Int. J. Exp. Path. (1996), **77,** 7–14

7,12-Dimethylbenz[*a*]anthracene-induced 'early' squamous cell carcinoma in the Golden Syrian hamster: evaluation of an animal model and comparison with 'early' forms of human squamous cell carcinoma in the upper aero-digestive tract

S. ANDREJEVIC*, J-F. SAVARY*, C. FONTOLLIET†, Ph. MONNIER* AND H. VAN DEN BERGH§

** Department of Otolaryngology, Head and Neck Surgery – CHUV Hospital CH-1011 Lausanne; † Institute of Pathology, University of Lausanne, CH-1005 Lausanne; and § Institute of Environmental Engineering, EPFL, CH-1005 Lausanne, Switzerland*

Received for publication 21 July 1995
Accepted for publication 30 October 1995

Summary. To test and optimize photodynamic therapy of early cancers in the upper aero-digestive tract and oesophagus, we sought an appropriate animal model, which was found in the 7,12-dimethylbenz[*a*]anthracene-induced early squamous cell carinoma in the Golden Syrian hamster. This chemically induced neoplasm is shown, by histology and immunohistochemistry, to pass through similar stages of early cancer development as its human counterpart. Its time sequence is highly reproducible, leading to a well differentiated carcinoma *in situ* and microinvasive carcinoma in the hamster cheek pouch over a period of 10 weeks.

Keywords: 7,12-dimethylbenz[*a*]anthracene (DMBA), squamous cell carcinoma (SCC), photodynamic therapy (PDT), hamster cheek pouch mucosa

Clinical trials have demonstrated that photodynamic therapy (PDT) (Kessel 1990; Henderson & Dougherty 1992; Van den Bergh 1994) is emerging as a possible alternative for treating early squamous cell carcinoma (SCC) in the upper aero-digestive tract, tracheo-bronchial tree, and oesophagus (Braichotte *et al.* 1995; Savary *et al.* 1994; Furuse *et al.* 1993; Monnier *et al.* 1990; 1991; 1992). In these trials it has become apparent that PDT with new photosensitizers admits significant improvement. This is because several parameters are involved, e.g. the drug dose, the light dose and rate, the time interval between dye administration and light application, and the wavelength of the laser light. Clearly, an appropriate animal model, if it can be found, will be essential in such a multiparameter optimization procedure. In the present paper we compare the well known 7,12-dimethylbenz[*a*]anthracene (DMBA)-induced carcinogenesis in the cheek pouch of the Golden Syrian hamster with the time sequence of premalignant and malignant histological and immunohistochemical changes which are observed in human squamous cell carcinogenesis in the upper aero- digestive tract and oesophagus. The results of most preclinical studies in PDT are more difficult to extrapolate to the clinical context for several reasons: (1) most tumour models are subcutaneously transplanted or implanted fast growing tumours. PDT is then often carried out when these tumours are bulky and well vascularized (Fingar *et al.*

Correspondence: Snezana Andrejevic, Department of Otolaryngology, Head and Neck Surgery, CHUV Hospital, BH 10, 1011 Lausanne, Switzerland.

1993; Ris *et al.* 1993; Whelpton *et al.* 1995). As an example, a 1-cm^3 sarcoma in a 30-g mouse, cannot easily be compared with a
1-mm^3 superficial SCC of the oesophagus, which may be or may not be vascularized at all, in a 70-kg human being. Furthermore, the latter sarcoma will generally grow quite slowly. (2) As most of the current second-generation photosensitizers are associated with lipoproteins in the blood during part of the transport after i.v. injection, the composition of the plasma lipoproteins should mimic the human case as closely as possible. This is much more the case in the Syrian hamster than in a mouse. (3) The histology, in most implanted or subcutaneously transplanted tumours, does not resemble that of the neoplasm of interest here. Hence, the Syrian hamster with DMBA-induced early squamous cell carcinoma in the cheek pouch, which shows a histology close to that of the human early squamous cell carcinoma in the upper aero-digestive tract, has a small mass relative to the total body weight of about 100 g, and is poorly vascularized in its earliest stages, appears a reasonable choice for this study.

This model was first described by Salley (1954). Although the hamster's cheek pouch is histologically quite similar to, but thinner than, the mucosa of the human upper digestive tract, the hamster carcinogenesis appears to go through the same stages as in humans. As seen in Figure 1, the normal cheek pouch of the hamster is composed of three or four cell layers of squamous epithelium with keratinization of the upper layer; the lamina propria contains connective tissue, small blood vessels and nerves; the deeper layers are made of a well vascularized muscular layer, fatty and glandular tissue surrounding the dermis and epidermis of the cutaneous side of the cheek.

As we shall see below, the carcinomas usually evolve in a multiple-step manner with dysplastic changes preceding *in situ* and microinvasive carcinomas. After 4 weeks of DMBA application, one observes an epithelial hyperplasia which progressively changes to mild, moderate or severe dysplasia after 8 weeks. Exceptionally, the dysplastic changes occur without being preceded by the epithelial hyperplasia during the tumour development, as is also seen in some instances in humans.

Materials and methods

Four groups of five pathogen-free male Golden Syrian hamsters weighing 80–100 g, 5–6 weeks old, were used. The animals were given food and drinking water *ad libitum* during this period. Using cotton swabs, a 0.5% solution of 7,12-dimethylbenz[*a*]anthracene (DMBA, Sigma) in mineral oil was applied topically 3 times weekly to the inferior frontal mucosal surface of the left buccal pouch. The right buccal pouch, which was not painted with DMBA, served as the control. The animals were checked regularly to document the development of neoplastic changes in the left cheek pouch mucosa and skin during 12 weeks. DMBA was chosen as the chemical carcinogen (Morris 1961; David *et al.* 1987; Slaga & Gimenez-Conti 1992) because it has been found that it could play the same aetiologic role in hamster SC carcinogenesis as do alcohol and tobacco in human oral and oesophageal SC carcinogenesis (Graham *et al.* 1990; Mufti *et al.* 1991; 1993; Shin *et al.* 1993). Other

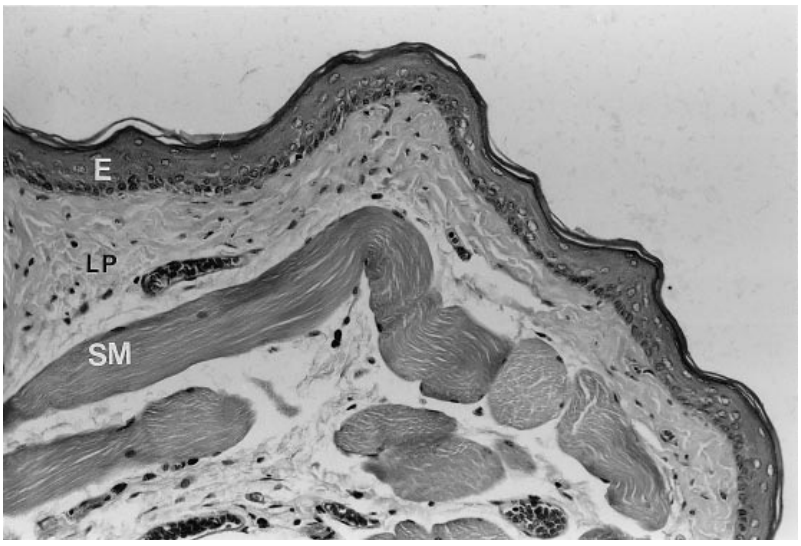

Figure 1. Healthy cheek pouch mucosa of the Syrian hamster. E, Epithelium; LM, lamina propria; SM, striated muscle. HE. ×70.

parameters, e.g. the tumour/full body volume ratio, low density lipoproteins (LPL) levels and vasculature also simulate the clinical context better than any other animal model.

Animals were killed by CO_2 inhalation at 6, 8, 10 and 12 weeks from the first DMBA application. The whole cheek pouch was resected and fixed in 5% buffered formalin (pH 7.0), paraffin embedded, sectioned in 5-μm thick slices which were stained with eosin and haematoxylin and analysed by conventional optical microscopy. Histopathologic findings were compared with premalignant and malignant changes in human carcinogenesis of the upper digestive tract. Immunohistochemical techniques were used to detect alterations of different keratin types in the carcinogenesis of the hamster cheek pouch and compared to the keratin patterns observed in the human carcinogenesis of the upper digestive tract and oesophagus (Hurlimann & Gardiol 1989; Gimenez-Conti 1990; Shuler *et al.* 1987; Tatemoto *et al.* 1987; Murase *et al.* 1986). Samples of the hamster buccal pouch mucosae were formalin fixed, paraffin embedded, and sectioned in 5-μm thick slices which were stained using standard immunoperoxidase techniques. Three different undigested antikeratin antibodies (MAB Lu5 (Hoffmann-La Roche Co, Basel), highly specific for stratified epithelia; MAB 35βH11 (Enzo Biochemicals, New York) obtained after immunization with cytoskeleton from human hepatoma specific for simple epithelia; and MAB 35βE12 (Enzo Biochemicals, New York) obtained after immunization with stratum corium, which is specific for stratified epithelia), diluted 1 : 500, were used to detect antigenic determinants of configuration present on acid and basic non-specific keratin.

Results

Histopathology

Between 6 and 9 weeks from the first DMBA application, different degrees of epithelial dysplasia were observed (Figure 2) and evolved to carcinoma *in situ* and microinvasive carcinoma after 10 weeks. Figure 3 shows the microscopic aspect of the early squamous cell carcinoma (SCC) in the hamster's cheek pouch after 10 weeks of DMBA application. In Figure 4 we can see invasive squamous cell carcinoma which appear 12 weeks after the first DMBA application. In some cases at this point in time we also meet papillary tumours in the cutaneous side of the cheek. The histopathologic changes observed during 12 weeks of DMBA-induced carcinogenesis are shown in the Table 1.

The mucosal changes induced by DMBA application in the left cheek pouch were always compared with the right cheek pouch which served as control. In the latter, up to 10 weeks of DMBA application did not induce any histopathologic changes. After 10 weeks, some microfoci of mild to moderate dysplasia are seen in the inferior part of the untreated right cheek pouch. This finding suggests that after a prolonged period of time, chemical carcinogens mixed in saliva induce malignant changes more diffusely in the oral cavity. The histopathologic changes occurring during this induced chemical

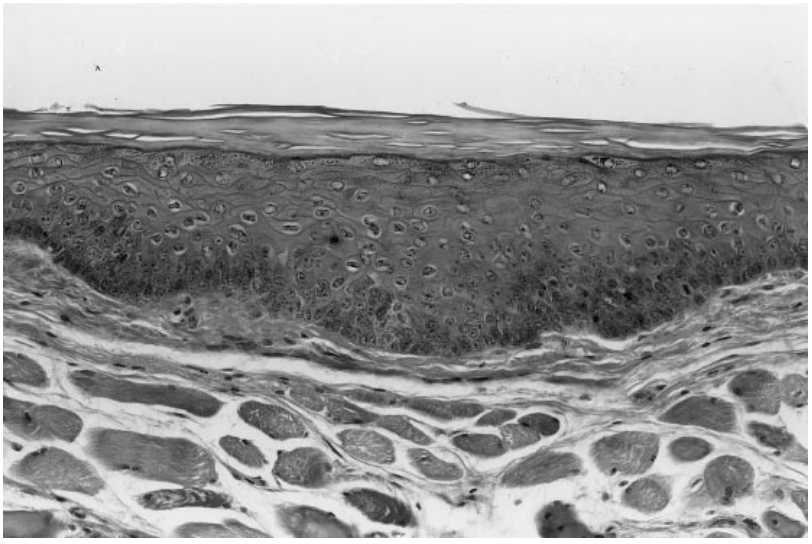

Figure 2. Transepithelial high-grade dysplasia of the hamster's cheek pouch, observed after 8 weeks of DMBA application. HE. $\times$70.

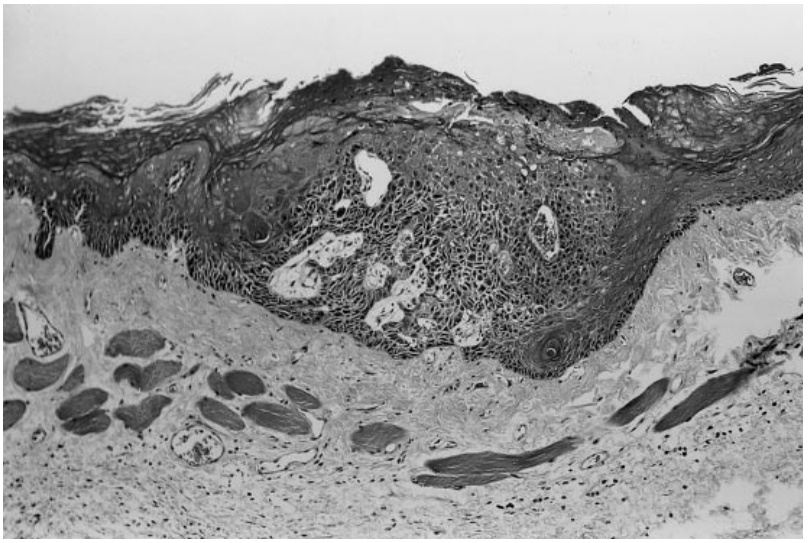

Figure 3. Well differentiated microinvasive SCC of the hamster cheek pouch, after 10 weeks of DMBA application. HE. ×35.

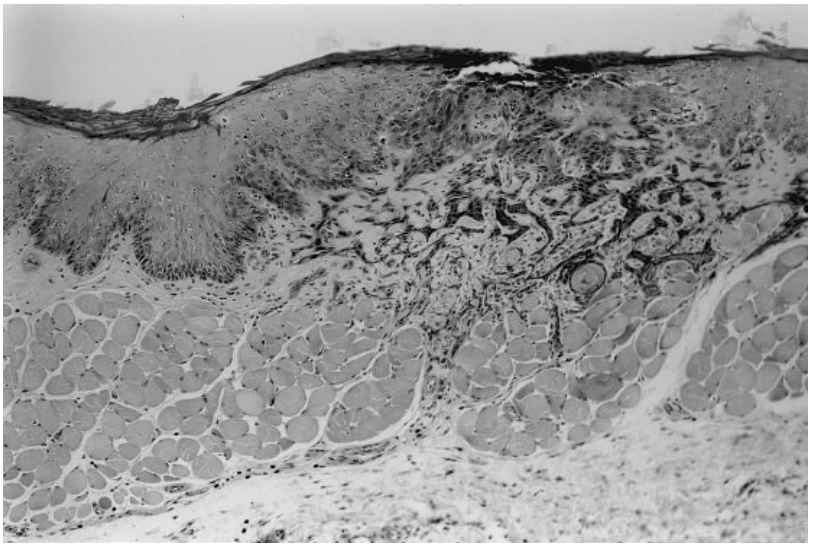

Figure 4. Invasive SCC of the hamster cheek pouch, which appear after 12 weeks of DMBA application. HE. ×35.

Table 1. The histopathologic changes observed during 12 weeks of DMBA-induced carcinogenesis

DMBA application (weeks)	Macroscopic observations	Microscopic observations
6	leukoplakia	hyperkeratosis and mild dysplasia
8	erythro-leukoplakia	hyperkeratosis, mild to severe dysplasia and carcinoma *in situ*
10	erythro-leukoplakia	hyper and dys-keratosis, intermediate and transepithelial high-grade dysplasia. Ca *in situ* and microinvasive carcinoma
12	erythro-leukoplakia and papillary Ca	hyper and dys-keratosis with multiple invasive SCC, papillary carcinoma and, in some cases, papillary Ca in the cutaneous part of the pouch

carcinogenesis were highly reproducible in different groups of animals.

Macroscopically and microscopically they are very close to the human forms of dysplasia, *in situ* and microinvasive squamous cell carcinomas in the upper digestive tract and oesophagus (Figure 5a and b).

Immunohistologic correlation between human and hamster keratin patterns

In the human oesophagus, two types of high-grade dysplasias are described (Hurlimann & Gardiol 1989); first, a transepithelial dysplasia, positive for Lu5 and 35βE12 antikeratin antibodies and specific for stratified epithelia. This form is associated with well differentiated carcinomas and is negative for the 35βH11 antikeratin antibody, which is specific for simple epithelia and associated with poorly differentiated or undifferentiated carcinomas. Human forms of oral and oesophageal squamous cell carcinomas are often associated with this type of transepithelial high-grade dysplasia. Second, a pagetoid dysplasia which is positive for 35βH11 and negative for 35βE12 antikeratin antibody, which is always associated with poorly differentiated or undifferentiated carcinomas. Sometimes intermediate types of high-grade dysplasia show characteristics of both types of dysplasia and carcinoma. In the hamster cheek pouch carcinogenesis we have seen intermediate and transepithelial high-grade dysplasias. During tumour development, more than 50% of the epithelial

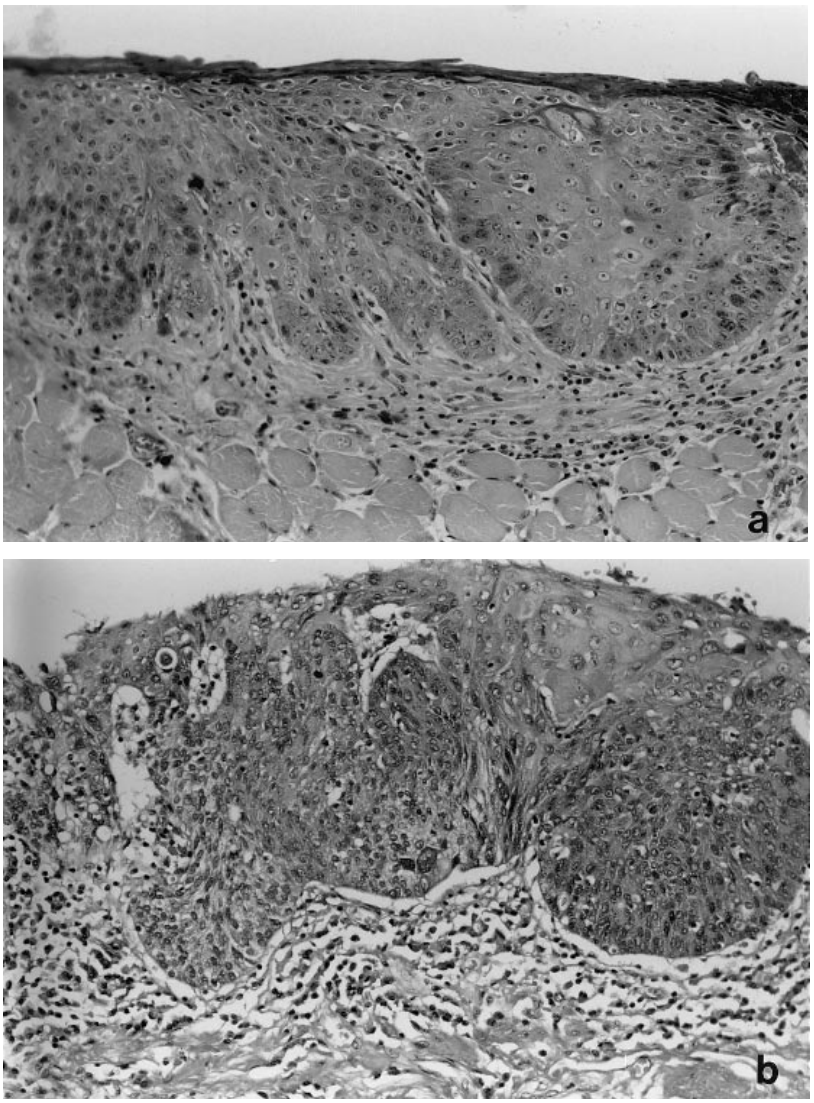

Figure 5. Histological comparison in a, the hamster's and b, human oesophageal early SCC. HE. ×70.

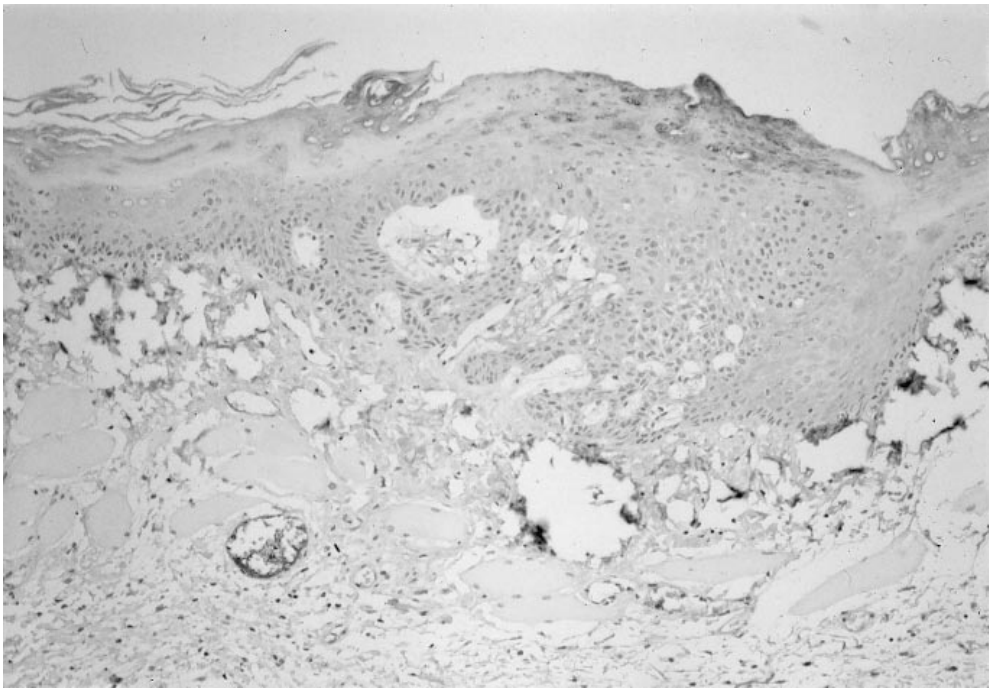

Figure 6. Early SCC of the hamster cheek pouch 10 weeks after DMBA application. Positive immunostaining with antikeratin antibody 35βE12, specific for stratified epithelia and well differentiated SCC. HE. $\times$80.

cells in the basal and suprabasal layers became positive for 35βE12 (Figure 6) and Lu5. After 12 weeks, squamous cell carcinomas are positive for keratin in the stratified epithelia. At the beginning of DMBA-induced carcinogenesis all epithelial layers are negative for 35βH11 antikeratin antibodies specific for simple epithelia. In other words this keratin pattern is very similar to the keratin pattern of human oral and oesophageal early squamous cell carcinogenesis.

Discussion

If we compare the early squamous cell carcinoma (SCC) in the hamster cheek pouch to transplanted tumours in most of the usual rodent animal models (Fingar *et al.* 1993; Reddi *et al.* 1987; Ris *et al.* 1993; Spirov *et al.* 1992; Whelpton *et al.* 1995), the following differences are seen (Table 2).

Early SCC in the Syrian hamster model is a superficial tumour, induced by topical application of chemical carcinogen over a 10-week period. After this time interval, we obtain in this model a well differentiated microinvasive squamous cell carcinoma, which is poorly vascularized and shows poor inflammatory reactions, without tumour necrosis. The tumour weight is negligible as compared to the full body weight.

On the other hand, the tumours in the more commonly used rodent animal models are transplanted or implanted fast growing, bulky invasive tumours. Often these are histologically well or poorly differentiated sarcomas, adenocarcinomas or mesotheliomas. They are highly

Table 2. Comparison between hamster 'early' SCC and other rodent animal models

Hamster 'early' SCC	Other rodent animal models
Chemically-induced during 10 weeks by topical application of 0.5% oily DMBA	Transplanted or implanted tumours (cell culture, grafts) growing rapidly during 2–3 weeks
Until 10 weeks controlled tumour growth (slow)	From the beginning, poorly controlled tumour growth (fast)
Superficial carcinomas (histologically: squamous cell)	Bulky invasive tumours (histologically: sarcoma, adenocarcinoma, mesothelioma or carcinoma)
Poor vascularization	High vascularization
Mild inflammation	Severe inflammation
No necrosis	Often central necrosis
Negligible tumour weight as compared to full body weight	Up to about 1 g tumour weight (i.e. a few per cent of the full body weight)

vascularized, and show an important inflammatory reaction, often with central necrosis. Tumour weight can be as high a proportion as 1/10 of the body weight of the animal.

The advantages of the hamster model are summarized as follows:

Histopathologically and immunohistochemically, DMBA-induced 'early' squamous cell carcinomas of the hamster cheek pouch are very similar to the human SCC of the upper-aero digestive tract.

The sequence from dysplastic changes to 'early' carcinoma is highly reproducible.

Tumours are well differentiated and keratin patterns are closely comparable to the keratin expression of human oropharyngo-oesophageal 'early' SC carcinogenesis.

Development of microinvasive carcinomas takes place over a short period of time (10 weeks).

The large surface (about 12 cm^2), of the hamster cheek pouch permits precise follow-up of malignant changes, easy observation and manipulation.

Conclusion

After 6–9 weeks from the first DMBA application, different degrees of epithelial dysplasia were observed and evolved to carcinoma *in situ* and microinvasive carcinoma after 10 weeks. Hyper and dys-keratosis are present at all stages of tumour development. The sequence of dysplastic changes to early carcinoma was reproducible in all groups of animals during the time dependent, chemically induced carcinogenesis.

It should be noted that we are particularly interested in 'early' stage cancers (*in situ* and microinvasive carcinomas) which appear 10 weeks after the first DMBA application, because they are potentially amenable to successful photodynamic therapy. As we have demonstrated above, the Syrian hamster's SCC model closely simulates the case of clinical interest to us. Hence we plan to use it to evaluate new photosensitizers and to optimize treatment modalities in photodynamic therapy of 'early' squamous cell carcinomas in the upper digestive tract.

Acknowledgements

We wish to acknowledge support from the following: the Swiss National Fund (Grant No. 31-43595); the 'Fonds de Service' and 'Fonds de Perfectionnement' of the Otolaryngology, Head and Neck Surgery Department, CHUV Hospital; the CHUV-UNIL-EPFL programme for collaborative research in biomedical technology; the Swiss priority programme in optics; and the Swiss commission for encouragement of scientific research (CERS).

References

Braichotte D., Savary J-F., Glauzmann Th., Westermann P., Folli S., Wagnieres G., Monnier Ph. & Van den Bergh H. (1995) Clinical pharmacokinetics studies of tetra(meta-hydroxyphenyl)chlorin in squamous cell carcinoma by fluorescence spectroscopy at 2 wavelengths. *International Journal of Cancer* **63**, 198–204.

David T.W. Wrong & Debajit K. Biswas (1987) Expression of c-erbB protooncogene during DMBA-induced tumorogenesis in hamster cheek pouch. *Oncogene* **2,** 67–72.

Fingar V., Wieman J. & Karavolos P. (1993) The effects of photodynamic therapy using differently substituted zinc phthalocyanines on vessel constriction, vessel leakage and tumor response. *Photochem. Photobiol.* **58,** 251–258.

Furuse K., Fukuoka M., Kato H., Horai T., Kubota K. & Kodama N. (1993) A prospective phase II study on photodynamic therapy with Photofrin II for centrally located early-stage lung cancer. *J. Clin. Oncol.* **11,** 1852–1857.

Gimenez-Conti I.B. (1990) Changes in keratin expression during 7,12-dymethylbenz[*a*]anthracene induced hamster cheek pouch carcinogenesis. *Cancer Res.* **50,** 4441–4445.

Graham S., Marsshall J., Haughey B., Brasure J., Freudenheim J., Zielezny M., Wilkinson G. & Nolan J. (1990) Nutritional epidemiology of cancer of the esophagus. *Am. J. Epidemiol.* **131,** 454–467.

Henderson B.W. & Dougherty T.J. (1992) *Photodynamic Therapy: Basic Principles And Clinical Applications.* Marcel Dekker Inc, New York.

Hurlimann J. & Gardiol D. (1989) Immunohistochemistry of Dysplasias and carcinomas of the esophageal epithelium. *Path. Res. Pract.* **184,** 567–576.

Kessel D. (1990) *Photodynamic Therapy of Neoplastic Disease.* Volume 3. CRC Press, Boca Raton FL USA.

Monnier Ph., Fontolliet C., Wagnières G., Braichotte D. & van den Bergh H. (1992) Photodétection et photothérapie des carcinomes épidermoïdes précoces du pharynx, de l'oesophage et des bronches. *Rev. Fr. Gastroentérol.* **27,** 103–112.

Monnier Ph., Savary M., Fontolliet C., Wagnières G., Châtelain A. & Cornaz P. (1990) Photodetection and photodynamic therapy of early squamous cell carcinomas of the pharynx, oesophagus and tracheo-bronchial tree. *Lasers Med. Sci.* **5,** 149–169.

Monnier Ph., Savary M., Fontolliet C., Wagnières G., Châtelain A., Cornaz P. & Depeursinge C. & van den Bergh H. (1991) Photodetection and photodynamic therapy of 41 'early' squamous cell carcinomas of the pharynx, oesophagus and tracheo-bronchial tree. In *Laser Tumour Therapy*, Volume 2. Ed. M.A. Trelles. Madrid, Spain. pp. 36–87.

Morris A.L. (1961) Factors influencing experimental carcinogenesis in the hamster cheek pouch. *J. Dental Res.* **40,** 3–15.

Mufti S.I., Eskelson C.D., Odeleye O.E. & Nachiappan V. (1993) Alcohol-associated generation of oxygen free radicals and tumour promotion. *Alcohol Alcoholism* **28,** 621–628.

Mufti S.I., Karimullah Z.I. & Garewal H.S. (1991) Precancerous lesions and biologic markers in esophageal cancer. *Cancer Detect. Prev.* **15,** 291–301.

Murase N., Fukui S. & Mori M. (1986) Heterogeneity of keratin

distribution in the oral mucosa and skin of mammals as determined using monoclonal antibodies. *Histochemistry* **85,** 265–276.

REDDI E., LO CASTRO G., BIOLO R. & JORI G. (1987) Pharmacokinetic studies with zinc(II)phthalocyanine in tumour-bearing mice. *Br. J. Cancer* **56,** 597–600.

RIS H-B., ALTERMAT H.J., NACHBUR B., STEWART CH., WANG Q., LIM CH.K., BONNETT R. & ALTHAUS U. (1993) Effect of drug-light interval on photodynamic therapy with meta-tetrahydroxyphenyl-chlorin in malignant meothelioma. *Int. J. Cancer* **53,** 141–146.

SALLEY J.J. (1954) Experimental carcinogenesis in cheek pouch of the Syrian hamster. *J. Dental Res.* **33,** 253–262.

SAVARY J-F. (1994) Preliminary clinical studies of photodynamic therapy with m-THPC as a photosensitizing agent for the treatment of early pharyngeal, oesophageal and bronchial carcinomas. SPIE, Bellingham WA USA **2078,** 330–340.

SHIN D.M., CHIAO P.J. & SACKS P.G. (1993) Activation of ribosomal proteins S2 gene expression in a hamster model of chemically induced oral carcinogenesis. *Carcinogenesis* **14,** 163–166.

SHULER C.F., MOSTOFI R., LIN L-M. & SCHWARTZ S.A. (1987) Keratin protein identification in DMBA-induced hamster cheek pouch squamous cell carcinomas. *J. Oral Pathol.* **16,** 59–163.

SLAGA TH.J. & GIMENEZ-CONTI I.B. (1992) An animal model for oral cancer. *J. Natl. Cancer Inst.* Monograph No. 13, pp. 55–59.

SPIROV K., JORI G. & RICCHELLI F. (1992) Comparative pharmacokinetic and photodynamic studies with zinc(II)phthalocyanine in hamsters bearing an induced or transplanted rhabdosarcoma. *Photochem. Photobiol.* **16,** 83–89.

TATEMOTO Y., FUKUI S., OSUMI H., HORIKE H. & MORI M. (1987) Expression of keratins during experimentally induced carcinogenesis in hamster cheek pouch visualized polyclonal and monoclonal antibodies. *Histochemistry* **86,** 445–452.

VAN DEN BERGH H. (1994) Photodynamic therapy and photodetection of early cancer in the upper aerodigestive tract, tracheobronchial tree, the esophagus and the urinary bladder. In *Proceeding of the First International Symposium on Hadrontherapy*. Como, Italie, 18–21 October 1993. Eds U. Amaldi & B. Larsson. Amsterdam: Elsevier. pp 577–621.

WHELPTON R., MICHEL-TITUS A., BASRA S. & GRAHN M. (1995) Distribution of Temoporfin, a new photosensitizer for the photodynamic therapy of cancer, in a murine tumor model. *Photochem. Photobiol.* **61,** 397–401.