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TR146 cells as a model for human buccal epithelium: II. Optimisation and use of a cellular sensitivity MTS/PMS assay

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Abstract

The optimisation and use of the cellular sensitivity MTS/PMS assay for TR146 cells are presented. The MTS/PMS assay is a colorimetric method based on reduction of a tetrazolium salt to the corresponding formazan including an electron acceptor as accelerator. The optimisation studies resulted in a final protocol for drug sensitivity testing of TR146 cells. The initial cell density was 2×10^4 cells/well, and after 4 h incubation with a MTS/PMS reagent (final concentrations: MTS, 240 μg/ml; PMS, 2.4 μg/ml), the optical density was recorded at 490 nm. The optical density of the TR146-generated MTS–formazan at room temperature and atmosphere was shown to be constant for a period of 30 min.

The final protocol for the MTS/PMS assay used for a series of β-adrenoceptor antagonists (propranolol, oxprenolol, metoprolol, and atenolol) in concentrations in the range 10^{-6}–10^{-2} M indicated that solutions of about 10^{-4} M caused a 10% decrease of the cellularly generated MTS–formazan (i.e. $IC_{10} = 10^{-4}$ M). The assay used for a series of morphine and morphine prodrugs (3-hexanoyl-, 3-propionyl- and 3-acetyl-morphine) in concentrations in the range 3.5×10^{-7}–3.5×10^{-3} M showed that only the most lipophilic substance, 3-hexanoyl-morphine, affected the TR146 cells ($IC_{10} = 10^{-4}$ M).

Keywords: β-Adrenoceptor antagonists; Cell line TR146; Cellular drug sensitivity MTS/PMS assay; Human buccal epithelium; Morphine and morphine prodrugs

Abbreviations: MTS, (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium; MTT, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide); XTT, (3′-[1-[(phenylamino)-carbonyl]-3,4-tetrazolium]-bis(4-methoxy-6-nitro)benzene-sulfonic acid hydrate; PMS, phenazine methosulfate; OD, optical density; Blank, wells without cells; OD_{Drug}, optical density of wells (with cells) with added drug and MTS/PMS reagent; $OD_{Drug\ blank}$, optical density of wells (without cells) with added drug and MTS/PMS reagent; $OD_{Control}$, optical density of wells (with cells) with added MTS/PMS reagent; OD_{Blank}, optical density of wells (without cells) with added MTS/PMS reagent; Corrected $OD = OD_{Control} - OD_{Blank}$; IC_{10} and IC_{50}, the drug concentrations causing a 10% and a 50% inhibition of cell-generated MTS–formazan compared to untreated cells.

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1. Introduction

There is a current interest in developing new drug delivery systems as alternatives to parenteral and peroral drug administration. The buccal route offers advantages, including excellent accessibility, rapid absorption due to relatively high blood flow, exhibition of epithelial robustness, bypass of first pass metabolism, and minimal exposure of drugs to the gastro-intestinal environment. A method to test buccal absorption was originally devised by Beckett and Triggs (1967) and modified by others (reviewed by Rathbone and Hadgraft (1991)). Later, some methods were based on oral in situ devices (e.g. Pimlott and Addy, 1985; Barsuhn et al., 1988) and a range of oral drug delivery systems (reviewed by Harris and Robinson (1992)). Other methods were based on in vitro permeability studies, e.g. using cultured hamster pouch buccal epithelium (Tavakoli-Saberi and Audus, 1989) or excised porcine buccal mucosa mounted in a diffusion cell (Le Brun et al., 1989; Hansen et al., 1992). In vitro cell culture methods, particularly based on human materials, are attractive supplements and/or alternatives to the methods mentioned above. Cell culture systems are particularly suitable for examination of the permeability and the metabolism of drugs, and the toxicity of drugs, pharmaceutical adjuvants, and implants.

Filter-grown TR146 cells, a continuous cell line originating from a human buccal carcinoma (Rupniak et al., 1985), have recently been proposed as a model for the human buccal epithelium (Jacobsen et al., 1995). The model has been used to study the cellular permeability of a series of β-adrenoceptor antagonists (Jacobsen et al., 1995).

Prior to the performance of a permeability study with filter-grown TR146 cells, the cellular drug sensitivity ought to be tested in order to ascertain the concentration of the test substance to be applied without affecting the cells. The optimisation of a colorimetric method, an MTS/PMS assay using TR146 cells, is presented. The assay is based on cellular enzymatic bioreduction of a practically colourless tetrazolium salt, MTS, to an intensively coloured MTS–formazan, and the number of viable cells is correlated to the quantity of the formazan produced (Mosmann, 1983; Promega Corporation, 1992; Carmichael et al., 1987a). Bioreduction of the tetrazolium salts has previously been applied to evaluate the cytotoxicity of chemotherapeutics (e.g. Carmichael et al., 1987a; Plumb et al., 1989) or chlorhexidine and nystatin (Zheng and Audus, 1994), cellular radiosensitivity (Carmichael et al., 1987b), the influence of growth factors on viability and pro-liferation of a variety of cell lines (e.g. Roehm et al., 1991; Loveland et al., 1992; Buttke et al., 1993), and the bactericidal activity of bovine neutrophils (Stevens and Olsen, 1993). Bioreduction of tetrazolium salts is equally suitable for drug sensitivity testing of anchorage-dependent and anchorage-independent cells originating from primary cultures of normal or tumorigenic cells, or from continuous cell lines established from various tissues (see references mentioned above). The tetrazolium salt/electron acceptor assay requires, however, optimisation of the assay for every cell line in order to achieve a high sensitivity (e.g. Carmichael et al., 1987a; Loveland et al., 1992). The objective of this paper is to describe the optimised MTS/PMS assay for TR146 cells and the usefulness of the assay as a drug sensitivity test for TR146 cells. Data for β-adrenoceptor antagonists, morphine, and morphine prodrugs are presented.

2. Materials and methods

2.1. Materials

The continuous cell line TR146, derived from a human neck node metastasis originating from a buccal carcinoma (Rupniak et al., 1985), was kindly provided by the Imperial Cancer Research Technology (London, UK) with generous help from Professor E.B. Lane (The University of Dundee, UK). The obtained passage was designated number one. Heat-inactivated foetal calf serum (FCS) was obtained from Sera-Lab (Sussex, UK). Dulbecco's modified Eagle medium (DMEM; containing sodium pyruvate, 4.5 g glucose/l, and Glutamax-1™), Hanks' balanced salt solution without phenol red (HBSS), Dulbecco's phosphate buffered saline (DPBS), gentamicin, trypsin–EDTA and other culture media were purchased from Gibco BRL (Paisley, UK). The an-

timycoticum *p*-hydroxybenzoic acid *n*-butyl ester, the fluorescent stain 2′-[4-hydroxyphenyl]-5-[4-methyl-1-piperazinyl]-2,5′-bi-1H-benzimidazole, Hoechst No. 33258 (cell culture tested), atenolol, DL-metoprolol D-tartrate salt, oxprenolol hydrochloride, DL-propranolol hydrochloride, and PMS (cell culture tested) were supplied by Sigma (St. Louis, MO, USA). Nunclon™ 25-cm^2 T-flasks and Nunclon™ MicroWell 96-well flat-bottomed culture plates (tissue culture treated, polystyrene) were obtained from A/S Nunc (Roskilde, Denmark). Morphine and morphine prodrugs were prepared as previously described (Drustrup et al., 1991) and kindly provided by L. Christrup (The Royal Danish School of Pharmacy, Copenhagen, Denmark). MTS (2.0 mg/ml) in DPBS (pH 6.0) and PMS (2.0 mg/ml) in DPBS were kindly provided by K. Berg (The Panum Institute, University of Copenhagen, Denmark). A kit CellTiter 96™ AQ_{ueous} Non-Radioactive Cell Proliferation Assay was purchased from Promega (Madison, WI, USA).

2.2. Apparatus

A Nikon Diaphot-TMD inverted microscope equipped with phase-contrast optics, Epi-Fluorescence, and UV-1A filter box for mycoplasma test were obtained from Nikon (Tokyo, Japan). A microplate reader $Thermo_{max}$ and software SOFTmax™ version 2.01d $THERMO_{max}$ 1.72 were obtained from Molecular Devices Corporation (Mento Park, CA, USA). A Shimadzu UV-160A spectrophotometer was purchased from Shimadzu Corporation (Kyoto, Japan).

2.3. Cell culture

Cultivation of the cell line TR146 has previously been described (Jacobsen et al., 1995). Briefly, TR146 cells were maintained in T-flasks and incubated at 37°C in a 98% humid atmosphere of 5% CO_2/95% air (standard conditions). The culture medium consisted of DMEM supplemented by 10% FCS, gentamicin (50 μg/ml), and *p*-hydroxybenzoic acid *n*-butyl ester (0.2 μg/ml). Cells of passage number 5 and 21 were used. TR146 cells were detached from the flasks by trypsin–EDTA treatment.

Bacterial and fungal contamination of TR146 cells was examined daily, either by visual inspection or phase-contrast microscopy. The TR146 cell cultures were monitored routinely for mycoplasma contamination by the Hoechst No. 33258 stain, according to a method described by Chen (1977) and slightly modified by Lind (1994).

2.4. Standard procedure

The MTS/PMS assay was initiated as follows: TR146 cells originating from a maintenance culture at the logarithmic phase of growth were seeded in a 96-well culture plate using 2×10^4 cells/well ($n = 4$) in 100 μl culture medium, if not specified otherwise. In the same plate, wells without cells ($n = 4$) were supplied with 100 μl culture medium. The plate was incubated for 24 h under standard conditions. After incubation, the culture medium was removed by inverting the plate and gently tapping on the bottom of the plate. Subsequently, optimisation of the MTS/PMS assay or a cellular sensitivity test was carried out. Generally, after addition of 125 μl MTS/PMS reagent to each well, the plate was protected against light and further incubated under standard conditions. The stated concentrations of the MTS/PMS reagents are final concentrations. The OD values were recorded at the absorbance maximum of 490 nm using a microplate reader.

2.5. Optimisation of the MTS/PMS assay for TR146 cells

All the experiments were initiated according to the standard procedure.

The absorbance maximum of the TR146-generated MTS–formazan was estimated. The added MTS/PMS reagent contained 240 μg/ml MTS and 2.4 μg/ml PMS. The period of incubation was 24 h. Supernatants from wells with TR146 cells (sample 1) and from wells without TR146 cells (sample 2) were each aspirated and pooled, respectively, then diluted 1 : 10 with HBSS. The absorbance spectrum was recorded for sample 1 and sample 2, respectively. HBSS constituted the reference.

The OD values of the references and the blanks were estimated. The added MTS/PMS reagent contained either (1) 240 μg/ml MTS in HBSS, (2) 2.4 μg/ml PMS in HBSS, or (3) 240 μg/ml MTS and 2.4 μg/ml PMS in HBSS. The period of incubation was up to 24 h, OD was recorded at 10 min, 4 h and 24 h.

2.5.1. Optimisation of the initial cell seeding density and the period of incubation

TR146 cells were seeded at cell concentrations ranging from 1×10^3 to 90×10^3 cells/well. The added MTS/PMS reagent contained 120 μg/ml MTS and either (1) 3.6 μg/ml PMS or (2) 60 μg/ml PMS. The period of incubation was up to 48 h, OD was recorded every 30 min up to 3 h, then at 24 h and at 48 h.

2.5.2. Optimisation of the PMS concentration in the MTS/PMS reagent and the period of incubation

TR146 cells were seeded at cell concentrations ranging from 2.5×10^3 to 30×10^3 cells/well. The added MTS/PMS reagent contained 120 μg/ml MTS and 0.6–3.6 μg/ml PMS. The period of incubation was up to 6 h and OD was recorded hourly up to 6 h.

2.5.3. Optimisation of the MTS concentration in the MTS/PMS reagent

TR146 cells were seeded at concentrations ranging from 5×10^3 to 30×10^3 cells/well. The added MTS/PMS reagent contained 120–360 μg/ml MTS and 2.4 μg/ml PMS. The period of incubation was 4 h; OD was recorded.

2.5.4. Stability testing of the MTS–formazan at room temperature

The stability at room temperature and atmosphere of the TR146 cellularly generated MTS–formazan was studied using ethanol as a test substance. After removing the culture medium, each well received 100 μl ethanol solution. The ethanol concentrations varied from 0.1% to 96%. The plate was incubated under standard conditions for 30 min. The ethanol solutions were then removed by inverting the plate. The wells received an MTS/PMS reagent containing 240 μg/ml MTS and 2.4 μg/ml PMS, and the plate was further incubated for 4 h under standard conditions. Thereafter, OD was recorded every 5 min for a period of 30 min, keeping the plate at room temperature.

2.5.5. Final protocol for testing the cellular drug sensitivity of the cell line TR146 by an MTS/PMS assay

The assay was initiated according to the standard procedure. After removing the culture medium, each well received 100 μl test solution. The plate was incubated for a period of time, e.g. corresponding to the duration of a permeability study. The test solution was then removed by inverting the plate. The wells received a MTS/PMS reagent containing 240 μg/ml MTS and 2.4 μg/ml PMS. The period of incubation was 4 h; OD was recorded.

The cellular sensitivity (%) was calculated according to the following equation:

$$\text{Cellular sensitivity (\%)} = \frac{(OD_{Drug} - OD_{Drug\ blank})}{(OD_{Control} - OD_{Blank})} \times 100. \quad (1)$$

IC_{10} and IC_{50} values were estimated by means of linear regression from a graph depicting cellular sensitivity (Eq. (1)) versus drug concentration.

2.6. Application of the optimised MTS/PMS assay

The cellular sensitivity of a series of four β-adrenoceptor antagonists (propranolol, oxprenolol, metoprolol and atenolol) in concentrations in the range 10^{-6}–10^{-2} M and a series of morphine and morphine prodrugs (3-hexanoyl-, 3-propionyl- and 3-acetylmorphine) in concentrations in the range 3.5×10^{-7}–3.5×10^{-3} M were tested according to the final protocol (see Section 2.5.5).

3. Results and discussion

3.1. Tetrazolium salt-based assays

A predictive screening of drug sensitivity is very useful, e.g. prior to cellular permeability and metabolism studies. The tetrazolium salt-based assays are colorimetric assays determining viability by means of cellular dehydrogenase activity and hence reduction of a tetrazolium salt to the corresponding formazan. The tetrazolium salt-based assays are rapidly carried out and they correlate with other in vitro sensitivity assays, including the dye exclusion test (Carmichael et al., 1987a,b; Loveland et al., 1992), the ^{3}H-thymidine uptake (Loveland et al., 1992; Buttke et al., 1993), and the clonogenic assay (Carmichael et al., 1987a,b). The assay with the tetrazolium salt, MTS, and the electron coupling reagent, PMS, displays some advantages associated with other tetrazolium salt-based assays, the MTT assay (Mosmann, 1983) and the XTT assay (Scudiero et al., 1988). MTS–formazan is water-soluble while MTT–formazan is water-insoluble and requires an additional solubilisation step, which is time consuming, increases the risk of microbial contamination of the assay, and involves potentially hazardous exposure of organic substances to personnel and environment (Stevens and Olsen, 1993). XTT–formazan is water-soluble, but the rate of formation of XTT–formazan is slower than that of MTT–formazan (Scudiero et al., 1988; Roehm et al., 1991).

Previous studies using different cell lines have indicated that the amount of formazan produced is dependent on various factors, e.g. the cell line, the number of cells, the initial concentration of tetrazolium salt, the cellular metabolic level, the pH of the medium, the presence of an electron coupling reagent, and the presence of serum (e.g. Altman, 1977; Carmichael et al., 1987a; Plumb et al., 1989; Hansen et al., 1989; Buttke et al., 1993). Careful optimisation of a tetrazolium salt-based assay is needed to obtain an effective drug sensitivity test, i.e. be capable of detecting a broad range of viable cell numbers.

The tetrazolium salt-based assays do not differentiate between static and lethal drug effect(s), or discriminate between the different sensitivity of subpopulations or of the entire cell population. However, these limitations are of no particular importance for the purpose of the assay as a screening tool for cellular sensitivity. Some drugs may initiate cellular production of various cytokines capable of accelerating or suppressing the cellular reductive capacity. This limitation has to be considered.

3.2. Optimisation of the MTS/PMS assay for TR146 cells

An optimisation of the MTS/PMS assay for TR146 cells was performed by altering the initial cell density, the concentration of MTS and PMS, and the period of incubation with the MTS/PMS reagent. This method of optimisation is generally applicable to all other cell lines. In addition, the stability of the generated cellular MTS–formazan at room temperature and atmosphere was studied.

The absorbance spectrum of the MTS–formazan generated by the TR146 cells exhibited a broad peak in the visual region with a maximum of about 490 nm. The absorbance spectrum of the MTS/PMS reagent in HBSS showed an absorbance maximum of 386 nm and very low OD in the visual region. Accordingly, further studies were performed at 490 nm.

The OD values of references and blanks of the MTS/PMS assays are presented in Table 1. After 4 h of incubation, the OD values were only slightly higher than the OD values after 10 min of incubation for the majority of the solutions (1–7). However, regarding solution 8, the OD values measured after 4 h were much higher than the OD values measured after 10 min of incubation. The OD values for all the solutions increased by a factor of 2–3 after 4–24 h incubation. The TR146-cells-formed MTS–formazan (solution 8: HBSS + MTS/PMS reagent + TR146 cells) showed a 9-fold higher mean OD value compared with the blank (solution 7: HBSS + MTS/PMS reagent) after 4 h incubation. The mean OD values for solution 8 was much higher than the mean OD value for the solution without PMS (solution 6: HBSS + MTS + TR146 cells). In conclusion, the incubation with MTS/PMS reagent

Table 1
MTS/PMS assay of TR146 cells. OD measurements at 490 nm of HBSS, PMS[a], MTS[b], MTS/PMS reagent[c] and TR146 cells[d] grown in 96-well culture plate. Number of replications (n) is indicated

Solution	OD_{490} (mean ± SEM (n))		
	Time of incubation		
	$t = 10$ min	$t = 4$ h	$t = 24$ h
1: HBSS	0.097 ± 0.0003 (3)	0.122 ± 0.005 (4)	0.308 ± 0.017 (4)
2: HBSS + TR146 cells	0.120 ± 0.0002 (4)	0.136 ± 0.009 (4)	0.265 ± 0.052 (4)
3: HBSS + PMS	0.113 ± 0.004 (4)	0.139 ± 0.004 (4)	0.343 ± 0.016 (4)
4: HBSS + PMS + TR146 cells	0.131 ± 0.004 (4)	0.152 ± 0.009 (4)	0.377 ± 0.018 (4)
5: HBSS + MTS	0.547 ± 0.05 (4)	0.160 ± 0.007 (4)	0.360 ± 0.022 (4)
6: HBSS + MTS + TR146 cells	0.148 ± 0.004 (4)	0.170 ± 0.008 (4)	0.452 ± 0.013 (4)
7: HBSS + MTS/PMS	0.138 ± 0.007 (14)	0.154 ± 0.003 (4)	0.380 ± 0.032 (4)
8: HBSS + MTS/PMS + TR146 cells	0.318 ± 0.03 (16)	1.333 ± 0.013 (16)	2.707 ± 0.026 (4)

[a] PMS in HBSS, final concentration 2.4 μg/ml.
[b] MTS in HBSS, final concentration 240 μg/ml.
[c] Final concentrations: MTS, 240 μg/ml; PMS, 2.4 μg/ml.
[d] Standard procedure (see Section 2). Subsequently, 100 μl HBSS and 25 μl of the indicated solutions were added to each well, and OD was recorded at the indicated time points.

must be for a specified time and the addition of PMS is required to speed up the TR146 generation of formazan, giving suitable OD values after a relatively short period, e.g. 4 h incubation.

3.2.1. Optimisation of the initial cell seeding density and the period of incubation

The relationship between the corrected OD values and the initial cell numbers at various times of incubation with the MTS/PMS reagent is depicted in Fig. 1 (data for 1.5, 2 and 2.5 h not shown). The SEM values were below 10% of the mean value, indicating a good reproducibility of replicates ($n = 4$) (data not shown). The curves show that approximately 3×10^3 cells/well are required in order to obtain measurable corrected OD values after 3 h incubation. The OD values after 24 and 48 h of incubation were practically constant at cell densities higher than 5×10^3 cells/well. The period of incubation ranging from 0.5 to 3 h generated an almost linear correlation of the corrected OD values versus the cell density over the range 3×10^3–30×10^3 cells/well and a plateau at 30×10^3–60×10^3 cells/well. The corrected OD values at 48 h incubation were slightly lower than the values at 24 h incubation, which may indicate a further reduction of the MTS–tetrazolium salt beyond the formazan step. Further optimisation of the assay was performed with cell concentrations in the range 2.5×10^3–30×10^3 cells/well, and a period of incubation up to 6 h with the MTS/PMS reagent.

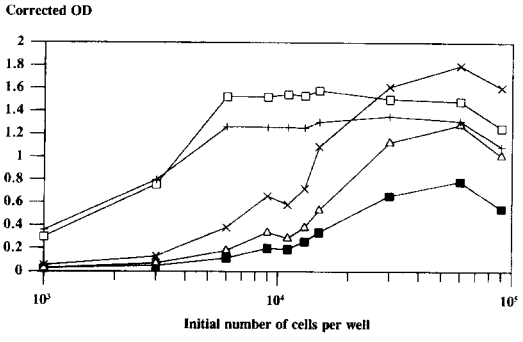

Fig. 1. MTS/PMS assay of TR146 cells. Corrected OD at 490 nm as a function of the initial cell density. OD was recorded at various times during incubation with the MTS/PMS reagent: (■) 0.5 h, (△) 1 h, (x) 3 h, (□) 24 h, and (+) 48 h. The data are expressed as mean ($n = 4$).

Table 2
MTS/PMS assay[a] of TR146 cells. Cellular sensitivity (%) for various concentrations of ethanol

	Concentration of ethanol (%)							
	0.096	0.96	4.8	9.6	24	48	72	96
Avg. cellular sensitivity ± SEM[b]	87.6 ± 1.9	76.1 ± 4.2	92.3 ± 1.0	84.4 ± 3.3	15.4 ± 0.7	18.3 ± 1.6	19.9 ± 0.4	26.8 ± 1.3

[a] Stability testing of the MTS–formazan (see Section 2).
[b] Average value ($n = 7$) of the cellular sensitivity calculated from the OD values ($n = 4$) measured every 5 min over a 30-min period for each concentration of ethanol.

3.2.2. Optimisation of the PMS concentration in the MTS/PMS reagent and the period of incubation

Different concentrations of PMS (0.6–3.6 μg/ml) in the MTS/PMS reagent (MTS 120 μg/ml) and various periods of incubation were studied in the range 2.5×10^3–30×10^3 cells/well. Corrected OD values as a function of different PMS concentrations after 4 h incubation showed a linear curve for 2.5×10^3 cells/well, a linear curve up to 2.4 μg/ml PMS for 5×10^3–10×10^3 cells/well, and a parabolic curve for 15×10^3–30×10^3 cells/well. In general, the two highest PMS concentrations, 2.4 and 3.6 μg/ml (final concentrations), generated similar OD signals for most of the different initial cell concentrations, indicating that the stimulating effect of PMS was not concentration dependent at concentrations above 2.4 μg/ml PMS.

The corrected OD values as a function of different PMS concentrations using a seeding density of approximately 20×10^3 cells/well showed an increase of the corrected OD values with increasing period of incubation. Final concentrations of 2.4 and 3.6 μg/ml PMS, and 4–6 h of incubation, showed suitable corrected OD values. A final concentration of 2.4 μg/ml PMS and a period of incubation of 4 h were chosen for further experiments.

3.2.3. Optimisation of the MTS concentration in the MTS/PMS reagent

Three concentrations of MTS in the MTS/PMS reagent (120, 240 and 360 μg/ml, expressed as final concentrations) were tested, with cell concentrations in the range 5×10^3–30×10^3 cells/well. The results indicate that the different tested MTS concentrations produced a similar amount of MTS–formazan for each cell concentration. Initial seeding densities of 5×10^3 and 10×10^3 cells/well resulted in low corrected OD values, 0.1–0.4, while 20×10^3 and 30×10^3 cells/well showed suitable corrected OD values, 1.2–1.7. A seeding density of 20×10^3 cells/well and 240 μg/ml MTS were chosen for the final protocol.

3.2.4. Stability testing of the MTS–formazan

The stability of the TR146-generated MTS–formazan was examined. Ethanol was applied at concentrations ranging from 0.1% to 96% and a cellular sensitivity assay was performed according to the final protocol (see Section 2) except that the plate was kept at room temperature and atmosphere, and the OD recording was repeated every 5 min for a period of 30 min. The cellular sensitivity (see Eq. (1)) versus the concentration of ethanol is given in Table 2. The results (SEM < 5%) indicated that the generated MTS–formazan product was stable in a period of 30 min at room temperature and atmosphere.

3.3. Final protocol for testing cellular drug sensitivity of the cell line TR146 by the MTS/PMS assay

Taking into account the results of the optimisation of the MTS/PMS assay and the stability of the generated MTS–formazan, the final protocol for drug sensitivity testing of TR146 cells comprises the standard procedure, an initial seeding density of 2×10^4 cells/well, and 4 h incubation with the MTS/PMS reagent, using final concentrations of 240 μg/ml MTS and 2.4 μg/ml PMS.

3.4. Application of the optimised MTS/PMS assay

The cellular sensitivity of 4-fold log concentrations of the tested β-adrenoceptor antagonists (propranolol, oxprenolol, metoprolol and atenolol) is shown in Fig. 2. The calculated IC_{10} and IC_{50} values for both series of compounds are displayed in Table 3.

According to Table 3, the calculated IC_{10} values for the β-adrenoceptor antagonists were greater than 10^{-4} M. This is in accordance with the results of previous transport studies using filter-grown TR146 cells. These results indicated that 10^{-4} M solutions of the same series β-adrenoceptor antagonists slightly affected the integrity of TR146 cell layers, shown by phase-contrast microscopy (Jacobsen et al., 1995).

The cellular drug sensitivity for the tested series of morphine and morphine prodrugs showed that cellular sensitivity was only evident for 3-hexanoyl-morphine. This prodrug has the highest lipophilicity (see Table 3).

The optimised MTS/PMS assay has recently been applied to test the cellular sensitivity of drugs and excipients of a novel mucoadhesive buccal drug delivery system (Burgalassi et al., 1996). Further studies using TR146 cells and the optimised MTS/PMS assay are currently being conducted prior to transport and metabolism studies with, for example, peptides and absorption enhancers.

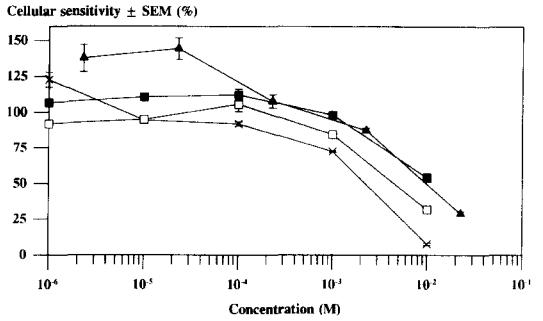

Fig. 2. MTS/PMS assay of TR146 cells testing β-adrenoceptor antagonists. The cellular sensitivity is expressed as a function of the concentration of drug. OD was recorded at 490 nm and the cellular sensitivity calculated according to Eq. (1) in Section 2. Specification of drug solutions: (x) propranolol, (□) metoprolol, (■) atenolol, and (▲) oxprenolol. The data are expressed as mean ± SEM ($n = 4$).

Table 3
MTS/PMS assay[a] of TR146 cells. IC_{10}[b] and/or IC_{50}[b] for a series of β-adrenoceptor antagonists and a series of morphine and morphine prodrugs

Drug	log P[c]	IC_{10}[b] (M)	IC_{50}[b] (M)
Propranolol, HCl	1.540[d]	2.0×10^{-4}	4.1×10^{-3}
Oxprenolol, HCl	0.4[e]	1.8×10^{-3}	1.3×10^{-2}
Metoprolol tartrate	0.068[d]	7.6×10^{-4}	6.9×10^{-3}
Atenolol	−1.397[d]	2.6×10^{-3}	$>1.0 \times 10^{-2}$
3-Hexanoyl-morphine	2.04[f]	1.0×10^{-4}	1.3×10^{-3}
3-Propionyl-morphine	0.66[f]	No inhibition	No inhibition
3-Acetyl-morphine	0.15[g]	No inhibition	No inhibition
Morphine, HCl	−0.06[f]	No inhibition	No inhibition

[a] Final protocol (see Section 2). The drug solutions were incubated for 30 min.
[b] 10% or 50% inhibition of control (IC), see list of abbreviations.
[c] P is the partition coefficient.
[d] Octanol/PBSA buffer (pH 7.4) at 37°C (Tavakoli-Saberi and Audus, 1989).
[e] Octanol/water, temperature not specified (Komiya et al., 1980).
[f] Octanol/phosphate buffer (pH 7.4) at 21°C (Druestrup et al., 1991).
[g] Unpublished data.

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