Effect of MCP-1 inhibitors on prostate tumor growth

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The MCP-1/CCR2 chemokine axis has been identified as an important signaling pathway involved in the development and progression of many types of tumors as multiple myeloma, breast and prostate cancers. MCP-1 has a direct effect on tumor cells stimulating proliferation, survival, and migration. Additionally MCP-1 contributes to the development of a metastatic niche by stimulating angiogenesis and local monocyte/macrophage recruitment. On the other hand, neutralizing antibodies targeting both human and mouse MCP-1 inhibited the PC-3 human prostatic cell line growth, which was accompanied by a decrease in macrophage recruitment to the tumor.
Small molecules able to inhibit MCP-1 were selected from in-house library following their *in vitro* characterization for their ability to reduce MCP-1 expression and production in LPS-stimulated MonoMac6 cells (IC50 range 20-300 μM).
Aim of the present study was to explore the potential of MCP-1 inhibitors on tumor growth and on MCP-1 production in a prostate cancer model. All animal-use procedures conformed to the guidelines of the European Community's Council for Animal Experiments.
Xenograft tumors were established by subcutaneous injection of 10^6 PC-3 prostate cancer cells in BALB/c nude mice. Body weight was assessed and tumor length and width were measured by caliber. AF13 and AF16, MCP-1 inhibitors, were orally given at 100 mg/kg, while cisplatin, used as reference drug, and the CCR2 antagonist RS504393 were intraperitoneally injected at 2 and 5 mg/kg, respectively. Treatment started at day 3 following cancer cells injection. Animals were sacrificed 4 weeks following PC-3 inoculum and tumor masses were harvested for MCP-1 quantification by ELISA. In addition, histological and immunohistochemical investigations were performed on tumor tissues.
No significant weight loss was observed following AF13, AF16 and RS504393 administration, while cisplatin significantly and markedly reduced body weight starting from day 8 post-dosing.
In mice treated with AF13 and AF16, tumor volume measurements revealed a 37% and 27% reduction, respectively, in tumor growth on day 29 with respect to control tumors. In the same experimental conditions, RS504393 showed a weak but significant reduction of tumor growth only at the end of experiment (33% inhibition). As expected, cisplatin was able to block tumor growth reaching a maximum effect of 90% tumor volume inhibition by the end of experiment.
Finally, no significant differences on mouse MCP-1 (mMCP-1) levels were observed in tumors between vehicle and AFs treated animals. On the contrary, when human MCP-1 (hMCP-1) was measured, AF13 and AF16 treatment produced a marked and significant inhibition of hMCP-1 levels, similarly to RS504393 and cisplatin.
To assess if the activity observed was related to an interference with MCP-1 expression and monocyte/macrophage recruitment, xenograft tumor samples were collected for histologic analysis. H&E stained sections showed necrotic regions that resulted greater in the AF16 treated group than in vehicle treated animals. In addition, immunohistochemistry performed on tumor sections from the same animals revealed in the vehicle receiving mice macrophage accumulation and a strong MCP-1 immunoreactivity that was reduced by treatment with AF16. Macrophages constitute an extremely heterogeneous population, schematically identified as M1 (or resident, classically activated) and M2 (or alternatively activated, with pro-tumoral functions promoting tumor cell survival, proliferation and dissemination). AF16 treatment reduced M2 subpopulation when compared to vehicle tumor tissues, while the presence of M1 macrophages was rare in all tumor sections.
Our results foster the role of MCP-1 on prostate tumor growth in vivo, but further investigations are necessary to explore mechanisms of action involved in this pharmacological activity and to clarify the therapeutic potential of MCP-1 inhibitors.