

## CHS 828 Kill Tumour Cells by Inhibiting the Nuclear Factor- $\kappa$ B Translocation but Unlikely through Down-regulation of Proteasome

SAADIA B. HASSAN<sup>1</sup>, HENRIK LÖVBORG<sup>1</sup>, ELIN LINDHAGEN<sup>1</sup>,  
MATS O. KARLSSON<sup>2</sup> and ROLF LARSSON<sup>1</sup>

<sup>1</sup>Division of Clinical Pharmacology, University Hospital, SE-751 85 Uppsala;

<sup>2</sup>Divisions of Pharmacokinetics and Drug Therapy, Faculty of Pharmacy,  
Uppsala University, SE-751 24 Uppsala, Sweden

**Abstract.** CHS 828 (N-(6-chlorophenoxyhexyl)-N'-cyano-N''-4-pyridylguanidine) has shown promising activity in many preclinical systems and in phase I/II clinical trials. The nuclear transcription factor kappa B (NF- $\kappa$ B) has been identified as a target for CHS 828. The aim of this study was to confirm the inhibitory effect of CHS 828 on NF- $\kappa$ B translocation and to explore its possible effect on the proteasome using 7 cell lines. Translocation of NF- $\kappa$ B from the cytoplasm to the nucleus was analysed using a quantitative cytometric system, ArrayScan<sup>®</sup>. The activity of the proteasome was assayed by monitoring the hydrolysis of a fluorogenic substrate. In parallel, the *in vitro* cytotoxic effect of CHS 828 was analyzed using a 72-h microtiter plate-based cytotoxicity assay (FMCA). CHS 828 inhibited NF- $\kappa$ B translocation in the cell lines where it was able to inhibit the tumour cell growth. However, the results did not prove any effect of CHS 828 on proteasome activity when compared to a proteasome inhibitor activity.

The nuclear factor kappa B family (NF- $\kappa$ B) is one of the main groups of transcription factors known to be important in autoimmune diseases and human cancer, as a regulator of cell growth, differentiation and cell death (1). In most cells, the inactive form of NF- $\kappa$ B is predominantly maintained in the cytoplasm in association with the inhibitory kappa B proteins I $\kappa$ B ( $\alpha$ ,  $\beta$ ) masking its nuclear localization sequence. In response to a large number of external stimuli, including the proinflammatory cytokine

TNF $\alpha$ , different serine kinases, such as inhibitory kappa B kinase and IKK, complex catalytic subunits ( $\alpha$ ,  $\beta$ ) are activated. These are implicated in the phosphorylation of I $\kappa$ B, marking it for ubiquitination and ultimately proteolytic degradation by proteasomes. The degradation subunits of NF- $\kappa$ B (p65 and p50) bind to form the active NF- $\kappa$ B transcription factor, which translocates to the nucleus, where it binds and participates in the transcriptional activity of a large variety of target genes (2). Proteasomes are the proteases responsible for selective degradation of the majority of endogenous cellular proteins targeted by the ubiquitination system (3). Blocking proteasome activity should lead to a decrease in NF- $\kappa$ B activity.

Growing evidence indicated that a variety of anticancer agents kill cells through apoptosis, programmed cell death, and resistance to anticancer therapies appeared to be mediated by resistance to apoptosis (4). Several anticancer agents may have an impaired ability to induce programmed cell death because of their activation of NF- $\kappa$ B (5). It is known that the NF- $\kappa$ B plays a role in preventing apoptosis (6), though it can also have pro-apoptotic effects depending upon cell type and the nature of stress (7). A tight control of the activity of NF- $\kappa$ B might be a reasonable approach against cancer. Possible therapeutic targets in the signalling pathway of NF- $\kappa$ B could be kinase activity, cytoplasmic proteasomes and nuclear NF- $\kappa$ B binding to DNA (8, 9).

CHS 828 (N-(6-chlorophenoxyhexyl)-N'-cyano-N''-4-pyridylguanidine) is a cyanoguanidine anticancer agent under development by LEO Pharmaceutical Products, Denmark, for which phase I/II clinical trials have now ended. It has shown an anti-proliferative effect on a variety of human cancer cells both *in vitro* and *in vivo* (10-12). Although its mechanism of action is not fully known, a recent report (13) showed that CHS 828 could inhibit the activity of NF- $\kappa$ B through inhibition of IKK $\beta$ .

*Correspondence to:* Saadia Bashir Hassan, Division of Clinical Pharmacology, Department of Medical Sciences, University Hospital, 751 85 Uppsala, Sweden. Tel: +46 18 611 52 50, Fax: +46 18 519237, e-mail: sadia.hassan@medsci.uu.se

**Key Words:** CHS 828, NF- $\kappa$ B, TNF $\alpha$ , translocation, proteasome, FMCA, ArrayScan<sup>®</sup>.

In the present study, the *in vitro* cytotoxicity of CHS 828 and its relation to NF- $\kappa$ B was further investigated using a high-content screening assay. Furthermore, whether the activity of the proteasome is influenced by CHS 828 in various tumour cell types was also examined.

## Materials and Methods

**Cell cultures and materials.** The human breast cancer (MDA 231) were a kind gift from Dr. Jonas Bergh at the Department of Oncology, Uppsala University, the myeloma (RPMI 8226/S) was kindly provided by Dr. W.S. Dalton, Department of Medicine, Cancer Centre Division, University of Arizona, Tucson, AZ, USA, the small cell lung cancer (NYH SCLC) from P. Buhl Jensen, Rigshospitalet, Copenhagen, Denmark, and the renal adenocarcinoma (ACHN) from the American Type Culture Collection (ATCC, Rockville, MD, USA). The cell lines were cultured in RPMI 1640 medium. The human prostate adenocarcinoma (PC-3) obtained from Leo Pharmaceutical Products, Ballerup, Denmark, was cultured in a mixture of HAM's F-12K and RPMI 1640 (50% each). The breast cancer (MCF-7), and the human cervix (HeLa) cell lines obtained from ATCC, were cultured in DMEM deprived of phenol red and Eagle's Minimal Essential medium with 1 mM sodium pyruvate medium, respectively. All medium were supplemented with 10% heat-inactivated fetal calf serum (FCS), 2-mM glutamine, 50  $\mu$ g/ml streptomycin and 60  $\mu$ g/ml penicillin (all from Sigma).

All materials were prepared according to the manufacturers' instructions. CHS 828 dissolved at 10 mM in DMSO was obtained from LEO Pharmaceutical, tumour necrosis factor alpha (10  $\mu$ g TNF $\alpha$ ) and fluorescein diacetate (FDA) were obtained from Sigma. The NF- $\kappa$ B Activation HitKit™, HCS (High Content Screening) was obtained from Cellomics, Inc., USA. The fluorogenic Proteasome Substrate III, SUC-Leu-Leu-Val-Tyr-AMC and the proteasome inhibitor MG-262, Z-Leu-Leu-Leu-B (OH) 2, were obtained from Calbiochem, Germany. The 20S Proteasome Assay Kit were obtained from BIOMOL Research Laboratories, Inc., PA, USA.

Throughout the study, CHS 828 was tested at three concentrations, 10, 1 and 0.1  $\mu$ M for all cell lines except for the NYH SCLC cell line, which was exposed to 0.1, 0.01 and 0.001  $\mu$ M CHS 828 for the TNF $\alpha$ -induced NF- $\kappa$ B translocation assay. All experiments were repeated independently 2-3 times. Ninety-six-well microtiter plates (Nunc, Roskilde, Denmark) were prepared with 20  $\mu$ l per well of drug solution at ten times the desired concentration with the aid of a pipetting robot (Biomek® 2000, Beckman Instrument, Inc., USA). Each concentration was prepared in triplicate. The plates were stored frozen at -70°C as stock drug plates.

**NF- $\kappa$ B translocation.** Cells (1500 cells/90  $\mu$ l/well of each cell line) were seeded in standard black 96-well clear bottom microplates (Packard, ViewPlate®, Packard, USA) and left to adhere overnight before the addition of 10  $\mu$ l CHS 828. The plates were incubated for 30 h at 37°C. In another set of experiments, PC-3, RPMI 8226/S and MDA 231 cells were exposed to 1  $\mu$ M CHS 828 for 24 h. At the end of the incubation time, treated cells and positive control cells were stimulated with 50 ng/ml of TNF $\alpha$  for 30 min. Negative control cells were used as a reference to quantify the extent of baseline NF- $\kappa$ B translocation in the cell lines studied.

The potential effect of CHS 828 to induce translocation of NF- $\kappa$ B, without stimulation of the cells with TNF $\alpha$ , was investigated in some of the experiments, where 1  $\mu$ M CHS 828 was added to the PC-3 and MDA 231 cells for 24 h and to the RPMI 8226/S and ACHN cells for 30 h.

NF- $\kappa$ B translocation was evaluated using the NF- $\kappa$ B Activation HCS HitKit™ utilising the ArrayScan® HCS reader (Cellomics, Inc., USA), as described earlier (14). The ArrayScan® reader automatically quantifies the mean nucleus-cytoplasm intensity difference of the amount of p65 immunofluorescence staining in a pre-defined number of cells. In the present study, the pre-selected number of cells to be analysed was set to 200-500 cells/well and it was found that increasing the cell number further did not affect the results. The mean nucleus-cytoplasm intensity difference was presented as NF- $\kappa$ B translocation %, defined as the mean nucleus-cytoplasm intensity difference in the experimental wells as a per cent of that in control wells.

**Proteasome enzyme activity in cellular and cell-free systems.** Tumour cells from the 7 cell lines were seeded at 50,000 cells/180  $\mu$ l in the 96-well stock CHS 828 plates and incubated at 37°C for either 24 or 30 h. Four hours before the end of the incubation time, 1  $\mu$ M of the proteasome inhibitor MG 262 was added to the pre-identified wells and was tested in triplicates to serve as a positive control. The plates were washed twice with PBS before incubation with 40  $\mu$ M fluorogenic proteasome peptide substrate for 2 h. The chymotryptic peptide hydrolysing activity of the proteasome enzyme was assayed by monitoring the hydrolysis of the fluorogenic substrate SUC-Leu-Leu-Val-Tyr-AMC (15) using a fluorescence plate reader (Fluostar Optima, BMG Labtechnologies GmbH, Germany). The enzymatic activity was expressed as fluorescence in experimental wells as a per cent of that in the negative control wells with blank values subtracted.

In one experiment, the enzymatic activity of the 20S proteasome in a cell-free system was measured by adding CHS 828 at different concentrations or 25  $\mu$ M of a known proteasome inhibitor, lactacystin, to the pure 20S enzyme in a 96-well plate and by subsequently adding SUC-Leu-Leu-Val-Tyr-AMC substrate supplied with the BIOMOL QuantiZyme™ Assay kit. The enzymatic activity was evaluated by monitoring the fluorescence generated from cleavage of the substrate over time.

**Cytotoxicity experiments.** To determine the cytotoxicity of CHS 828, in parallel to NF- $\kappa$ B translocation and proteasome activity analysis, tumour cells were seeded at 20,000 cells/180  $\mu$ l in the 96-well stock drug plates. The plates were incubated at 37°C for 72 h and at the end of the incubation period, the cell survival was determined with the fluorometric microculture cytotoxicity assay (FMCA), as described previously in detail (16). The FMCA is based on the measurements of fluorescence generated from hydrolysis of FDA to fluorescein by cells with intact plasma membrane and the fluorescence signal is proportional to the number of viable cells. Cell survival was presented as survival index (SI), defined as the fluorescence in experimental wells as a per cent of that in control wells, with blank values subtracted.

**Statistical analysis.** One-way ANOVA tests for comparing 3 groups or more and the correlation analysis, using Pearson's correlation co-efficient, were performed using GraphPad Prism software (GraphPad Software, San Diego, CA, USA).

## Results

**NF- $\kappa$ B translocation.** The percentage of NF- $\kappa$ B translocation and the percentage of cell survival in all cell lines tested are presented in Figure 1. Exposure to 0.1, 1 or 10  $\mu$ M CHS 828 for 30 h significantly inhibited TNF $\alpha$ -induced NF- $\kappa$ B translocation in a dose-dependent manner in both the PC-3 and MDA 231 cell lines ( $p < 0.05$ ). The RPMI 8226/S and NYH SCLC cells showed a constitutively high level of NF- $\kappa$ B activity. CHS 828 did not inhibit TNF $\alpha$ -induced NF- $\kappa$ B translocation in the RPMI 8226/S cells; however, a tendency could be seen. Exposing NYH SCLC cells to TNF $\alpha$  did not stimulate NF- $\kappa$ B translocation any more (data not shown); interestingly, 0.1  $\mu$ M CHS 828 significantly inhibited the constitutive NF- $\kappa$ B translocation ( $p < 0.05$ ). CHS 828 was not able to inhibit TNF $\alpha$ -induced NF- $\kappa$ B translocation in HeLa, ACHN or MCF-7 ( $p > 0.05$ ). A 24-h exposure to CHS 828 did not inhibit the TNF $\alpha$ -induced NF- $\kappa$ B translocation in the 3 cell lines tested (data not shown).

The potential of CHS 828 to induce NF- $\kappa$ B translocation could not be proved in any of the cell lines at any exposure time-points tested (Figure 2).

**Proteasome activity.** Neither at 30 h nor at 24 h, did CHS 828, at any concentration tested, inhibit the chymotrypsin-like activity of the proteasome in any of the cell lines studied ( $p > 0.05$ ), while exposure to 1  $\mu$ M MG-262 for 4 h significantly inhibited proteasome activity (data not shown).

In a cell-free system, CHS 828 was not able to inhibit the proteasome enzyme activity, whereas lactacystin induced an immediate inhibition of the activity as measured from the fluorescence generated from the cleavage of 20S enzyme (data not shown).

**Cell survival.** The survival of PC-3, MDA 231, NYH SCLC and RPMI 8226/S cells was inhibited by 72-h exposure of CHS 828 in a concentration-dependent manner, while the HeLa, ACHN and MCF-7 cells were not affected when assayed in parallel to NF- $\kappa$ B translocation and proteasome activity experiments. CHS 828 potency in the NF- $\kappa$ B translocation and cell survival assays was in the same range for the sensitive cell lines (Figure 1).

**Correlations between NF- $\kappa$ B translocation, proteasome activity and cell survival.** The percentages of NF- $\kappa$ B translocation and cell survival of each cell line exposed to CHS 828 were highly correlated ( $R^2 = 0.81, 0.64$  and  $0.81$  at  $0.1, 1$  and  $10 \mu$ M CHS 828, respectively) when data from six out of seven cell lines were included in the analysis. However, when results from the RPMI 8226/S cell line were included in the analysis, the correlation co-efficients were reduced ( $R^2 = 0.36, 0.09$  and  $0.61$ ). The correlation between the percentages of proteasome activity and cell survival was found to be very low

( $R^2 = 0.27, 0.32$  and  $0.14$  at  $10, 1, 0.1 \mu$ M, respectively). The correlation analysis between the percentages of NF- $\kappa$ B translocation and of the proteasome activity revealed no relationship between the two parameters as indicated by very low coefficients ( $R^2 = 0.39, 0.07$  and  $0.14$  at  $10, 1, 0.1 \mu$ M, respectively).

## Discussion

The aim of the present study was to examine the activity of CHS 828 on the TNF $\alpha$ -induced NF- $\kappa$ B translocation and on proteasome activity, and also intended to investigate the relationship between those two parameters and the cytotoxic activity of CHS 828. The results indicated an inhibition of NF- $\kappa$ B activity in the tumour cell lines PC-3, MDA 231 and NYH SCLC and a tendency to inhibit NF- $\kappa$ B activity in RPMI 8226/S in response to a 30-h pre-incubation with CHS 828, whereas the experiments proved no effect of CHS 828 on proteasome activity. The study also demonstrated a close relationship between the anti-tumour effect of CHS 828 and the inhibition of NF- $\kappa$ B translocation. Only the RPMI 8226 cell line diverged from this pattern, as documented by the increase of correlation co-efficients when RPMI 8226 data were removed from the analysis. It is possible that the NF- $\kappa$ B mechanism is not the only mechanism behind the CHS 828 cytotoxicity seen in the RPMI 8226/S cell line and that other still-unknown mechanism(s) for cell death by CHS 828 remain to be elucidated. Indeed, this is in line with our previous speculation that CHS 828 could have two mechanisms of action (17). It should be noted that in the cell lines where CHS 828 was not able to induce cytotoxicity under our experimental conditions, no effect on the level of TNF $\alpha$ -induced NF- $\kappa$ B translocation was observed. This observation is compatible with previous observations (13), where there was a close correlation between the inhibition of NF- $\kappa$ B activity and the reduction of xenografts in nude mice. The observed NF- $\kappa$ B inhibition induced by CHS 828 at 30-h but not at 24-h is in agreement with what is known about the CHS 828 dynamic process. Macromolecular synthesis is not affected until after 24 h and the viability was reduced in a time-dependent manner (18).

The transcriptional factor NF- $\kappa$ B is a key anti-apoptotic factor in mammalian cells. Recent reports have demonstrated the critical role of the NF- $\kappa$ B in protecting cells from apoptosis induced by various anticancer drugs (4), and suggested that apoptotic response to conventional chemotherapy may be increased by inhibition of NF- $\kappa$ B activation in drug-resistant cancer cells (19). Tumour cells, in which NF- $\kappa$ B is constitutively active, are highly resistant to chemotherapeutic agents and ionising radiation. Inhibition of NF- $\kappa$ B activity in these cells may greatly increase their sensitivity to such treatment (20). The myeloma cells, RPMI

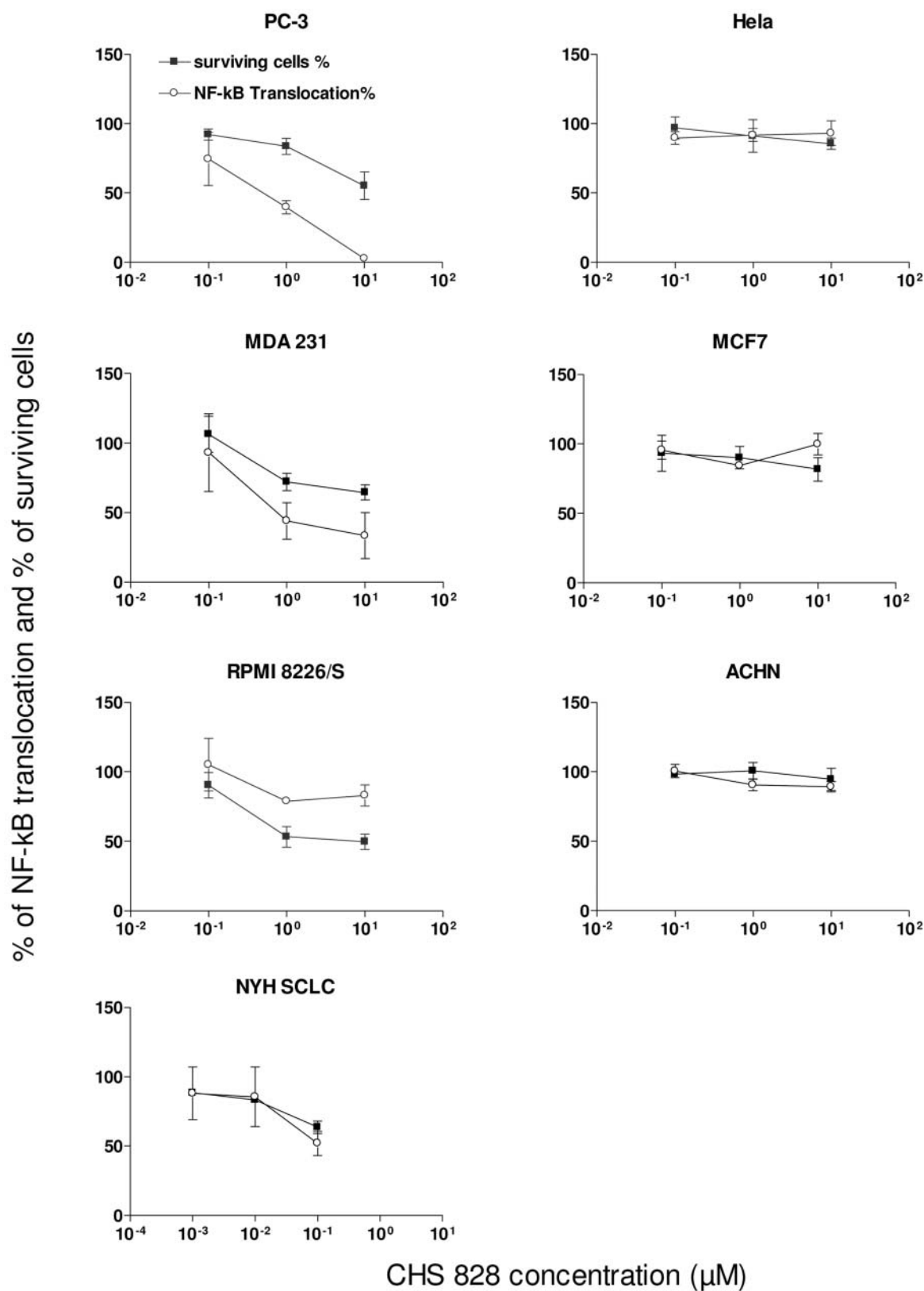


Figure 1. CHS 828 effect on TNF $\alpha$ -induced NF- $\kappa$ B translocation and on cell survival in the 7 cell lines tested. The percent of NF- $\kappa$ B translocation and of the viable cells are plotted versus CHS 828 concentration (three experiments mean  $\pm$  SEM).



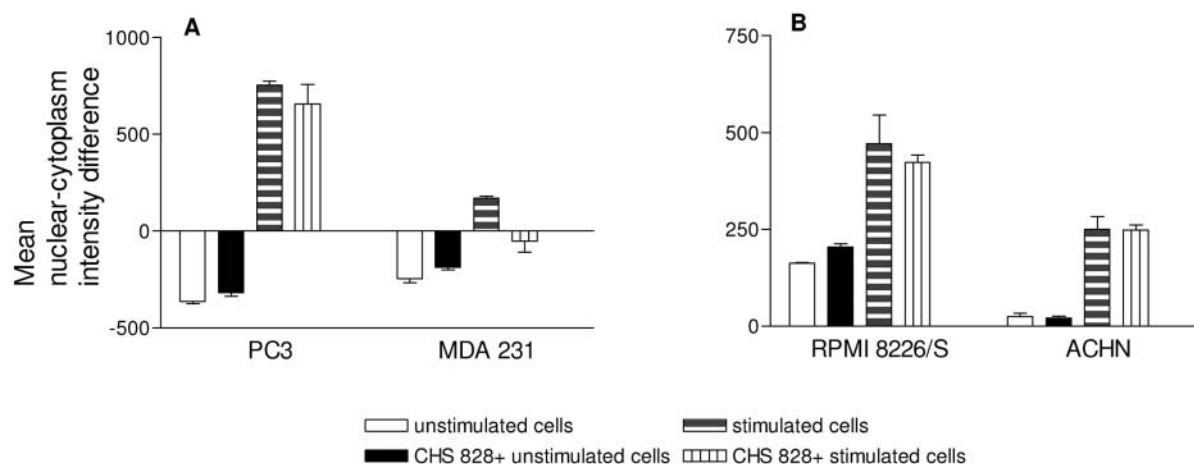


Figure 2. The potential effect of 1  $\mu$ M CHS 828 on the translocation of NF- $\kappa$ B on the PC-3 and MDA 231 cell lines at 24-h exposure (A) and on RPMI 8226/S and ACHN cell lines at 30-h exposure (B).

8226/S, and the small cell lung cancer cells, NYH SCLC, showed constitutive NF- $\kappa$ B activity under our experimental conditions. A high level of NF- $\kappa$ B was not reported before in the NYH SCLC cell line, however it is known that lung carcinoma express constitutively active NF- $\kappa$ B (21). It is interesting to note that NYH SCLC was the most sensitive cell line in our study. CHS 828 tended to reduce the NF- $\kappa$ B activity in RPMI 8226/S cells despite the high level of NF- $\kappa$ B expression observed in these cells by others (22, 23).

The preliminary observation that CHS 828 showed no ability to induce NF- $\kappa$ B activation, even, for example, in ACHN cells, where it did not cause any significant reduction in cell growth, could indicate that the lack of cytotoxicity might not be due to the counteraction of the therapeutic effect of CHS 828 by activation of NF- $\kappa$ B.

Activation of NF- $\kappa$ B during cancer therapy is common for many anticancer agents and is one mechanism of tumour cell resistance to chemotherapeutic agents (20). The above observations might indicate that CHS 828 could be used to treat tumours which are resistant to other chemotherapeutic agents due to their expression of constitutive NF- $\kappa$ B activity. It might also be interesting to use CHS 828 in combination with other chemotherapeutic agents to enhance their antitumour activity. CHS 828 has shown previously preclinical synergistic results with etoposide (24), amiloride and mitomycin C (25). Studies of CHS 828 combinations with other standard cytotoxic drugs are currently conducted in our laboratory.

The failure of CHS 828 to affect the proteasome activity in all cell lines studied was not associated with its activity on the cell survival, as presented by the very low co-efficients of the correlations between these parameters. Our data in a cell-free system excluded any inhibitory effect of CHS 828 on the proteasome activity. A recent study (26) have also

shown that CHS 828 had no correlation with other known proteasome inhibitors with respect to the activity pattern and shape of concentration-response curves in a 10 cell-line panel excluding mechanistic similarity. Additionally, there was no correlation found between NF- $\kappa$ B and proteasome activities in the present study. Taken together, it is unlikely that CHS 828 induces its inhibitory effect on the NF- $\kappa$ B activity and on cell survival through the regulation of proteasome activity. The current result is different from a previous observation (26), in which CHS 828 was found to induce a dose-dependent inhibition of the proteasome activity in the lymphoma cell line, U-937 GTB, after 24 h of exposure. However, cell type dependence and/or different experimental conditions cannot be excluded as possible explanations for this discrepancy.

In summary, although treatment with CHS 828 did not down-regulate the proteasome activity, we demonstrated an inhibitory effect of CHS 828 on the NF- $\kappa$ B pathway in a variety of human tumour cell lines. Our data suggest a correlation between the inhibition of NF- $\kappa$ B translocation and the cytotoxic activity of CHS 828. This could support the notion (13) that the NF- $\kappa$ B pathway plays an important role in the anticancer effect of this compound. However, the precise role of proteasome activity as a possible mechanism behind CHS 828 cytotoxicity in some tumour types remains to be further elucidated.

## References

- Garg A and Aggarwal BB: Nuclear transcription factor- $\kappa$ B as a target for cancer drug development. *Leukemia* 16: 1053-1068, 2002.
- Baldwin AS Jr: The NF- $\kappa$ B and I  $\kappa$ B proteins: new discoveries and insights. *Annu Rev Immunol* 14: 649-683, 1996.

- 3 Hilt W and Wolf DH: Proteasomes: destruction as a programme. *Trends Biochem Sci* 21: 96-102, 1996.
- 4 Baldwin AS: Control of oncogenesis and cancer therapy resistance by the transcription factor NF-kappaB. *J Clin Invest* 107: 241-246, 2001.
- 5 Wang CY, Mayo MW and Baldwin AS Jr: TNF- and cancer therapy-induced apoptosis: potentiation by inhibition of NF-kappaB. *Science* 274: 784-787, 1996.
- 6 Gibbs JB: Mechanism-based target identification and drug discovery in cancer research. *Science* 287: 1969-1973, 2000.
- 7 Kaltschmidt B, Kaltschmidt C, Hofmann TG, Hehner SP, Droge W and Schmitz ML: The pro- or anti-apoptotic function of NF-kappaB is determined by the nature of the apoptotic stimulus. *Eur J Biochem* 267: 3828-3835, 2000.
- 8 Darnell JE Jr: Transcription factors as targets for cancer therapy. *Nat Rev Cancer* 2: 740-749, 2002.
- 9 Karin M, Yamamoto Y and Wang QM: The IKK NF-kappa B system: a treasure trove for drug development. *Nat Rev Drug Discov* 3: 17-26, 2004.
- 10 Hjarnaa PJ, Jonsson E, Latini S, Dhar S, Larsson R, Bramm E, Skov T and Binderup L: CHS 828, a novel pyridyl cyanoguanidine with potent antitumor activity *in vitro* and *in vivo*. *Cancer Res* 59: 5751-5757, 1999.
- 11 Hassan SB, de la Torre M, Nygren P, Karlsson MO, Larsson R and Jonsson E: A hollow fiber model for *in vitro* studies of cytotoxic compounds: activity of the cyanoguanidine CHS 828. *Anticancer Drugs* 12: 33-42, 2001.
- 12 Jonsson E, Friberg LE, Karlsson MO, Hassan SB, Nygren P, Kristensen J, Tholander B, Binderup L and Larsson R: *In vivo* activity of CHS 828 on hollow-fibre cultures of primary human tumour cells from patients. *Cancer Lett* 162: 193-200, 2001.
- 13 Olsen LS, Hjarnaa PJ, Latini S, Holm PK, Larsson R, Bramm E, Binderup L and Madsen MW: Anticancer agent CHS 828 suppresses nuclear factor-kappaB activity in cancer cells through down-regulation of IKK activity. *Int J Cancer* 111: 198-205, 2004.
- 14 Ding GJ, Fischer PA, Boltz RC, Schmidt JA, Colaienne JJ, Gough A, Rubin RA and Miller DK: Characterization and quantitation of NF-kappaB nuclear translocation induced by interleukin-1 and tumor necrosis factor-alpha. Development and use of a high capacity fluorescence cytometric system. *J Biol Chem* 273: 28897-28905, 1998.
- 15 Rock KL, Gramm C, Rothstein L, Clark K, Stein R, Dick L, Hwang D and Goldberg AL: Inhibitors of the proteasome block the degradation of most cell proteins and the generation of peptides presented on MHC class I molecules. *Cell* 78: 761-771, 1994.
- 16 Larsson R, Kristensen J, Sandberg C and Nygren P: Laboratory determination of chemotherapeutic drug resistance in tumor cells from patients with leukemia, using a fluorometric microculture cytotoxicity assay (FMCA). *Int J Cancer* 50: 177-185, 1992.
- 17 Hassan SB, Jonsson E, Larsson R and Karlsson MO: Model for time dependency of cytotoxic effect of CHS 828 *in vitro* suggests two different mechanisms of action. *J Pharmacol Exp Ther* 299: 1140-1147, 2001.
- 18 Martinsson P, Liminga G, Dhar S, de la Torre M, Lukinius A, Jonsson E, Bashir Hassan S, Binderup L, Kristensen J and Larsson R: Temporal effects of the novel antitumour pyridyl cyanoguanidine (CHS 828) on human lymphoma cells. *Eur J Cancer* 37: 260-267, 2001.
- 19 Cusack JC Jr, Liu R and Baldwin AS Jr: Inducible chemoresistance to 7-ethyl-10-[4-(1-piperidino)-1-piperidino]-carbonyloxycamptothecin (CPT-11) in colorectal cancer cells and a xenograft model is overcome by inhibition of nuclear factor-kappaB activation. *Cancer Res* 60: 2323-2330, 2000.
- 20 Wang CY, Cusack JC Jr, Liu R and Baldwin AS Jr: Control of inducible chemoresistance: enhanced anti-tumor therapy through increased apoptosis by inhibition of NF-kappaB. *Nat Med* 5: 412-417, 1999.
- 21 Batra RK, Guttridge DC, Brenner DA, Dubinett SM, Baldwin AS and Boucher RC: IkappaBalpha gene transfer is cytotoxic to squamous-cell lung cancer cells and sensitizes them to tumor necrosis factor-alpha-mediated cell death. *Am J Respir Cell Mol Biol* 21: 238-245, 1999.
- 22 Feinman R, Koury J, Thames M, Barlogie B, Epstein J and Siegel DS: Role of NF-kappaB in the rescue of multiple myeloma cells from glucocorticoid-induced apoptosis by bcl-2. *Blood* 93: 3044-3052, 1999.
- 23 Ni H, Ergin M, Huang Q, Qin JZ, Amin HM, Martinez RL, Saeed S, Barton K and Alkan S: Analysis of expression of nuclear factor kappa B (NF-kappa B) in multiple myeloma: downregulation of NF-kappa B induces apoptosis. *Br J Haematol* 115: 279-286, 2001.
- 24 Martinsson P, Ekelund S, Nygren P and Larsson R: The combination of the antitumoural pyridyl cyanoguanidine CHS 828 and etoposide *in vitro* from cytotoxic synergy to complete inhibition of apoptosis. *Br J Pharmacol* 137: 568-573, 2002.
- 25 Ekelund S, Persson I, Larsson R and Nygren P: Interactions between the new cytotoxic drug CHS 828 and amiloride and mitomycin C in a human tumour cell line and in tumour cells from patients. *Chemotherapy* 48: 196-204, 2002.
- 26 Lövborg H: Cellular Pharmacology of the Novel Antitumor Cyanoguanidine CHS 828 (Thesis). Faculty of Medicine. Uppsala University. Sweden. P. 31. 2004. Available from: <http://publications.uu.se/theses/abstract.aspx?dbid=4088>.2004

Received April 13, 2006

Revised July 31, 2006

Accepted September 1, 2006