IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

REVIEW ON REPORTED ACTIVITIES OF CHLORAMBUCIL AS ANTICANCER AGENT

[1]Pooja B. Jaiswal, [2]Chetan R. Mahale, [3]Siddhant S. Mukkirwar, [4]Radhakumari U. Rai, [5]Priyanka T. Bhusnar

[1]Student, [2]Student, [3]Student, [4]Student, [5]Student,

[1]Department of Pharmacology,

[1]IVM's Indrayani Institute Of Pharmaceutical Education And Research, Talegaon Dabhade, Maval, Pune, India - 410507

Abstract: Glutathione (GSH) levels and glutathione S-transferase (GST) activities were measured in the leukemia cells of 12 patients with chronic lymphocytic leukemia. Both were correlated with prior clinical exposure to alkylating agents and with DNA cross-link formation by chlorambucil in these cells in vitro. No correlation was observed between prior exposure to alkylating agents and GSH level or GST activity.

Keywords: alkylating agents, cancer, chlorambucil, glutathione, glutathione s-transferase, dna,

I. INTRODUCTION

An inverse correlation was observed between GST activity and cross-linking by chlorambucil, which was enhanced if both GST activity and GSH level were related to cross-linking. These findings suggest that the combination of GST and GSH protects the DNA of leukemia cells from chlorambucil, but the role of this combination in clinical resistance remains to be determined.[1]

The purpose of this study was to assess the toxicoses and antitumor activity of metronomic chlorambucil at a dosage of 4 mg m^{-2} daily in dogs with naturally occurring cancer. Thirty-six dogs were enrolled in the study. The protocol was well tolerated with no grade 3 or 4 toxicoses noted. Complete remission was achieved, and lasted over 35 weeks in three dogs (mast cell tumour, soft tissue sarcoma and thyroid carcinoma). Partial remission was noted in 1 dog with histiocytic sarcoma (39 weeks duration) for an overall remission rate of 11% (4 of 36). Stable disease was noted in 17 dogs (47%) with various other cancers. The median progression-free interval was 61 days, and the median survival time was 153 days. Chlorambucil given in a metronomic protocol showed antitumor activity in dogs with a variety of naturally occurring cancers.[2]

The clinical applications of nitrogen mustard antitumor drugs are limited by their poor aqueous solubility, poor cellular uptake, lack of targeting, and severe side effects. Cyclen could be protonated under physiological conditions, which may be beneficial for increasing cell membrane affinity and cellular uptake. Herein, a novel self-assembling peptide–drug conjugate was developed by conjugating chlorambucil (CRB)

and cyclen to a self-assembling peptide. The resultant supramolecular hydrogel was prepared *via* a heating–cooling process and displayed improved aqueous solubility. Rheology, CD spectra, and transmission electron microscopy measurements indicated that the hydrogel with a β-sheet configuration and a nanofiber structure had favorable rheological properties. A cellular uptake experiment demonstrated that cyclen effectively increases the uptake of the resulting hydrogel by tumor cells. MTT results indicated that the hydrogel exhibited favorable inhibitory activities against A549, HeLa, and MCF-7 cancer cell lines and was less toxic towards 3T3 (normal cells). The results of γ-H2AX experiments showed that the obtained nanomedicine could induce significantly more DNA damage compared with free chlorambucil. Hematology analysis experiments revealed that the obtained nanomedicine has good biocompatibility. Our findings indicate that the self-delivery nanodrug system has clinical potential for cancer treatment.[3]

The objective of this study was to formulate chlorambucil, a lipophilic DNA alkylating agent, in soybean oil-containing nanoemulsion formulations for improved delivery efficiency in solid tumors. The nanoemulsions were prepared using a high pressure homogenization method with a Microfluidizer® M-110EH processor. The optimized nanoemulsion formulations were investigated for cellular uptake, cytotoxicity, and apoptotic activity in SKOV3 human ovarian adenocarcinoma cells. The Microfludizer® processing conditions were optimized (12,500 psi for 30 sec) to obtain the average oil droplet size below 150 nm in diameter. The control, poly(ethylene glycol) (PEG)-modified, and cationic lipid (i.e., DOTAP)-modified nanoemulsion formulations had excellent chlorambucil encapsulation efficiency (>97%). The cytotoxicity and the pro-apoptotic activity of chlorambucil were significantly enhanced when administered in the nanoemulsion formulations relatively to aqueous solution. The results of this study show that stable oil-in-water nanoemulsion formulations can be prepared with a commercially-available Microfluidizer® processor. The nanoemulsion formulations were effective in intracellular delivery of chlorambucil, which enhanced the therapeutic effect in tumor cells.[4]

A Walker 256 rat mammary carcinoma cell line (WR) resistant to bifunctional nitrogen mustards has been shown to have an approximate twofold increase in bulk glutathione-S-transferase activity compared to the parent cell line. Substrate specificity studies suggest that higher levels of Yb subunit contribute to the increased activity. By exposing WR cells to additional chlorambucil, either as a single concentration (50 micrograms/ml) or at 5 micrograms/ml for 10 days, transferase activity was further increased by up to three times the normal WR level. By using colony-forming assays, mitotic index depression, or trypan blue exclusion, the increased transferase activity could be correlated with an increase in resistance of these cells to either subsequent chlorambucil or a different bifunctional nitrogen mustard, phosphoramide mustard.[5]

Glycosylated antitumor ether lipids (GAELs) kill cancer cells and cancer stem cells via a novel, apoptosis-independent mechanism. In contrast, chlorambucil, a drug in clinical use for the treatment of chronic lymphocytic leukemia reacts with nucleophiles within the major groove of DNA, leading to apoptosis. We hypothesized that hybrid molecules that combine apoptosis-dependent and apoptosis-independent mode of actions in a single molecule may lead to enhanced antitumor activity. Here, we describe the antitumor

activities of chlorambucil-linked glucosamine-derived glycerolipid hybrids and investigate the role of the chlorambucil moiety and the effect of cationic charge on the hybrid molecule. Three hybrids and two control GAELs were synthesized and their activities against breast (JIMT1, MDA-MB-231, BT474), pancreas (MiaPaCa2) and prostate (DU145, PC3) cancer cell lines were determined using MTS assay. Hybrid **3** displayed the most potent activity on DU145 at CC_{50} of 6.0 μM while hybrid **4** displayed the best activity on JIMT1 at 7.5 μM. Hybrid **5** exhibited no activity at the highest concentration tested (CC_{50} >20 μM), underscoring the significance of the cationic charge at *C*-2 position as previously reported. Although chlorambucil **(2)** itself showed very little activity against all the six cell lines (CC_{50} >150 μM), GAELs **6** and **7** which lack the chlorambucil moiety were consistently less active than **3** and **4**, suggesting that the chlorambucil moiety contributes to the overall activity. The hybrids were however not as active as the parent GAEL **1** against MiaPaCa2 whereas **6** restored activity comparable to **1**.[6]

Equimolar doses of chlorambucil (10 mg/kg) and the lipophilic chlorambucil derivative, chlorambuciltertiary butyl ester (13 mg/kg), were given i. v. to rats. Plasma and brain concentrations of chlorambucil and its active metabolites, 3,4-dehydrochlorambucil and phenylacetic mustard, as well as of chlorambucil-tertiary butyl ester were then determined by HPLC between 2 and 240 min after drug administration. Chlorambucil demonstrated a monophasic disappearance from plasma following its administration, with a half-life of 28 min. Significant amounts of phenylacetic mustard were detected after 15 min, and this agent maintained high levels of active compounds in plasma throughout the study. Only low concentrations of chlorambucil and phenylacetic mustard were detected in brain between 2 and 120 min. Following equimolar chlorambucil-tertiary butyl ester administration, it rapidly disappeared from plasma, with a half-life of approximately 2 min, and maintained low plateau concentrations between 15 and 120 min after treatment. It was not detected thereafter, although significant amounts of chlorambucil and phenylacetic mustard were detected throughout the study. Significant amounts of chlorambucil-tertiary butyl ester entered and remained within the brain, achieving a peak concentration at 15 min and disappearing thereafter with a half-life of 37 min. Low levels of chlorambucil and phenylacetic mustard were also detected. Calculated from the areas under the concentration vs time curves of total active compounds derived from chlorambucil and chlorambucil-tertiary butyl ester in brain and plasma, the brain: plasma concentration integral ratios were 0.018 and 0.68, respectively. Following equimolar doses of chlorambucil and chlorambucil-tertiary butyl ester, a 7-fold greater concentration integral was achieved by chlorambucil-tertiary butyl ester in brain at a 5-fold lower plasma concentration integral. Chlorambucil-tertiary butyl ester may be of value in the treatment of brain-sequestered tumors.[7]

Chemotherapeutics for the treatment of tumorigenic conditions that feature novel modes of action are highly sought after to overcome the limitations of current chemotherapies. Herein, we report the conjugation of the alkylating agent chlorambucil to the RAPTA scaffold, a well-established pharmacophore. While chlorambucil is known to alkylate DNA, the RAPTA complexes are known to coordinate to amino acid side chains of proteins. Therefore, such a molecule combines DNA and protein targeting properties in a single molecule. Several chlorambucil-tethered RAPTA derivatives were prepared and tested for their cytotoxicity,

stability in water and reactivity to protein and DNA substrates. The anticancer activity of the complexes is widely driven by the cytotoxicity of the chlorambucil moiety. However, especially in the cisplatin-resistant A2780R cells, the chlorambucil-functionalized RAPTA derivatives are in general more cytotoxic than chlorambucil and also a mixture of chlorambucil and the parent organoruthenium RAPTA compound. In a proof-of-principle experiment, the cross-linking of DNA and protein fragments by a chlorambucil–RAPTA derivative was observed.[8]

The human carcinogen and mouse germ cell mutagen chlorambucil is reported here to give a strong positive response in the mouse bone marrow micronucleus assay (4 mg/kg; single intraperitoneal injection). An increased incidence of micronucleated bone marrow white cells was also observed. It is concluded that chlorambucil is a broad spectrum mutagen that possesses subtle and specific mutagenic activities in some assay systems.[9]

At present, there is an urgent necessity for the discovery of new chemotherapeutic agents with novel molecular skeleton structures that exhibit wide spectrum antitumor activity. In this work, a cationic pentathiophene (5T) is synthesized and discovered to have both anticancer activity and molecular imaging property. 5T can selectively accumulate in mitochondria to exhibit organellar imaging and efficiently induce cell apoptosis associating with JNK pathway activation. Additionally, complexes are prepared through electrostatic interactions between 5T and sodium chlorambucil (a widely used anticancer drug) with varying molar ratios. The complexes form nanoparticles in water with the size of about 50 nm. The 5T-chlorambucil nanoparticles enhance anticancer activity by 2–9 fold due to the synergistical anticancer activity of 5T and chlorambucil. 5T is therefore a promising multifunctional anticancer agent that incorporates optical monitoring capability and anticancer activity that targets mitochondria.[10]

We have reported previously the isolation and characterization of a Chinese hamster ovary cell line, designated CHO-Chl^r, which exhibits resistance to bifunctional nitrogen mustards while maintaining sensitivity to a range of other alkylating agents and chemotherapeutic drugs. This enhanced drug resistance is associated with a greater than 40-fold increase in the level of expression of an alpha class (YcYc) glutathione *S*-transferase (GST) as compared to the parental, CHO-K1, cell line. Here, we have purified GST from CHO-Chl^r cells and show that the nonsteroidal antiinflammatory drug indomethacin acts as an inhibitor of enzyme activity. Indomethacin at 500 μM causes no significant decrease in colony forming ability of either CHO-K1 or CHO-Chl^r cells. However, the cytotoxicity of chlorambucil is potentiated 5.5-fold in CHO-Chl^r cells, but only 2.5-fold in CHO-K1 cells following preexposure to 500 μM indomethacin. In contrast, the antiinflammatory agent acetylsalicylic acid failed to inhibit the activity of purified GST and caused no potentiation of chlorambucil toxicity, suggesting that the potentiation by indomethacin is not due to the effects of this drug on prostaglandin synthesis. These studies provide further evidence that GSTs may be involved in the development of resistance to bifunctional alkylating agents and suggest that indomethacin, or agents with similar activities, may be of value as an adjunct to chemotherapy in some patients with tumors resistant to treatment with alkylating agents.[11]

Cell-surface localizing heterologous antibodies against the mouse EL4 lymphoma and a human malignant melanoma could be bound to chlorambucil without causing the loss of the alkylating activity of chlorambucil or interfering with the reactivity of the antibodies with their respective tumour cells. When given to mice preinoculated with tumour cells 2, 24, 72, and 120 hours before the beginning of treatment the chlorambucil-bound antibody was a much more effective tumour inhibitor than chlorambucil or the antibody alone. In a patient with disseminated malignant melanoma injection of the chlorambucil-bound anti-melanoma antibody first locally into a few metastatic nodules and then by the intravenous route was followed by the regression of all the metastatic nodules.[12]

REFERENCES

1. Glutathione S-Transferase Activity, Sulfhydryl Group and Glutathione Levels, and DNA CrossLiking Activity With Chlorambucil in Chronic Lymphocytic Leukemia

   James B. Johnston, Lyonel G. Isrels, Gerals J. Goldenberg, Cynthia D. Anhalt, Linda Verburg, Michael R. A. Mowat, Asher Begleiter

2. Prospective trial of metronomic chlorambucil chemotherapy in dogs with naturally occurring cancer

   T. N. Leach, M. O. Childress, S. N. Greene, A. S. Mohamed, G. E. Moore, D. R. Schrempp, S. R. Lahrman, D. W. Knapp

3. A peptide–drug hydrogel to enhance the anti-cancer activity of chlorambucil†

   Qingxiang Guo,[a] *Yifan Liu*,[b] *Ganen Mu*,[a] *Lijun Yang*,[a] *Wei Wang*,[b] *Jinjian Liu* *[a] *and Jianfeng Liu* *[ab]

4. Microfluidic Preparation of Chlorambucil Nanoemulsion Formulations and Evaluation of Cytotoxicity and Pro-Apoptotic Activity in Tumor Cells

   Ganta, Srinivas; Devalapally, Harikrishna; Baguley, Bruce C.; Garg, Sanjay; Amiji, Mansoor

5. Increased glutathione-S-transferase activity in a cell line with acquired resistance to nitrogen mustards. Wang AL,Tew KD

6. Design, synthesis and antitumor properties of glycosylated antitumor ether lipid (GAEL)-chlorambucil-hybrids

   panelTemilolu Idowu [a], Pranati Samadder [b], Gilbert Arthur [b], Frank Schweizer [a]

7. Pharmacokinetics of chlorambucil-tertiary butyl ester, a lipophilic chlorambucil derivative that achieves and maintains high concentrations in brain

   Nigel H. Greig, Eileen M. Daly, Daniel J. Sweeney & Stanley I. Rapoport

PROTEIN RUTHENATION AND DNA ALKYLATION: CHLORAMBUCIL-FUNCTIONALIZED RAPTA COMPLEXES AND THEIR ANTICANCER ACTIVITY

8. Alexey A. Nazarov *[ab], Samuel M. Meier [c], Olivier Zava [a], Yulia N. Nosova [b], Elena R. Milaeva [b], Christian G. Hartinger *[d] and Paul J. Dyson *[a]
9. Clastogenicity of chlorambucil to the mouse bone marrow: consideration in relation to its genetic specificity of action in some assays J. Ashby, H. Tinwell
10. A Multifunctional Cationic Pentathiophene: Synthesis, Organelle-Selective Imaging, and Anticancer Activity Gaomai Yang, Libing Liu, Qiong Yang, Fengting Lv, Shu Wang
11. IMMUNOCHEMOTHERAPY OF CANCER WITH CHLORAMBUCIL-CARRYING ANTIBODY *T. GHOSE, S. T. NORVELL, A. GUCLU, D. CAMERON, A. BODURTHA, A. S. MACDONALD*
12. CURRENT AND EMERGING TREATMENTS FOR CHRONIC LYMPHOCYTIC LEUKAEMIA TADEUSZ ROBAK, KRZYSZTOF JAMROZIAK & PAWEL ROBAK