Br. J. Cancer (1986), **53**, Suppl. VII, 12–15

Radiation response of the mouse tongue epithelium

R. Moses[1] & J. Kummermehr[2]

[1]*Strahlenbiologisches Institut, Universität München, and* [2]*Abteilung für Strahlenbiologie, Gesellschaft für Strahlen-und Umweltforschung, D-8042 Neuherberg, FRG.*

Mucositis is often a limiting complication in the radiotherapy of advanced ENT tumours in that it necessitates a delay in the completion of treatment. More recently, the introduction of regimes with multiple fractions per day was found to alter the response of mucosa (Van der Schueren *et al.*, 1983). The biological principles of how delivery of a greater dose in a given time will affect the tolerance or the response kinetics of a rapidly turning-over tissue are understood. The detailed mechanisms and the possible ways of optimising treatment, however, will have to be worked out and suitable animal models are needed. Mouse lip mucosa has shown to meet the requirements better than mouse skin (Parkins *et al.*, 1983; Xu *et al.*, 1984). In this paper we propose mouse tongue mucosa as a further model. The ventral surface of mouse tongue is covered by a multilayered squamous epithelium which is fairly similar in its structure to human oral mucosa, although the latter does not keratinize.

Materials and methods

For local tongue irradiation, female C3H mice were anaesthetised with Enflurane and placed in a prewarmed aluminium block that held one animal in a central bore. The head was gently positioned against the top by a wedge and the tongue was carefully pulled out through an overlying small hole. It was fixed horizontally on the surface by sellotape, so as to expose the proximal 5 mm of the ventral surface to a vertical X-ray beam. The machine used was a Siemens Dermopan, operated at 29 kV, with 0.3 mm Al filtering. The HVL thus was about 3 mm of tissue; this gave good homogeneity in the epithelium facing the beam while the opposite surface received only 80% of the entrance dose.

In pilot expierments the tongue reaction was observed daily, under Enflurane anaesthesia. It soon became obvious that the severe reaction occurred regularly between days 8 and 13 and the daily inspections were restricted to this period. An arbitrary scoring system including erythema, oedema and ulceration was initially applied. As it turned out, ulceration gave the least equivocal response and hence the percentage of animals that developed an ulcer was used to quantitate the dose dependence of mucositis.

For histology, tongues were excised daily between days 4 and 14 after a single dose of 20 Gy, routinely embedded in methacrylate and sections stained with haemalum and eosin. For cell kinetic studies, controls and animals irradiated with 20 Gy six or 12 days previously, were pulse-labelled with [^{3}H]-TdR over a 24 h-period. Tongues were excised 45 min after injection, divided in midline, processed as above and autoradiographs made.

Results

Following doses greater than 14 Gy, acute symptoms in the tongue develop within 6 days. At this time oedema and erythema may become visible, reaching a maximum on days 8 and 9 and then slowly subsiding. With doses over 15 Gy ulcers develop on days 9 to 10 and reach a maximum on days 10 and 11. They are mostly located on the tip and the lateral borders and rarely extend over the entire irradiated area. Healing is always rapid and even after high doses is complete on day 13 or 14. Body weight was also regularly recorded. As shown in Figure 1, maximum loss occurred on day 10, thus slightly preceding the maximum mucosal reaction. In some protocols weight loss was strongly correlated with dose, while in others it was not. Major histological damage can be detected before the clinical symptoms. On day 4 the basal cell nuclei show distinct swelling and by day 6 become grossly atypical. By day 8 a defined basal layer is no longer present; cell number is about halved, but due to the enlargement of individual cells the epithelial thickness is almost maintained. Denudation, however, follows quickly. On day 9 patches of single-layered atypical cells and complete denudation are seen. Deeper erosions prevail on day 10 or 11. This is followed by rapid regeneration leading transitorily to a hyperplastic epithelium on days 13 and 14.

The proliferation pattern in normal epithelium and in tongues at days 6 and 12 after 20 Gy is depicted in Figure 2, where the number of labelled cells per surface length is plotted against time of day. A marked diurnal fluctuation emerges in the control, with a maximum of cells in S-phase around 2.00 a.m. and a minimum around 4.00 p.m. Prior to epithelial loss in irradiated tongue, i.e. 6 days after 20 Gy, there is still remarkable proliferation, but

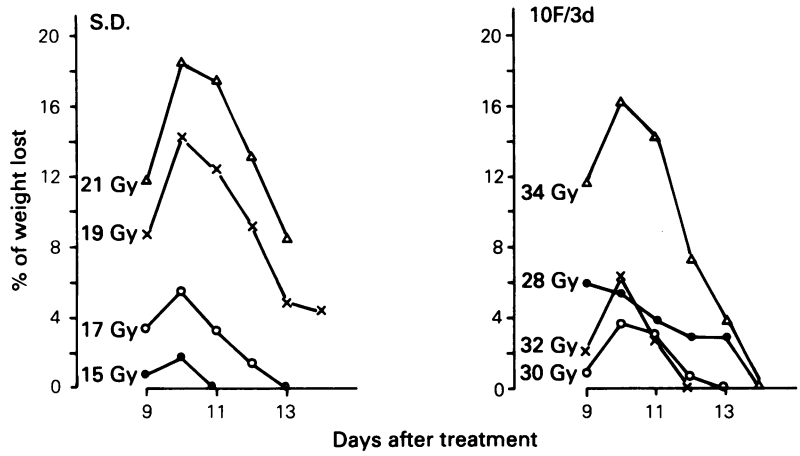

Figure 1 Percent loss of body weight during acute mucositis following local tongue irradiation with 29 kV X-rays.

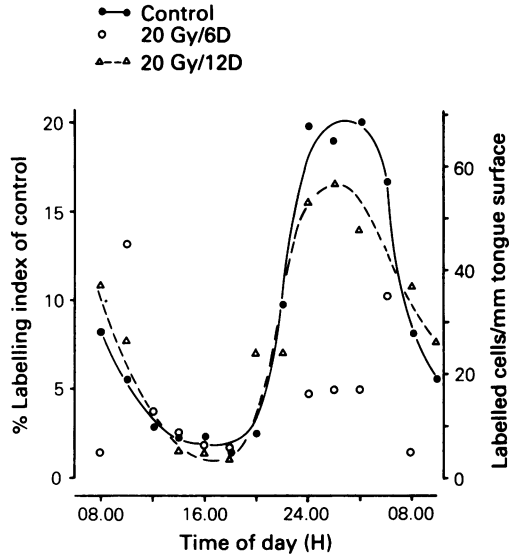

Figure 2 Time course of cells in S-phase in the bottom surface epithelium of mouse tongue.

a diurnal rhythm is questionable. On day 12 after 20 Gy, when scored close to tongue midline, i.e. in areas that usually just escape denudation but become hyperplastic, the number of labelled cells per mm surface and thus cell production per unit area have almost returned to normal and the circadian rhythm is fully restored (open triangles in Figure 2).

The proportion of animals that developed an ulcer as a function of single or fractionated dose is shown in Figure 3. For single doses the threshold is between 14 and 15 Gy, and the ED_{50} is 16.8 Gy. With 2, 4, and 10 fractions the ED_{50} is raised to 20.1 Gy, 24 Gy and 31 Gy, respectively. The ED_{50} values were analysed by the linear-quadratic model, plotting reciprocal total dose against dose per fraction. As shown in Figure 4, this resulted in an α/β ratio of 14.4 Gy.

Discussion

Mouse tongue mucosa appears to be a useful model to study dose responses to local irradiation. The irradiation procedure is certainly less feasible and more time-consuming than e.g. snout irradiation (Xu *et al.*, 1984). On the other hand, tongue is the only location where a reasonable area of intraoral, multilayered epithelium in the mouse can be locally treated and scored. A further advantage is the relatively small burden that is imposed upon the animal.

The dose levels that produce a severe acute reaction are only slightly higher than in lip mucosa (Parkins *et al.*, 1983; Xu *et al.*, 1984). In pilot experiments with external 300 kV X-irradiation just tolerated by the lip we have not seen critical damage to the tongue. A future technique will be to use 300 kV X-rays to the snout to produce a subclinical effect, supplemented with local top-up doses of 29 kV X-rays.

Radiobiologically, erosive mucositis of the tongue is a typically acute effect. This also agrees with the response to fractionated irradiation. The α/β ratio derived is the highest reported for early effects (Fowler, 1984); this is partly due to the 29 kV radiation which probably has a higher linear component of damage than conventional X-rays.

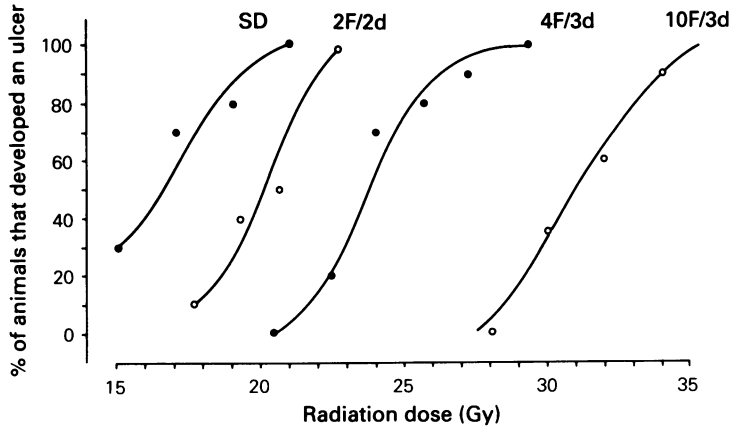

Figure 3 Dose response curves of tongue ulcer frequency to single dose and fractionated irradiation with 29 kV X-rays.

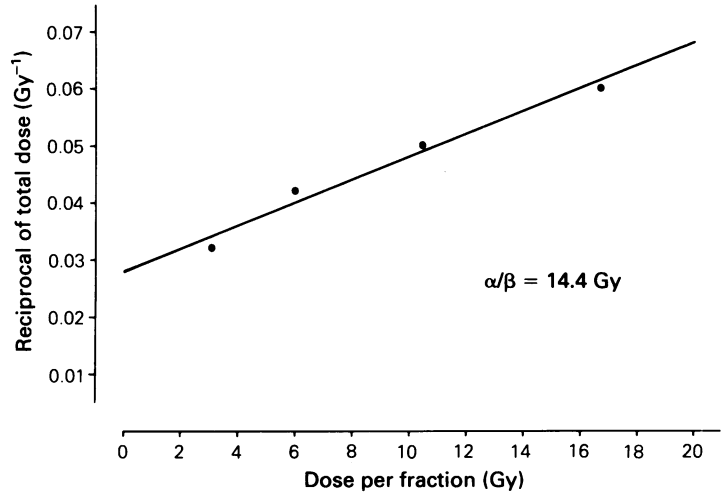

Figure 4 Reciprocal total doses required to give 50% incidence of tongue ulcer plotted *vs.* dose per fraction.

Whatever the exact value, lowering the dose per fraction will probably give no relative increase in tolerance compared to tumours and more therapeutic gain can be expected from accelerated fractionation (Ang, 1985). This, however, requires detailed knowledge of the kinetics of damage expression and regeneration in mucosa following various treatment modalities. An interesting finding in this regard is that in the present model the onset of denudation was not correctly predicted by the normal turnover time of the tissue. In Figure 2, the diurnal fluctuations in the control labelling index can be interpreted as being due to a subpopulation of 20 to 25% of cells that gather in a synchronised S-phase around 2.00 a.m. and subsequently divide (Wright & Alison, 1984); the resulting turnover time would be less than 5 days. This value grossly understimates the time to complete loss of nucleated cells (and hence mechanical loss of epithelial lining) which is at least 9 days. The most likely explanation of the discrepancy is continuing cell production by abortive divisions. Little is known about the response of this residual proliferation to unconventional fractionation regimes and studies in relevant animal models are clearly needed.

References

ANG, K.K., XU, F.X., VANUYTSEL, L. & VAN DER SCHUEREN, E. (1985). Repopulation kinetics in irradiated mouse lip mucosa: The relative importance of treatment protraction and time distribution of irradiations. *Radiat. Res.*, **101**, 162.

FOWLER, J.F. (1984). Review: Total doses in fractionated radiotherapy – implications of new radiobiological data. *Int. J. Radiat. Biol.*, **46**, 103.

PARKINS, C.S., FOWLER, J.F. & YU, S. (1983). A murine model of lip epidermal/mucosal reactions to X-irradiation. *Radioth. Oncol.*, **1**, 159.

VAN DER SCHUEREN, E., VAN DEN BOGAERT, W. & ANG, K.K. (1983). Radiotherapy with multiple fractions per day. In *The Biological Basis of Radiotherapy*, Steel, *et al.* (eds) p. 195. Elsevier: Amsterdam.

WRIGHT, N. & ALISON, M. (1984). eds. Circadian variation in stratified squamous epithelia. In *The Biology of Epithelial Cell Populations*. Vol. 1, ch. 9, p. 346. Clarendon Press: Oxford.

XU, F.X., VAN DER SCHUEREN, E. & ANG, K.K. (1984). Acute reactions of the lip mucosa to fractionated irradiations. *Radioth. Oncol.*, **1**, 369.