Introduction

Craniopharyngioma is a benign neoplasm of epithelial origin located in the sellar and suprasellar region. The adamantinomatous craniopharyngioma represents around 2/3 of all craniopharyngiomas and is the most common pathology in this region in children [1,2]. Other histological subtypes are the squamous papillary and the xanthogranulomatous type [3]. The former occurs almost exclusively in adults. The main treatment options are surgery and conventional radiotherapy (CRT). Surgical cure is hampered by the eloquent surrounding structures and is accomplished in few cases. The acknowledgement of the devastating effect of hypothalamus damage on quality of life has led to more conservative surgery and increased use of adjuvant RT in children [4,5]. CRT has proved its value in controlling craniopharyngiomas; however considerable irradiation to the surrounding tissues is unavoidable by this technique and not without sacrifices.

The decline in intellectual function in children [6], inability to treat the youngest patient group, radiation induced malignant gliomas [7] and 10-years recurrent free survival rates of 32 % - 49 % [8,9] in children stresses the limits in the current CRT.

Gamma Knife radiosurgery (GKRS) has been used as an alternative to CRT to reduce the radiation load on the surrounding tissues. Reports on low morbidity from the anterior visual pathways using this technique, 3.1%(10), underlines the power of photon beam focusing. The main drawbacks are the target volume restrictions and higher risk of geometrical misses compared to CRT. The ultimate limitation of the single session...
GKRS is that the most effective GKRS-dose, 11.7 - 12.7 Gy, is also the critical toxicity dose for the anterior visual pathway [10,11].

The majority of craniopharynoma patients receive radiation treatment during their diseased life and around one third of them experience tumor progress [12]. The hazard of repeated irradiation precludes further radiation in most cases and the danger of repeated surgical trauma is well known. The gravity of the situation of late recurrences is reflected in a very high mortality after salvage treatment [13,14]. Thus, improvement in the radiation treatment is clearly needed. No clear data exists on the radiosensitivity of craniopharyngiomas and the optimal dose has not been defined. The lack of detailed dose-response data and standardized way of reporting endpoints disables a thorough interpretation of radiobiological data from the literature. Furthermore in vitro radiosensitivity data, that could be useful in designing new fractionation regimens, is lacking.

The purpose of this study is first to assess the in vitro radiosensitivity of adamantinomatous craniopharyngiomas and second to address the alpha beta ratio (α/β) specifically from a clinical perspective.

**Methods and Materials**

**Cell cultures**

Primary cultures of human craniopharyngioma cells were isolated and prepared from tumour samples in a similar manner as for keratinocytes according to the methods described elsewhere [15,16]. The obtained cultures used for the (irradiation) experiments were plated without feeder cells in passages between 3 and 9 (median 5). All patients harboured histological verified adamantinomtous type of craniopharyngioma. The procedures were in accordance with the ethical standards of the institutional committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 2000.

**Clonogenic survival**

Appropriate cell numbers were plated for survival using the clonogenic assay technique described previously [17]. The single-cell suspensions were plated into 35 mm plastic petri dishes (Corning, New York) in triplicates to a final medium volume of 3 ml and then left in the incubator for 3-4 h to attach before irradiation.

The cells were irradiated at room temperature with doses of 0-10 Gy at a dose rate of 0.5 Gy/min from a $^{137}$Cs-source (Scanditronix, Uppsala, Sweden). The cultures were then incubated for 10-14 days, with a change of medium after 5-7 days. Thereafter colonies were fixed, stained and counted. Radiation survival curves were constructed from two independent experiments. The mean PE for un-irradiated cells was 43, 55, 85, 12, 10, 42 and 36% for case 1 to 7, respectively.

*Dose-response models for clonogenic cell survival.* The LQ model[18] was used to fit the data with the Maximum Likelihood method, where the probability for clonogenic cell survival $S$ at a dose $D$ is given by [19]:

$$S = e^{-\alpha D - \beta D^2}$$  \hspace{1cm} (1)

The surviving fraction at 2 Gy (SF2) was computed from the whole survival curve and was used as a unique measure of cellular radiation sensitivity. The mean inactivation dose, $\bar{D}$, was calculated according to Taylor [20]. The $\bar{D}$ was chosen since it has been shown to keep the scattering of data smaller than for other parameters such as SF2 and D0 [21].

**Assessment of α/β in vivo**

From in vivo data, the LQ model can be used to calculate the α/β from isoeffective fractionation regimens. This is based on the assumption that the biological effective dose (BED) is the same if two fractionation regimens result in an equivalent clinical effect [22]. In this study, the same tumour control rates were assumed with 2.0 Gy daily fractions with a total dose of 50 Gy [23] and 11.5 Gy prescription dose at the 50% isodose line using GKRS [10]. For the GKRS, it was assumed as a first reasonable approximation, that the tumor response was related to the mean dose to the tumor which is generally 30 - 50% higher than the dose to the periphery. Thus for a prescribed dose of 11.5 Gy, the mean dose will be in the range from 15 Gy to 17 Gy. Calculation were done for two cases; 1) without- and with correction for repopulation during the time of fractionated treatment, in the second case, two values of Tk (the time after start of the treatment when repopulation starts) were used; for keratinocyte-related malignancies such as head and neck tumors (Tk=21 days) and craniopharyngioma related normal tissues such as mucosa (Tk=7 days) [24]. For each of the two Tk values, the cell doubling time (Tp) was estimated in patient C1 and C2 using the formula

$$Tp=0.693*t/\ln(N/N_0).$$

![Figure 1: Clonogenic survival curves for 7 craniopharyngioma cell strains as a function of single absorbed doses. The experimental data are fitted to the LQ survival model. Error bars indicate S.E.](image)
The clonogenic cell survival curves and the radiosensitivity parameters from the seven craniopharyngioma cell strains are presented in Figure 1 and Table 1, respectively. The mean values and the coefficient of variation (CV) for SF2 and C2 were 0.401 (SE +/- 0.021) and 2.04 (SD +/- 0.08) and 17% and 15%, respectively. The $\alpha/\beta$ value ranged from 10.3 to 29.9 Gy with CV of 33%. The mean value was 19 Gy (SE +/- 2.4). Acknowledging the fact that other survival models will fit the data in the lower dose region better, we used the entire dose range when fitting the data. This was done since doses in the range of 8-13 Gy are being used for these types of tumors.

In Figure 2, $\alpha/\beta$ values are given as a function of the mean dose to the tumor in GKRS. Estimated mean doses to the tumor, for a prescription dose of 11.5 Gy will be in the range from 15 Gy to 17 Gy. Results are shown, without correction for repopulation, as well as corrected with two Tk values, with two sets of Tp and $\alpha$ values.

Table 1: Parameters from the clonogenic survival curves are shown. Mean values from two repeat experiments for each case are indicated.

<table>
<thead>
<tr>
<th>Case</th>
<th>$\alpha$ (Gy$^{-1}$)</th>
<th>$\beta$ (Gy$^{-1}$)</th>
<th>$\alpha/\beta$ (Gy)</th>
<th>SF2</th>
<th>D1(Gy)</th>
<th>D2(Gy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.3492</td>
<td>0.0145</td>
<td>24.1</td>
<td>0.471</td>
<td>9.51</td>
<td>2.44</td>
</tr>
<tr>
<td>2</td>
<td>0.3282</td>
<td>0.0322</td>
<td>10.3</td>
<td>0.457</td>
<td>7.91</td>
<td>2.23</td>
</tr>
<tr>
<td>3</td>
<td>0.3273</td>
<td>0.0266</td>
<td>14.6</td>
<td>0.468</td>
<td>8.45</td>
<td>2.44</td>
</tr>
<tr>
<td>4</td>
<td>0.5294</td>
<td>0.0254</td>
<td>20.8</td>
<td>0.313</td>
<td>6.60</td>
<td>1.65</td>
</tr>
<tr>
<td>5</td>
<td>0.5210</td>
<td>0.0187</td>
<td>29.9</td>
<td>0.328</td>
<td>7.10</td>
<td>1.73</td>
</tr>
<tr>
<td>6</td>
<td>0.4427</td>
<td>0.0274</td>
<td>16.4</td>
<td>0.371</td>
<td>7.20</td>
<td>1.88</td>
</tr>
<tr>
<td>7</td>
<td>0.4057</td>
<td>0.0249</td>
<td>19.3</td>
<td>0.403</td>
<td>7.73</td>
<td>2.04</td>
</tr>
<tr>
<td>Mean</td>
<td>0.4148</td>
<td>0.0242</td>
<td>19.3</td>
<td>0.401</td>
<td>7.79</td>
<td>2.04</td>
</tr>
<tr>
<td>SE</td>
<td>±0.0269</td>
<td>±0.0026</td>
<td>±2.4</td>
<td>±0.021</td>
<td>±0.21</td>
<td>±0.08</td>
</tr>
<tr>
<td>CV (%)</td>
<td>21</td>
<td>24</td>
<td>33</td>
<td>17</td>
<td>12</td>
<td>15</td>
</tr>
</tbody>
</table>

*Dose to achieve 1% survival (S); *Mean inactivation dose (Dbar)

Discussions

In this study we present a novel data set on radiosensitivity of adamantinomatous craniopharyngioma cell strains, based on clonogenic survival assays. More than 25 years ago Fertil et al. [25] pointed out a correlation between the radiosensitivity of human cancer cell lines in vitro and the radiocurability of the corresponding tumor types in vivo. Since then, histological groups of human cell lines have been characterized by an intrinsic radiosensitivity in vitro [26,27] and this data has been shown to be useful in predicting tumor response to RT [28].

The high variation of $\alpha/\beta$ within specific tumor group as for adamantinomatous craniopharyngiomas in this study is commonly noted in reports for different human cancer lines in vitro. Taghian et al. [29] reported data on glioblastoma, which is among the most radiosensitive tumors. The $\alpha/\beta$ ranged from 3.7 to 48 Gy. Weichselbaum et al. [30] reported mean $\alpha/\beta$ for human cancer cell lines with large SD values, reflecting a high variation within each tumor group.

According to SF2 and C2 craniopharyngiomas cell strains are slightly more radiosensitive than the average radiosensitive cancer cell line [27] and the variation is not higher than reported in other cell lines [21,27]. Comparative in vitro data for other benign intracranial tumors is lacking. However, from in vivo data, and with the method used here $\alpha/\beta$ for meningiomas and vestibular schwannomas have been calculated to be 3.3 Gy and 2.3 Gy, respectively [31]. In this study, the $\alpha/\beta$ will be in the range of 4-6 Gy for adamantinomatous craniopharyngiomas, without correction for repopulation and from 6 Gy to more than 20 Gy if reasonable parameters for correction for repopulation are applied (Figure 2). Those values are still much lower than the in vitro values in this study. The reason for this discrepancy could be explained by a lack of correlation between the in vivo and in vitro data, by paucity of reliable in vivo data or both.

Arguments for craniopharyngiomas having high $\alpha/\beta$ can be found when growth kinetics is considered. It is known that the growth kinetics of the tissue irradiated dominates the radiation response. A higher proliferation indices [32-35] and more dramatic response to irradiation [10,36-39] compared to meningiomas and acoustic neuromas support our findings of a high $\alpha/\beta$ for craniopharyngiomas. The $\alpha/\beta$ for craniopharyngioma related tissues, such as mucosa and epithelium is in the range for early responding tissues or 7 - 15 Gy [40-42]. Since there is often a close similarity between the mean lethal dose of tumour and the normal tissues from which they arise, it is likely that $\alpha/\beta$ for craniopharyngioma is in this high range. If this is the case, one should consider the repopulation time factor in calculating the $\alpha/\beta$ from clinical data. This results in higher $\alpha/\beta$ values (Figure 2) [43,44].

The above estimations of $\alpha/\beta$ values based on in vivo data have to be viewed with caution since the differences in patient and treatment characteristics between clinical studies make it difficult to compare results from different radiation regimens. The main obstacle is that certain parameters that can influence endpoints, such as histological subtypes and tumor volume, are generally not considered in reports on treatment results.
The comparison of iso effective doses for GKRS and CRT could be flawed by differences in tumour volumes treated with those techniques. The general principle is that large tumours (more clonogenic cells) require higher doses than small ones (fewer clonogenic cells) to obtain the same tumour control probability (all clonogenic cells killed, Figure 1). This is also partly explained by the differences in micro-environment between large and small tumors [45,46]. The lack of information on tumour size is more a rule than an exception in studies using CRT for craniopharyngiomas. In viewing the few studies available, it seems that the tumour volume tends to be substantially larger in the CRT studies compared to GKRS studies [10,47-49]. This is a reasonable assumption regarding the radiobiological volume restrictions of GKRS. The implication of this phenomenon in CRT for craniopharyngioma is probably less than for more solid tumours types due to the large fluid component in craniopharyngiomas.

Taking the arguments above together:

A. CRT studies tend to include substantially larger tumour volumes and

B. Larger tumour volumes require higher doses for local control, it is tempting to conclude that a somewhat lower CRT dose than 50 Gy would give the same local control as GKRS for similar tumour volumes. If this line of arguments is correct, the α/β from clinical data, will be larger than the ones presented in Figure 2 and hence even more close to the α/β presented in this study from in vitro data.

It is debated whether benign intracranial tumours benefit from fractionation since the α/β is thought to be similar as for neural tissue or 2 Gy. This is generally supposed to reduce the beneficial effects of fractionation but if the clinical α/β is as high as observed in our study we have strong arguments to apply fractionated radiation regimens to improve the therapeutic ratio. It is however important to notice that improvements in RT are clearly needed.

Even though CRT has been used for craniopharyngiomas for more than 40 years, the toxicity risk for the anterior visual pathway and the hypothalamus has not been reduced with recent improvements in this technique. The major part of those structures is still included in the high dose field prescribed for the tumour and receives doses just below the maximum tolerance level [6,39,48]. Furthermore structures involved in neurocognition are still at risk [6]. Improvements could be made by using fractionation with Gamma Knife® or similar technique. By this approach one could overcome the target volume restrictions of single fraction treatment, avoid critical doses to structures involved in neurocognition and reduce the radiation load on the visual pathway and hypothalamus. Radiobiological data such as presented in this study would then be needed to design new fractionation regimens.

Conclusion

The high α/β in this in vitro study indicates that the adamantinomatous craniopharyngioma is among early responding tissues and supports the clinical praxis of fractionated radiation. The radiosensitivity parameters, SF2 and $\alpha/\beta$, indicate that these cell strains are slightly more radiosensitive than the average radiosensitive cancer cell line. Although some correlation with in vivo data is found, improvements in presenting treatment parameters and results after radiation treatment and further in vitro studies are encouraged to validate this data.

References

15. Green H, Kehinde O, Thomas J (1979) Growth of cultured human...


