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Anhydrotetracycline, a novel effector for tetracycline controlled gene expression systems in eukaryotic cells

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Tetracycline (Tc) controlled gene expression has been demonstrated in a variety of eukaryotic systems such as tobacco (1, 2, 3), *Saccaromyces cerevisiae* (4), *Saccaromyces pombe* (5) and *Dictyostelium discoidum* (6). In these systems the repressor of the *E.coli* Tn*10* tetracycline (Tc) resistance operon (7) is utilized to interfere with transcription initiation of RNA polymerase II or III by binding to operators properly placed within a promoter region. This interference is abolished by Tc.

By contrast, we have converted the Tet repressor into an efficient tetracycline controlled transcriptional transactivator (tTA) whose function can be controlled by Tc. In double-stable human HeLa cell lines such as the X1 cells (8), which contain both an expression unit for tTA and a luciferase gene controlled by a tTA dependent promoter, gene activity can be regulated over up to five orders of magnitude depending on the Tc concentration in the medium (8).

The general applicability of Tc controlled systems (e.g. in transgenic situations) will largely depend on the effector's toxicity, its chemical and biological half-life time, etc. Thus, although Tc has excellent general pharmacological properties its very low but distinct cytotoxicity to mammalian cells may not be negligible under all circumstances. On the other hand among the numerous well studied tetracyclines and their derivatives there may be compounds with superior properties in Tc regulated systems. One such example is anhydrotetracycline (ATc) which binds the Tet repressor more efficiently than Tc (10^{11} M^{-1} vs. 3×10^{9} M^{-1}, ref. 9) and which has a lower antibiotic activity towards *E.coli* (10). Moreover, it was suggested that ATc acts at a different level than Tc, which interferes with the translational process (10). Here we describe the properties of ATc as an effector in our tTA dependent regulatory system. We incubated X1 cells at different concentrations of either Tc or ATc and monitored the luciferase activity produced under these conditions. Our results show that ATc is much more effective than Tc in inactivating tTA and that it completely abolishes tTA mediated luciferase activity at concentrations as low as 3 ng/ml (Figure 1a). In good correlation with the relative affinities of Tc and ATc towards the Tet repressor (9), this concentration is ten fold below that required for Tc. More importantly, the concentration at which the prolonged presence of ATc begins to affect the growth rate of HeLa cells in culture (ATc conc. >3 μg/ml; Figure 2) is more than thousand fold above the effective concentration. Finally, the slope of the dose response curve indicates that like Tc, ATc is well suited to attenuate gene expression at intermediate levels of induction.

These properties together with the high functional stability of ATc in cell culture make ATc a most attractive alternative effector for Tc-controlled gene expression in higher eukaryotic systems.

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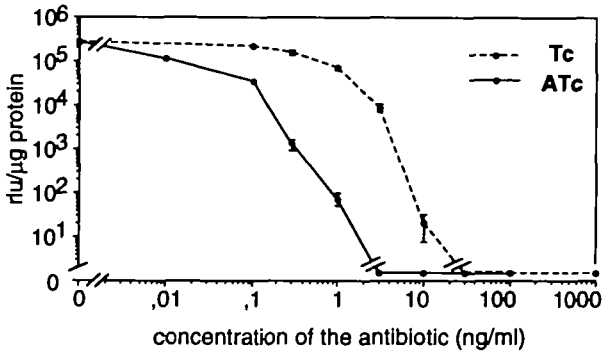

Figure 1 Dose response of anhydrotetracycline (ATc) and tetracycline (Tc) on the activity of luciferase in the HeLa cell line X1. HeLa X1 cells grown in medium containing 100 ng/ml Tc were seeded with a density of about 10 000 cells/35 mm culture dish and incubated with the antibiotics at the concentrations indicated. After .wo days the antibiotic containing medium was renewed. After two further days the cells were harvested and luciferase activity and protein content of the extracts were determined. The antibiotics used were tetracycline hydrochloride (dashed line) from Sigma Chemical Co., St Louis (stock 1 mg/ml in H_2O, stable at 4°C for at least 2 weeks when light protected), and andydrotetracycline hydrochloride (continuous line) from Janssen Chimica, Geel (stock 0.1 mg/ml in H_2O, stable at 4°C for at least 2 weeks when light protected). Data shown are from two independent experiments. Cell culture, luciferase assays and protein determinations were carried out as described previously (8).

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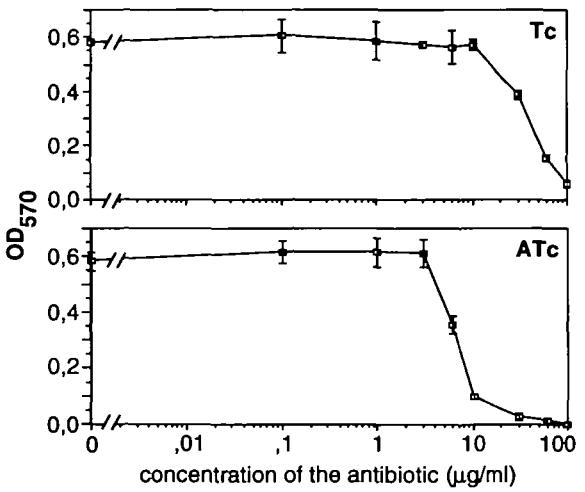

Figure 2. Cytotoxic effect of tetracycline and anhydrotetracycline on HeLa cells. The cytotoxicity of elevated concentrations of the antibiotics on HeLa cells were determined according to Mossman (11). Cultures of 1000 cells/dish in 96 well plates were incubated for 3 days with concentrations of the antibiotics indicated and growth was analysed by the MTT-assay. Values given are the mean of four experiments.