RADIATION RESEARCH **179**, 610–614 (2013)
0033-7587/13 $15.00

DOI: 10.1667/RR3130.1

A Mechanistic Model for Medulloblastoma Induction in Mice

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Heidenreich, W. F., Saran, A., Atkinson, M. and Pazzaglia, S. A Mechanistic Model for Medulloblastoma Induction in Mice. *Radiat. Res*. 179, 610–614 (2013).

Medulloblastomas in *Patched* heterozygous mice (*Ptc1*$^{+/-}$ mice) are induced with high probability by ionizing radiation applied in the immediate post-natal period. A mathematical model is described here that accommodates the dependence of the medulloblastoma incidence on dose, age at exposure and age. The model assumes that the first step in the development of the cancer is already present in all cells of the patched mouse due to germ-line inactivation of one allele of the patched tumor suppressor gene. The subsequent rate-limiting step is dependent linearly on dose at least up to 3 Gy. The observed strong decrease in carcinogenic effect of radiation between exposure on day 1 and day 10 is described by a physiological elimination of target cells during post-natal maturation of the brain. A single malignant cell develops into a tumor following a gamma-distribution with mean of about 160 days. The multiplicity of medulloblastomas is predicted.

INTRODUCTION

Mice with one copy of the *Ptc1* gene already inactivated (patched mice) develop medulloblastoma after exposure to ionizing radiation in the first days after birth. Tumorigenesis is rapid and frequent (*1*), and this experimental system is well suited to investigate details of the process of radiation carcinogenesis, and of carcinogenesis in general (*2*). Because cells in the cerebellum of *Ptc1*$^{+/-}$ mice only express reduced levels of the wild-type Ptc1 protein (*2, 3*), and tumors show loss of all wild-type activity, the rate-limiting event to cancer appears to be the inactivation of the remaining wild-type Ptc1 allele.

Here we investigated how medulloblastoma incidence is altered by age, age at exposure and dose of ionizing radiation with the aim of developing a biologically based mathematical model for this system. The model is built with the following assumption: (1) It is assumed that other potentially competing lethal end points are not similarly radiation sensitive in the patched mouse. (2) Age-adjusted rates of medulloblastoma incidence are calculated to obtain a crude description of the data. (3) The dose-dependence of the fraction of all animals, which develop medulloblastomas is compared, with the assumption that one malignant cell is sufficient to induce the cancer and that malignant cells arise after radiation by a Poisson process. (4) A waiting time distribution is fitted to give a complete description of the data by allowing for tumor growth to give detectable tumor mass.

Medulloblastoma is the most frequent malignant brain tumor in children. In about 15% of human medulloblastomas (27% among those aged less than 5 years), mutations in at least one allele of the *PTCH1* gene were found (*4*). For this subset of human medulloblastomas, the *Patched* heterozygous mice are a useful tool for investigation of malignant progression in medulloblastoma.

MATERIALS AND METHODS

We use data from 7 groups of mice, treated with a range of full-body X-ray doses from experiments done by the Italian coauthors, Pazzaglia *et al*. (*1*), see Table 1. The Kaplan-Meier plots for the end point medulloblastoma and the survival function for all other end points (competing risk) are plotted in Fig. 1.

The medulloblastoma incidence is very high after exposure on day 1 (P1) and it is strongly decreasing with age at exposure within the first 10 days of life. The survival function for the competing risk falls roughly into two categories, the groups with a dose of 3 Gy and all others. Therefore medulloblastoma is the most radiation sensitive cancer in this animal model. Since medulloblastoma is also shortening the life of the mice, therefore by definition must be considered a fatal cancer.

The building blocks used in developing the mathematical model are standard parts of biologically based cancer models. In particular, age adjusting for given competing risks follows the technique described in ref. (*5*), and the waiting time distribution for the growth of a malignant cell to an observable tumor described in ref. (*6*) was employed. Other parts of the model are described in the Results section, when the model can be justified more clearly.

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TABLE 1
Characteristics of the Experimental Groups of Mice

AAE (days)	Dose (Gy)	Number	Medulloblastomas
Controls	0	42	4
1	0.10	41	6
1	0.25	43	12
1	0.50	36	18
1	3.0	21	17
4	3.0	49	26
10	3.0	33	1

Note. Shown for each experimental group is the age at exposure (AAE), the dose, the number of mice for which a pathological classification was performed, and the number of mice with at least one medulloblastoma.

RESULTS

The raw fractions of medulloblastoma incidence do not allow for a good comparison of the risk, as the survival times are very different. This suggests that age-adjusted fractions would be more appropriate. For the groups exposed on day 1, especially at the higher doses most animals develop a medulloblastoma. A technique for age-adjustment that can deal with such a situation does not adjust to ''same survival'' (*7*), but to ''same competing survival'' (*5*). Depending on the chosen function for ''same competing survival'', different rates were calculated. We adjust to the competing survival of the group with day 10 and 3 Gy of exposure (short survival), and to no competing risk. The raw rates and the adjusted rates with standard error boundaries are shown in Table 2. When adjusted to the shorter period the fractions increase almost linearly for doses up to 0.5 Gy.

One hypothesis for the induction of medulloblastoma suggests a time point during which a pool of undifferentiated cells present in the cerebellum are the target cells for medulloblastoma. If one of these cells acquires an inactivating mutation in the remaining *Ptc1* allele, then medulloblastoma develops within a maximum of about 400 days. This is the age after which no further medulloblastoma occurred in the given dataset.

This hypothesis also allows us to predict the fraction of animals free of cancers from two numbers: A certain fraction f_0 of animals develop a cancer cell spontaneously (about 10%). Radiation induces an additional fraction of *Ptc1*-mutations. A linear dose dependence is described as a Poisson process with expectation values λD. For exposure on day 1 the parameter $\lambda \approx 1$/Gy, and it rapidly decreased with advancing age at exposure. With these assumptions the fraction of animals free of medulloblastoma at 400 days after exposure to dose D is

$$S = P(0, f_0 + \lambda D), \tag{1}$$

where $P(n,\lambda)$ describes the Poisson-probability for n events. In this expression no competing risks are considered.

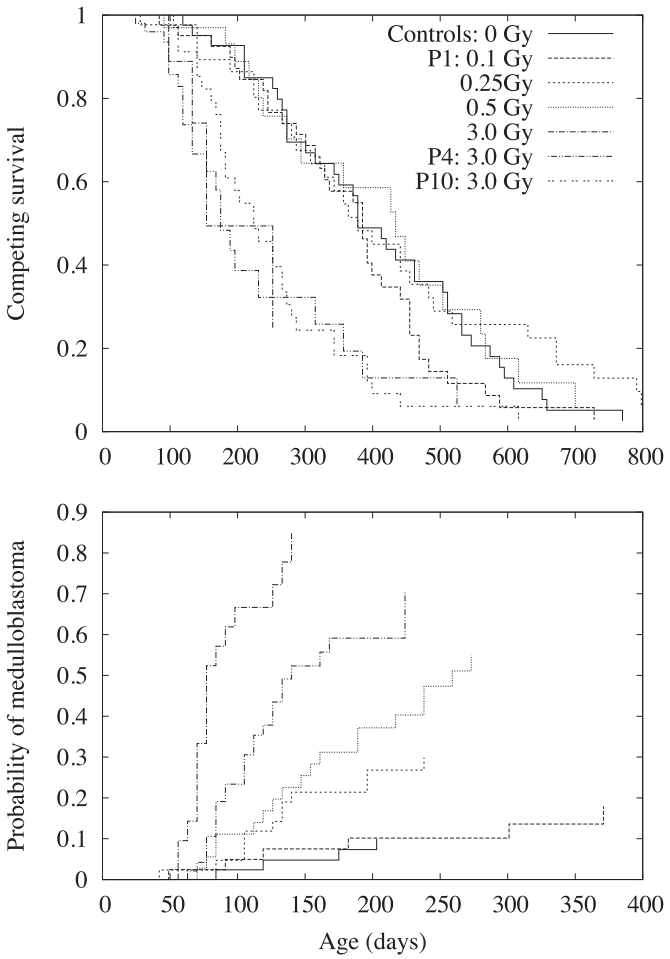

FIG. 1. Kaplan-Meier plot of survival function for all risks competing with medulloblastoma (upper panel). Kaplan-Meier plot for medulloblastoma incidence (lower panel). The P10 group is not shown as there is only 1 case.

Figure 2 compares the medulloblastoma incidence corrected to no competing risk from Table 2 with the expectation from Eq. (1). The maximal incidence of the groups exposed at day 1 is described well with the linear dose-response.

The model also predicts the probability for any number n of independently created malignant cells as $P(n, f_0 + \lambda D)$. The predicted multiplicity of radiation-induced medulloblastomas in the mice is shown in Table 3. It has not been determined experimentally and requires some means of establishing clonality in the tumor tissue.

When inspecting Fig. 1, one can see that the groups with higher doses (or higher tumor fraction) tend to have shorter times to tumor. This could be explained by the occurrence of a larger average number of malignant cells initially (several clones, of which only the fastest one is recorded). To test this hypothesis, we use a waiting time distribution for the time from a first malignant cell to the observable cancer. Specifically we used the 2-dimensional gamma-distribution

TABLE 2
Raw Percentages for Medulloblastoma Incidence and Age-Adjusted Percentages with Standard Errors

			Adjusted to short survival		No competing risk	
AAE (days)	Dose (Gy)	Raw	Estimate	Range	Estimate	Range
Controls	0	9.5	8.0	(3.8–12.2)	9.9	(5.2–14.6)
1	0.10	14.6	10.7	(5.7–15.7)	17.9	(11.0–24.8)
1	0.25	27.9	25.2	(18.5–32.0)	29.9	(22.6–37.2)
1	0.50	50.0	40.2	(32.1–48.4)	54.9	(45.9–63.8)
1	3.0	80.9	83.5	(74.7–92.4)	85.2	(76.5–93.9)
4	3.0	53.1	62.6	(54.0–71.1)	70.8	(61.7–79.9)
10	3.0	3.0	3.0	(0.0–6.0)	3.3	(0.06–6.6)

$$f(a,b,t) = \frac{1}{b\Gamma(a)}(t/b)^{a-1}e^{-t/b}, \quad (2)$$

that has been previously used by other to smear out lag time in the two-stage clonal expansion model (*6*). With this gamma-distribution correction the medulloblastoma survival function for mice with one initial malignant cell at $t=0$ is

$$S(t) = \int_{t/b}^{\infty} f(a,b,t')dt' = Q(a,t/b), \quad (3)$$

where Q is the incomplete gamma-function. For n independently created initial malignant cells the survival function is Q^n. This assumes that the malignant clones do not influence one another. The probability of not having acquired a medulloblastoma up to waiting time t for mice with a probability $P(n)$ for n malignant cells at time $t=0$ is

$$S(t) = \sum_{n=0}^{\infty} P(n)Q(a,t/b)^n. \quad (4)$$

The hazard function becomes

$$h(t) = -\frac{dS(t)/dt}{S(t)} = \frac{\sum_{n=1}^{\infty} nP(n)Q(a,t/b)^{n-1}f(a,b,t)}{S(t)}. \quad (5)$$

For the waiting time t in these formulas we use the age of the animal, as even the last age-at-exposure studied (10 days) is short compared to the waiting time, and the spontaneously occurring malignant cells are also assumed to be present before that date.

The parameter values estimated from the data are given in Table 4. The quality of the agreement of the final model with the data can be seen in Fig. 3. The model has three main mechanistic features: (1) A linear-dose response up to 3 Gy for inactivation of the remaining *Ptc1*-gene, where at an age of 1 day, 75 mGy produce about the same number of mutated cells, as are created spontaneously. (2) A strong decrease of this mutation probability with age at exposure shown in Fig. 4. (3) A gamma-distribution $f(a,b,t)$ for waiting time from a malignant cell to the observable tumor shown in Fig. 5.

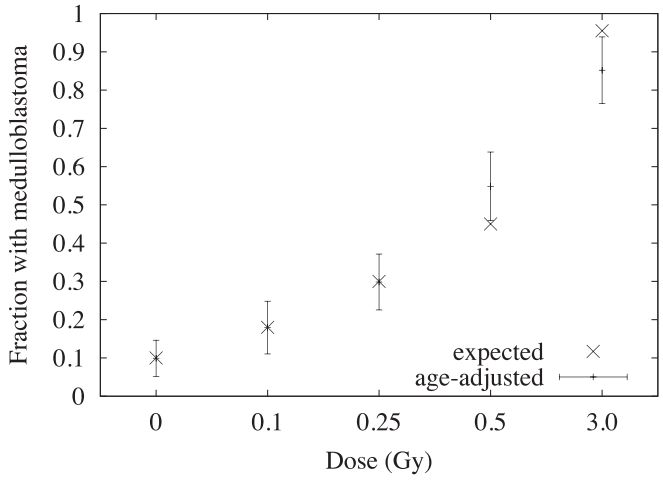

FIG. 2. Comparison of the medulloblastoma probability and its standard errors, with the probability expected from Eq. (1) for the controls and the groups exposed at P1. Both of these probabilities are corrected to no competing risk.

DISCUSSION

Our mathematical model of medulloblastoma induction is based on the Knudson two-step mechanism of cancer (*8, 9*), and assumes that inactivation of both copies of the *Ptc1* gene are the rate-limiting events for the induction of medulloblastoma in wild-type mice. In the $Ptc1^{+/-}$ mice, one copy is already inactivated in all cells by the germ line inactivaton. So compared to a wild-type mouse, all sensitive cells form a premalignant clone for medulloblastoma.

TABLE 3
Probabilities for 0, 1 or More Medulloblastomas, Calculated from the Poisson-Distribution

	Probability for medulloblastoma		
Dose (Gy)	0	1	More
0.0	0.90	0.09	0.01
0.1	0.82	0.16	0.02
0.25	0.70	0.25	0.05
0.5	0.55	0.33	0.12
3.0	0.04	0.14	0.82

TABLE 4
Estimated Parameter Values and Standard Errors from Profile Likelihood for the Mutation-Model

Parameter	Estimate	Standard error
f_0	0.09	0.04
λ(P1) (Gy^{-1})	1.2	0.2
λ(P4) (Gy^{-1})	0.43	0.10
λ(P10) (Gy^{-1})	−0.01	0.02
a	5.4	0.8
b (days)	30	6

Therefore is only one rate-limiting step left for the development of medulloblastomas, and this step is the inactivation of the wild-type copy of *Ptc1* in one of the target cells of medulloblastoma. This step can occur spontaneously, or be induced by radiation. For the development of an observable tumor from a malignant cell with two inactivated *Ptc1* alleles, a standard waiting time distribution is used. With these mechanistic assumptions our model gives a good fit with the available data. The quantitative results obtained with this model can be compared with values from the literature for other systems.

The dose response for the inactivation of the wild-type *Ptc1*-gene is approximately linear up to 3 Gy. This agrees well with data from mutation induction to thioguanine-resistance in HF19 human cells; however for V79 hamster cells a slightly larger increase with increasing dose was observed (*10*).

We have shown that the effect of radiation is strongly dependent on age at exposure. One interpretation within the model could be, that the number N of susceptible cells is decreasing by a factor of about 3 between day 1 and day 4, and that no susceptible cell remains at day 10. The small decrease of medulloblastoma after exposure at day 10 could be due to cell killing by irradiation, but this decrease is not statistically significant. The decrease in the number of susceptible cells is consistent with developmental patterns of the tissue (*11*). For example during the first 10 days after birth the target cells can undergo differentiation beyond a state where they can become malignant by a single step. The age-at-exposure dependence here is compatible with the one found for medulloblastoma induction in Math1-creER/$Ptc^{c/c}$ mice, see ref. (*2*). By treating the mice with tamoxifen at various ages, the authors reported that both *Ptc1*-alleles can be inactivated in Math1-expressing cells, which include granule neuron precursor (GNP) cells. The authors also demonstrate that medulloblastoma is rare when mice are treated about 10.5 days before birth, or 10 days after birth. But when the tamoxifen treatment is given between 6.5 days before birth and 8 days after birth all animals developed medulloblastoma. However, at the age of 10 days, only 2 out of 7 animals developed medulloblastoma. The effect of

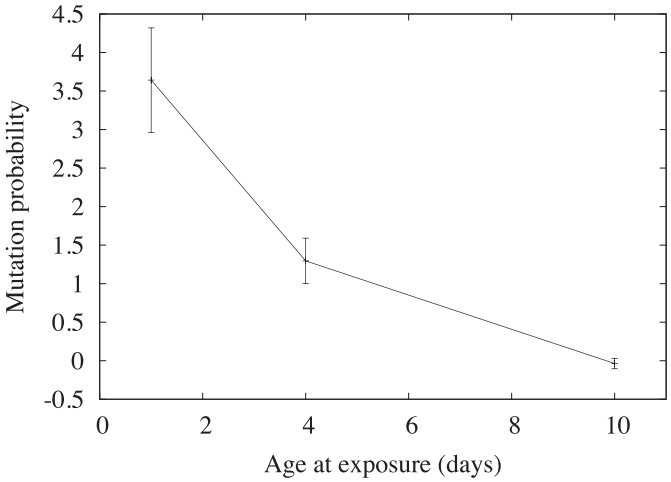

FIG. 4. Age-at-exposure dependence of the probability of a malignant cell being created by a dose of 3 Gy. The line is drawn only to guide the eye.

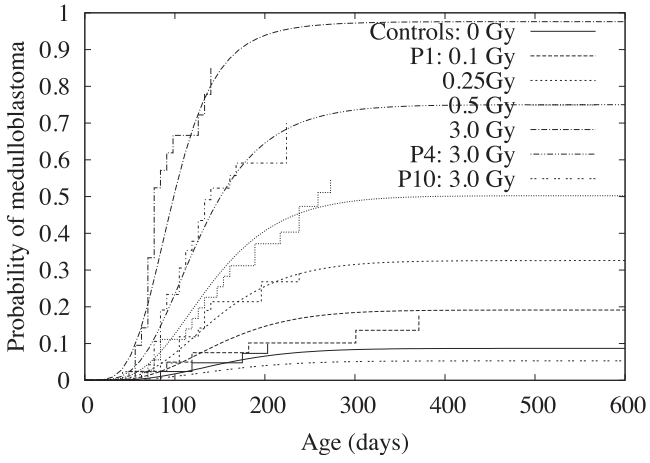

FIG. 3. Survival functions for medulloblastoma-induction with the mutation-model and Kaplan-Meier plots for medulloblastoma incidence.

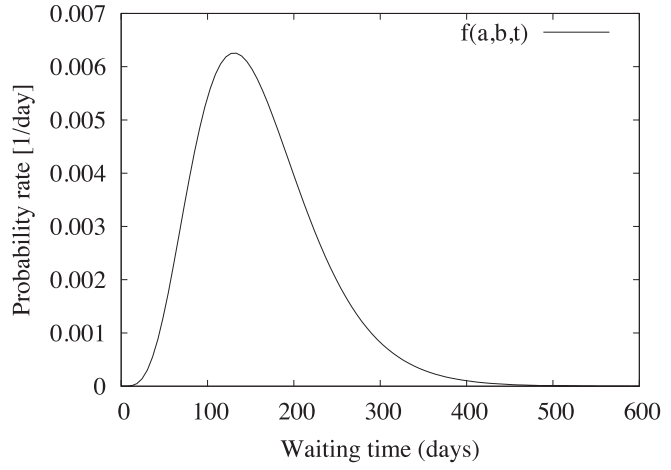

FIG. 5. Estimated waiting time distribution for one malignant cell to an observable medulloblastoma. The parameters, a = 5.4 and b = 30 days are fitted values for the gamma distribution f.

the tamoxifen treatment deletes the patched genes in all GNP cells, and therefore is much larger than any of the radiation exposures here. Nevertheless, the survival curve given for day 4 in ref. (*2*) is close to the strongest effect curve (3 Gy on day 1), we observed, which supports our concept that at this exposure time several cells are mutated.

In our present model the total spontaneous inactivation of the *Ptc1* gene has the same induction as about 75 mGy on day 1 or 200 mGy on day 4. The target size for spontaneous medulloblastoma induction can be described as the number of target cells times the period they exist as target cells, i.e., the integral over age a

$$\mathrm{T} = \int N(a)da \qquad (6)$$

The number of spontaneously produced malignant cells would therefore be $\lambda_0 T$, where λ_0 is the spontaneous mutation rate.

For lung cancer in mice it was estimated that about 10 mGy produce as many initiating mutations as would be expected spontaneously in one day (*12*). If we use this estimate here, a target size T of 7.5 days times N(day 1) would be consistent with the present results. Figure 4 would suggest a T of about 3 days $\times$ N(day 1) from an age of 1 day. Depending on the size of the prenatal target, this could give a good agreement with earlier published results. The age-dependence of the prenatal target from ref. (*2*) described above is consistent with this comparison.

The waiting time distribution describes heuristically the time from a malignant cell to the observed tumor. The shape of the waiting time distribution we utilized has been previously used, for a model of lung cancer induction in humans (*6*). The values for mice are surely different. For lung cancer induction in adult mice, a fixed waiting time of 170 days after neutron exposure, and 250 days for gamma-exposure were estimated (*12*). These earlier estimates are consistent with the distribution here, which has a mean of about 162 days. During this waiting time, additional processes can occur than just deterministic growth of the malignant cell, but these would be expected to occur rapidly, and they therefore are not anticipated to be rate limiting. The mathematics of our model is also applicable if some of the malignant clones randomly die out. In that case the creation rate refers to the surviving clones only.

In summary, the large relative risks of radiation-induced medulloblastoma can be understood using a combination of existing modeling assumptions, estimated radiation action quantities, and a short time window for some of the GNP-cells to become malignant by a single step. The distribution in age of medulloblastoma agrees with existing waiting time distributions observed in other systems. These results suggest that the mouse model can be used to study some of the later steps of radiation carcinogenesis with a very sensitive and fast experimental system.

Received: September 12, 2012; accepted: December 12, 2012; published online: April 5, 2013

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