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ABSTRACT

Purpose

There is growing evidence demonstrating the role of inflammation in cancer initiation, progression and metastasis. Drugs that are able to inhibit both inflammation and cancer are of great interest for clinical use. In the present study, we demonstrate that PBI-1308, a dimeric 1,3,5-triazine derivative, inhibits both inflammation and human prostate cancer cell proliferation.

Experimental Design

Anti-cancer activity of PBI-1308 (proliferation, cell cycle and NF-κB activation) was assessed *in vitro* using the androgen-insensitive human prostate carcinoma PC-3 cells. Anti-inflammatory effect of PBI-1308 was tested in an LPS-induced inflammation in the rat air pouch model.

Results

Anti-cancer activity: PBI-1308 inhibits the proliferation of human prostate cancer cells PC-3 with an IC_{50} of 2.31×10^{-6} M at 24 hours. This inhibition is mediated by a partial G0/G1 and G2 cell cycle block. Western blot analysis shows a down regulation of cyclin D1 and D3 expression. Also, NF-κB activation was assessed in both PC-3 and Jurkat cells transiently transfected with the p-NF-κB-Luc plasmid. The results show a dose-dependent inhibition of NF-κB activation. Furthermore, PBI-1308 induces a significant reduction (T/C between 16% to 84%) of intradermal xenograft human PC-3 tumor growth.

Anti-inflammatory activity: Treatment with PBI-1308 (*iv*, 50 mg/kg) prior to LPS induction inhibits recruitment of neutrophils and accumulation of inflammatory mediators in the pouch. PGE_2 and LTB_4 are significantly inhibited after 2 hours. Inhibition of TNF-α is observed after 12 hours post-LPS induction.

Conclusion

These results suggest that PBI-1308 inhibits the activation of NF-κB and some NF-κB-regulated genes, especially cyclin D and TNF-α. In addition, PBI-1308 inhibits PGE_2 and LTB_4 production. PBI-1308 is a valuable candidate for the treatment of NF-κB-dysregulation observed in inflammation and cancer.

INTRODUCTION

There is growing evidence demonstrating the role of inflammation in cancer initiation, progression and metastasis. Nuclear factor kappaB (NF-κB), a transcription factor, plays an important role in carcinogenesis as well as in the regulation of the inflammatory response. NF-κB regulates the expression of a plethora of genes that modulate apoptosis, cell survival as well as inflammation, proliferation, differentiation, stress response and other physiological processes. Furthermore, many cancer cells show aberrant or constitutive NF-κB activation. Therefore, targeting intrinsic NF-κB activation, as well as its upstream or downstream regulators, offers a potential cancer therapy strategy. This study demonstrates that PBI-1308 is a potential candidate in pathological conditions (inflammation or cancer) where NF-κB is dysregulated.

METHODS AND RESULTS

Cell Viability Assay

Human prostate carcinoma PC-3 cells were treated with PBI-1308 at different concentrations. Cell viability was measured by the MTT method at 24 hours.

Figure 1: PBI-1308 inhibits the proliferation of human prostate carcinoma PC-3 cells with an IC_{50} of 2.31×10^{-6} M as determined by MTT assay.

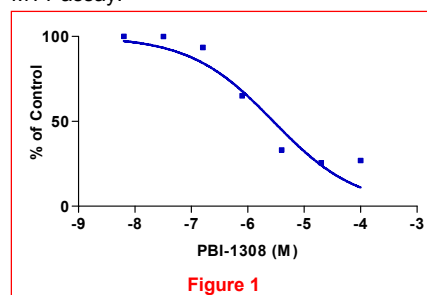


Figure 1

Xenograft Human Prostate PC-3 Tumor Model

PC-3 cells were grown in RPMI-1640 with 10% FBS. At day 0, 2×10^6 viable PC-3 cells were injected subcutaneously to produce localized tumors in male CD1 nu/nu mice (6- to 8-week old). When the tumors reached a satisfactory volume (~80 mm³), mice were randomized and then treated every other day for 3 weeks with intravenous injections of vehicle (negative control), cyclophosphamide (positive control, 100 mg/kg) or PBI-1308 (50 mg/kg). Mice were sacrificed between days 56 to 65.

Figure 7: PBI-1308 inhibits PC-3 tumor growth in CD1 nu/nu mice.

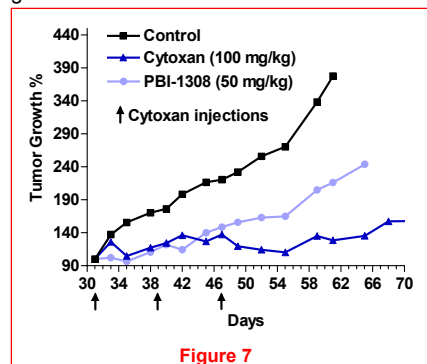


Figure 7

Cell Cycle Analysis

PC-3 cells were plated and allowed to attach overnight in complete medium. Cells were starved for 24 hours and then treated with PBI-1308 in complete medium for 24 hours. After incubation, cells were resuspended in lysis buffer (0.1% sodium citrate, 0.02 mg/ml RNase A, 0.37% NP-40, 0.05 mg/ml PI) for 30 minutes on ice. Cells were analyzed using a Coulter flow cytometer.

Figure 2: PBI-1308 induces G0/G1 and G2 cell cycle partial blocks in PC-3 cells as visualized by PI analysis determined by flow cytometry.

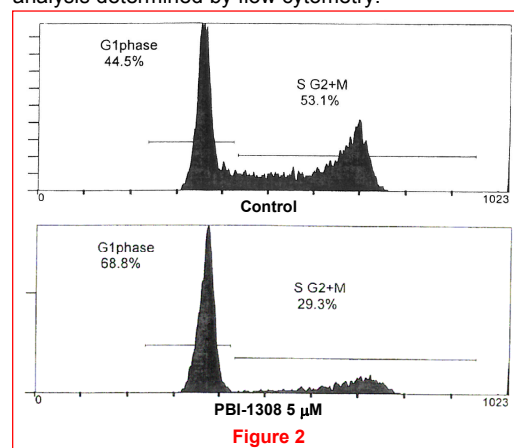


Figure 2

Dorsal Air Pouch

Air pouches were raised in the dorsum of Lewis rats by subcutaneous injection of sterile air on day 0 and 3. On day 6, 1 hour before LPS stimulation (2 μg/ml into the air pouches), indomethacin (6 mg/kg, per os) or PBI-1308 (50 mg/kg, *i.v.*) was administered to the animals. Two and 12 hours after LPS stimulation, exudates were collected and leukocytes were counted. TNF-α, PGE_2 , LTB_4 and MCP-1 levels in exudates were determined using specific ELISA kits, according to manufacturer's instructions.

Figure 8: Effect of PBI-1308 on leukocyte recruitment after LPS induction.

Figure 9: Effect of PBI-1308 on inflammatory markers in exudates after LPS induction.

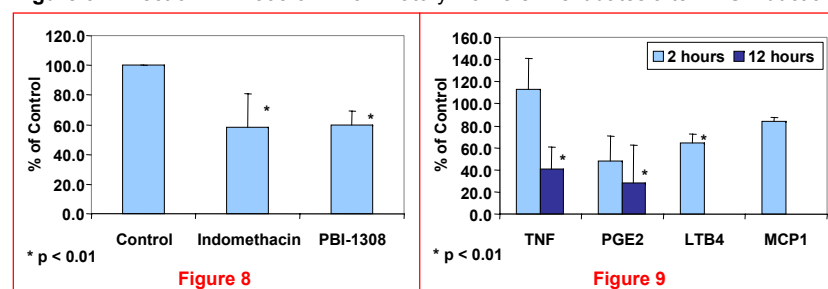


Figure 8

Western Blot Analysis of Cyclin D1 and D3

PC-3 cells were plated and allowed to attach overnight in complete medium. Cells were starved for 36 hours and treated with PBI-1308 for 18 hours in RPMI-10% FBS. Proteins were extracted from cells in ice-cold lysis buffer (Cell Signaling). 40 μg of proteins were resolved over 10% SDS-polyacrylamide gels and transferred to a nitrocellulose membrane. Anti-cyclin D1 and anti-cyclin D3 antibodies are from BD BioSciences.

Figure 3: PBI-1308 down regulates cyclin D1 expression in PC-3 cells.

Figure 4: PBI-1308 down regulates cyclin D3 expression in PC-3 cells.

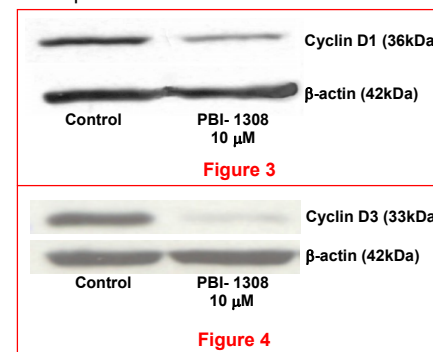


Figure 3

Figure 4

NF-κB Assay

Jurkat or PC-3 cells were transiently transfected using Lipofectamine 2000 (Invitrogen) with 1 μg of NF-κB-Luc (PathDetect Cis-Reporter Plasmid, Stratagene). After transfection, cells were cultured at 37°C overnight. Cells were plated in 96-well plates at 2×10^5 cells/well. PBI-1308 was added to cells. Induction of transcription was induced by a mixture of PMA (200 ng/ml) and PHA (4 μg/ml) in Jurkat cells. Complete medium was added to PC-3 cells. After 6 hours, 100 μl of Bright-Glo luciferase assay system (Promega) was added to each well. Luminescence was then measured. MG-132 was used as a positive control for the inhibition of NF-κB.

Figure 5: Effect of PBI-1308 on NF-κB constitutive activation in PC-3 cells.

Figure 6: Effect of PBI-1308 on PMA/PHA activated NF-κB in Jurkat cells

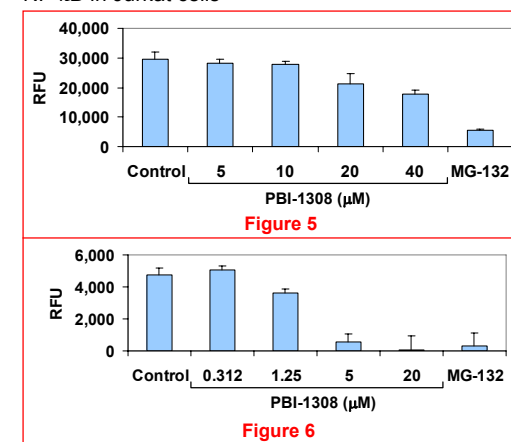


Figure 5

Figure 6

CONCLUSION

PBI-1308, a dimeric 1,3,5-triazine compound, demonstrates both anti-cancer and anti-inflammatory properties.

- Anti-cancer activity: PBI-1308 inhibits NF-κB activation as well as the expression of genes regulated by NF-κB such as cyclin D1. Increased NF-κB activity in transformed cells is linked to cell cycle progression through transcriptional activation of cyclin D1 gene.
- Anti-inflammatory activity: PBI-1308 inhibits the production of pro-inflammatory cytokine TNF-α and chemokine MCP-1, whose respective genes are regulated by NF-κB. PBI-1308 inhibits the production of PGE_2 , a product of the inducible COX-2 enzyme which is also regulated by NF-κB. In addition, PBI-1308 inhibits the production of inflammatory mediator LTB_4 which could be the result of the down regulation of other mediators.