whereas in this case, the number of brain tumors is less than when the carcinogen is administered on day 15 of intrauterine development (Kahle, 1970).

4. The prenatal administration of these compounds increases the neurospecific carcinogenic effect. After transplacental administration tumors are located almost exclusively in the CNS. Postnatal application also produces a significant number of extraneural tumors (Schreiber et al, 1972).

3.2.3 Dosage and application
The incidence and latency period of experimental tumors is highly influenced by the dose of carcinogen. The number of tumors transplacentally induced by ENU can vary between 100% and 63% when the carcinogen dose is reduced from 80 mg / kg to 5 mg / kg. Moreover, the latency period is increased from 180 days to 500 days, when the dose of ENU administered in neonatal rats is reduced in the same way. The mode of application of the carcinogen plays a key role in the location and type of tumor that will be induced. Thus, local application of nitrosoureas can induce the formation of local tumors, but when these compounds are administered by intravenous injection, they can produce tumors that are spread throughout the body.

3.2.4 Hormonal and immunological factors
The possible influence of hormones on chemical carcinogenesis was first indicated by Ivankovic (1969) and Alexandrov (1973). They found that pregnant mice, when injected one or more doses of MNU, developed a high incidence of tumors of the uterus, vagina and breast cancer, however, when similar doses were administered in non-pregnant rats the results were different. Schreiber et al. (1972) found an increase in the number of extraneural tumors induced by MNU in rats to which previously had undergone ovariectomy. However, neither the execution of ovariectomy, or the application of testosterone or other oral contraceptives, have altered the oncogenic results (Schreiber et al. 1972; Thomas et al. 1972).
Regarding the role of immunological factors in the development of nervous system tumors, there is very little data. Delinger et al. (1973) studied the effect of the suppression of cell-mediated immunity in carcinogenesis with MNU in Fischer rats. They used a treatment with anti-lymphocyte serum and observed no change in the incidence of neurogenic tumors.

3.3 Morphology and biology of nitrosourea-induced brain tumors
There are a number of compounds able to induce tumors in the nervous system. However, all studies have been directed toward understanding the morphology of tumors induced by repeated doses of MNU in adult animals, or just for perinatal injection of ENU (Schiffer et al. 1970; Koestner et al. 1971; Lantos, 1972; Swenberg et al. 1972, Jones et al. 1973).

3.3.1 Tumor location
Regardless of the type of carcinogen used, preferably tumors develop in a number of specific regions of the nervous system. For example, in the brain, they are located mainly in
3.3.2 Morphology

Tumors induced by chemical carcinogenesis in the nervous system are tumors with similar morphology to that presented gliomas and malignant schwannomas in humans. After numerous histological studies on a large number of tumors induced in rats by nitrosoureas (Wechsler et al. 1969, Druckrey et al. 1970), unequivocal neuronal tumors were not found. After studies by light and electron microscopy of neurogenic tumors induced by ENU, Koestner et al. (1971) and Swenberg et al. (1972) established a classification for them that correlated with human tumors, but using different terminology. Thus, these authors classified the experimental brain tumors induced by ENU as: 1) Mixed gliomas (oligodendro-astrocytomas). 2) Anaplastic gliomas, tumors that show great cellular pleomorphism with high mitotic activity and regressive changes. 3) Gliopependymomas, tumors with ependymoma features that contained pleomorphic glial cells. 4) Gliosarcomas, containing neoplastic glial cells and mesodermal cells. In 1973, Jones et al. provide another classification showing distinct groups (in order of frequency of occurrence) of ENU-induced tumors: 1) Gliomas of periventricular subependymal plate, they are divided in turn into ependymomas and ependymoma-oligoastrocytomas. 2) Astrocytic and oligodendrocytic tumors. 3) Neural tumors of the spinal cord and intracranial nerve ganglia. 4) Neuronal-like tumors and 5) Meningeal tumors.

Gliomas of periventricular subependymal plate are the first tumors that develop, they are identical to the anaplastic gliopependymomas, and almost equivalent to the periventricular pleomorphic gliomas originating from the undifferentiated cells of the subependymal plate described by Lantos (1972). The presence of true ependymomas between the ENU-induced tumors is controversial, and generally they have been considered as such, either by their intraventricular location, or due to their histological features, reminiscent of ependymomatous tumors of humans. Unequivocal ependymomas were not seen in series of mice exposed transplacentally to ENU, but according to accepted classifications, approximately 20% of the ENU-induced brain tumors could be diagnosed as ependymomas, anaplastic ependymomas, or mixed glial tumors with ependymoma areas (Mandybur and Alvira, 1982). In many classifications, ependymomatous tumors were termed as "anaplastic gliopependymomas" due to the presence of ependymoma-like cells, but these tumor cells coexist with other glial-like cells, pleomorphic cells and generally with rounded cells being very similar to those of human oligodendrogliomas. In any case, the histopathological diagnosis of the ENU-induced ependymomas is based on the existence of tumor cells arranged in rosettes around blood vessels. Ultrastructurally, there are two cell types: a small undifferentiated cell, and a larger type, more differentiated. Transitional forms between these two cell types can be seen. Undifferentiated cells are small, with a relatively large nucleus and little cytoplasm. The more differentiated tumor cells have a pleomorphic nucleus in an eccentric position, surrounded by abundant cytoplasm. Overall neoplastic ependymal cells do not possess cilia or blepharoplasts, and are not equipped with junctional complexes. Studies by Mandybur and Alvira (1982) supported that the named
“ENU-induced ependymomas” are not true ependymal tumors and that differ from the human ependymomas, because none of the ultrastructural features of normal or tumoral ependymal cells were present. Therefore these authors suggested that these tumors may actually be regarded as undifferentiated tumors, with some features of ependymomas. On the other hand, the histology of tumors induced by ENU and MNU are similar. There are however some differences that were highlighted by Swenberg et al. (1972). These authors found that ENU-induced gliomas are better differentiated than the MNU-induced tumors, and that ENU produces a greater number of anaplastic schwannoma-like tumors. In animals treated with MNU, gliosarcoma can be found in 10% of cases, however, this type of tumor is completely absent in the treatments with ENU, a carcinogen that produced almost exclusively oligodendroglioma-like tumors and malignant schwannoma-like tumors, as was pointed out by Schiffer et al. (1970). This criterion has been confirmed in numerous studies later and most of the reviews about the morphology of the ENU-induced brain tumors reflected the observation that most tumors can be considered as malignant oligodendrogloma-like tumor or malignant schwannoma-like tumors (Vaquero et al, 1994). The oligodendroglioma-like tumors are characteristically located at the subcortical white matter of the cerebral hemispheres, showing macroscopic appearance of well-defined tumors, often with hemorrhagic characteristics and foci of necrosis. Sometimes these tumors develop large cystic cavities.

In light microscopy studies, the oligodendroglioma-like tumors show a fairly uniform cell population. They are composed of small cells, which show a dark and small nucleus, and a clear cytoplasm. Regressive changes are absent and there are small hemorrhagic foci. Outlying areas of these tumors have a cellular isomorphism, which is not appreciated in the central areas, where the existing cell population shows more pleomorphism, containing giant cells, occasionally multinucleated. Ultrastructural studies reveal the presence of neoplastic cells with an elongated or oval dark nucleus, and a small, clear cytoplasm, poor in organelles. However, some neoplastic cells show a dark nucleus and a dense cytoplasm. These findings suggest that these tumors are primitive undifferentiated tumors with some oligodendroglial features, and their undifferentiated character is supported by immunohistochemical studies. On immunohistochemistry, there is a concordance between our results and those of other authors regarding the expression of the protein S-100, PGA and vimentin (Conley, 1979, Mauro et al. 1983; Mennel and et al. 1990; Raju, 1990; Reifenberg et al. 1989) but importantly we have obtained strong synaptophysin positivity in most of these tumors. Considering that in human pathology, this marker is useful for the recognition of primitive neuroectodermal tumors such as medulloblastoma (Molenaar et al, 1991) and also for the neuronal characterization of brain tumors, it is logical to suppose that the majority of ENU-induced brain tumors can be regarded as undifferentiated neuroectodermal tumors with possible neuronal differentiation, regardless of their morphological appearance. Furthermore, in our studies, most of the ENU-induced oligodendrogloma-like tumors show immunopositivity to the neuroblastic marker NB-84. This finding agrees with some of the previous classifications of these neoplasms, such as that of Jones et al. (1973), who first identified the ENU-induced tumors as neuroblastomas. The schwannoma-like tumors generally developed at the skull base, on the zone of the Gasser ganglion. They can be also located in the spinal root, with usually solid and sometimes cystic consistency. In our studies, these malignancies began to show neurological symptoms after a latent period ranging between 3 and 7 months after carcinogen administration. After 8 months of postnatal life, the development of these tumors is more
infrequent, and after this time, intracerebral neoplasms, mainly of oligodendroglioma-like type, started to become symptomatic.

The microscopical study of these tumors with hematoxylin-eosin technique suggests that they can be classified "malignant schwannomas". They generally show a cell population highly isomorphous, consisting of small cells with dark and more or less rounded nucleus, usually in a central position, with the typical appearance of undifferentiated cells. Furthermore, a great number of mitotic figures can be seen. Moreover, in these tumors there is a large blood supply, with hyperplasia of the vessels and the formation of large cystic spaces. Sometimes is possible to find areas of necrosis. Despite the large cellular isomorphism that characterizes these tumors, is possible see compact areas showing cells with fusiform aspect, arranged in palisade, or sometimes areas with looser reticular aspect. When the tumors are located in the region of trigeminal ganglion, is frequent the presence of large neuron-like cells interspersed with the small undifferentiated cells, which supposedly correspond to trigeminal ganglion neurons that are trapped between tumor cells, but the possibility of actually correspond to a gangliocytic differentiation of the tumor can not be ruled out.

In the ultrastructural study of these tumors, at least two cell types can be found. On the one hand, there was a cell type with dark nucleus, whose chromatin is condensed to form a ring around the nuclear membrane and cytoplasmic features suggesting a neoplastic Schwann-cell. The other cell type shows a small, dark and round nucleus usually with a central position and chromatin that was condensed at the nuclear periphery. These cells showed a dense cytoplasm, with abundant rough endoplasmic reticulum, free ribosomes and polyribosomes, microtubules, primary lysosomes and a large quantity of mitochondria with dense matrix. Some of these cells show cytoplasmic granular vesicles, suggesting neuronal differentiation.

Fig. 1. Macroscopic appearance of an ENU-induced intraparenchymatous brain tumor
Finally, interspersed with these two cell types, it is possible to observe the existence of small cells, with scant cytoplasm and much more irregular nuclear configuration, which were interpreted as undifferentiated tumor cells.

The immunohistochemical study of these tumors shows a clear positivity for S-100 protein and synaptophysin. Furthermore, neuroblastic specific markers, such as NB-84 are positive in all cases. Vimentin is strongly positive in only some cases, and finally, the detection of GFAP is negative in all cases.

Fig. 2. Microscopic aspects of ENU-induced brain tumors. A: Tumor with oligodendroglial aspect showing abundant mitoses. B: Expression of GFAP in astrocytes trapped in the tumor. C: Expression of synaptophysin in an ENU-induced brain tumor with oligodendroglial appearance. D: Tumor cells showing positivity to the neuroblastic marker NB-84.
Fig. 3. Macroscopic appearance of ENU-induced tumors at level of trigeminal ganglion (A) and lumbar roots (B).

Fig. 4. A: Microscopic appearance of an ENU-induced tumor at level of trigeminal ganglion. Mature neurons can be seen, generally interpreted as trapped neurons from trigeminal ganglion. B: Ultrastructural aspect of tumor cells show undifferentiated aspect with dense granules (arrows), suggesting the neuroblastic nature of the tumor cells.
3.3.3 Development
The first sequence of the development of brain tumors induced by transplacental administration of ENU in rats was studied by Lantos and Cox in 1976. There is a latency period that it is the time between birth and the first neurological manifestations. This period has generally been estimated between 5 or 6 months. When animals are killed during this period of time, tumor lesions can be observed with different levels of development.

The different stages of development of tumors induced by administration of ENU transplacentally in rats were also studied by Shiffer in 1991. This author adopted the terms proposed by Koestner et al (1971), and reported various lesions which differed in size and that called as "early neoplastic proliferations" (less than 300 microns), microtumors (between 300 - 500 microns) and "tumors" (greater than 500 microns in diameter). The first "early neoplastic proliferations" appear about two months after birth. These lesions represent early stages of tumor development, and are generally located in the white matter at the level of the lateral ventricles, and the angle of the ventricle, between the caudate nucleus and corpus callosum, or in the subcortical white matter. The tumors that develop from these microtumors retain their morphology, including proliferation centers, but occasionally may have an increased cellular pleomorphism. At four-five months, they show a polymorphic aspect. In many proliferative centers, the cells develop a cytoplasm showing a clear appearance of astrocytes, which subsequently can be gemistocytes. In these neoplasms a central zone showing avascular necrosis, and a peripheral vascular zone can be observed. In most vessels, hyperplasia at level of endothelial cells (Nishio et al. 1983), and an increase in the number of capillaries can be seen.

3.3.4 Possibility of diagnostic "in vivo"
In our studies, we have obtained clear evidence that ENU-induced brain tumors in Wistar rats can be detected in vivo using conventional Magnetic Resonance Image (MRI). With this technique, the experimental brain tumors are characteristicaly hypointense on T1-phase, and hyperintense on T2-phase. They show intense and homogeneous enhancement after paramagnetic contrast administration (gadolinium). It is obvious that using this experimental model, MRI can identify effectiveness of different experimental therapeutic protocols with potential application to human neuro-oncology.

3.3.5 Transplantation
Transplantation and culture in vitro of chemically induced brain tumors have provided important information about their biological characteristics. In addition, transplantation on syngeneic newborn animals can get a great number of brain tumor-bearing animals in a short period of time. On the other hand, tumor lines derived from chemically induced brain tumors are often used, especially for studies of drug response, such as the glioma C-6 of rats, the 9L gliosarcoma, the T9 tumor, the RG2 and F98 gliomas, or the RN-2 glioma.

The C-6 tumor is a glioma induced by methyl nitrosourea in Wistar-Furth rats by Benda et al, in 1968. Usually it shows S-100-positivity. However, this line has the disadvantage of its frequent sarcomatous degeneration, so it is rarely used as a transplantable tumor model, although it has been used occasionally with success in the nude mouse.

The murine 9L gliosarcoma possibly developed natively in an animal crossing Wistar rats and CD Fischer, through the administration of methyl-nitrosourea.

The T9 tumor was induced in F344 rats by methyl-nitrosourea and do not have enough information about its stability in successive passes or transplants.
Fig. 5. Magnetic Resonance Images showing ENU-induced brain tumors. A, B and D were intraparenchymatous oligodendrogial-like tumors. C and D were extraparenchymatous tumors with features of malignant schwannomas.

The RG2 and F98 gliomas were both chemically induced by administering ENU to pregnant rats, the progeny of which developed brain tumors that subsequently were propagated in vitro and cloned. They have an invasive pattern of growth and uniform lethality, which make them particularly attractive models to test new therapies.

The murine glioma RN-2 derived from the induction by ethyl-nitrosourea in F344 rats. It transplanted well and has a stable glial population, many expressing antigenic markers. These and other models are commonly used in experimental neuro-oncology, but it is essential to know the limitations of each of the experimental brain tumor models, and depending upon the nature of the study to be conducted, it is important that the appropriate model be selected (Barth and Kaur, 2009). In any case, the achievement of stable tumor cell lines, capable of growing in immunocompetent animals, is of great importance to study the efficacy of new antitumor drugs or biological agents capable of modifying the biological response in presence of a brain tumor.

3.4 Biological similarities of enu-induced brain tumors with human tumors

Although the ENU-model of neurocarcinogenesis offers the possibility to study many aspects of the biology of brain tumors, the fact is that there are many differences when establishing morphological similarities between the ENU-induced brain tumors and the different types of brain tumors in man. In human neuropathology it is accepted that certain tumors of neuronal nature, such as the so-called "central neurocytoma" may present an appearance of oligodendroglioma, but immunohistochemical and ultrastructural studies provide the correct classification (Hassoun et al. 1982). It is very similar to what happens in the case of ENU-induced oligodendroglioma-like tumors, and similar considerations can be
applied to the ENU-induced malignant schwannomas. In our opinion, the immunostain and ultrastructural pattern of these experimental tumors suggests that, regardless of their histologic appearance with conventional hematoxylin-eosin staining, ENU-induced tumors can be regarded as undifferentiated neuroectodermal tumors with a tendency to neuronal differentiation.

On the other hand, we consider interesting the discussion about the etiological relationships between human brain tumors and experimental ENU-induced tumors. Although there are no reliable data on the etiological factors that determine the beginning of a human brain tumor, and the possibility of a multifactorial mechanism is considered, is interesting the finding that prenatal exposure to a carcinogen can lead to tumor development several months after birth. Moreover, considering the lifetime of rodents, the age that experimental brain tumors become manifest (mean age of the life of the rat) corresponds to the higher frequency of brain tumor development in humans (adult age). If some types of human brain tumor may be caused by exposure to certain carcinogens in the prenatal period, is an open question (Huncharek, 2010).

Fig. 6. A: Subcutaneous transplantation of an ENU-induced brain tumor in immunocompetent newborn rat. B: Tumor growth one month later.
4. References


Brain Tumors: Current and Emerging Therapeutic Strategies focuses on tumor models, the molecular mechanisms involved in the pathogenesis of this disease, and on the new diagnostic and treatment strategies utilized to stage and treat this malignancy. A special section on immunotherapy and gene therapy provides the most up-to-date information on the pre-clinical and clinical advances of this therapeutic venue. Each chapter in Brain Tumors: Current and Emerging Therapeutic Strategies is authored by international experts with extensive experience in the areas covered.

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