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ORIGINAL ARTICLE

Preclinical evaluation of amiodarone for the treatment of murine leukemia P388. *In vivo* and *in vitro* investigation

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Summary

Purpose: *The purpose of the present study was the investigation of antileukemic effect of amiodarone in leukemia P388 BDF$_1$ bearing mice and its genotoxic and cytostatic effect in cultured normal human lymphocytes.*

Methods: *Leukemia P388 was used in this study. BDF$_1$ mice were used for chemotherapy evaluation in vivo. The antitumor activity was assessed by the oncostatic parameter T/C, representing the increase of life span of drug-treated animals vs. controls. Lymphocyte cultures were used to study the genotoxic and cytostatic effect in vitro, expressed by enhanced sister chromatid exchange (SCE) and reduced proliferation rate indices (PRI$_S$).*

Results: *Amiodarone was found to exert antileukemic potency against leukemia P388 bearing mice at all three different treatment schedules used, yielding T/C values of 155%, 163% with one cure and 230%. In the in vitro cytogenic experiments, significant increase of SCE rates by amiodarone was observed at 0.2 μM, while at the same concentration significant suppression of PRI$_S$ was achieved.*

Conclusion: *According to the National Cancer Institute (NCI), a compound is characterized as potential chemotherapeutic deserving further evaluation if it produces T/C values ≥125%. On the other hand the SCE assay has predictive value as a clinical assay for drugs exhibiting a strong correlation between cell killing and induction of SCEs. Further studies are warranted to clarify the structure-activity relationship of amiodarone.*

Key words: amiodarone, experimental chemotherapy, leukemia P388, sister chromatid exchange

Introduction

Amiodarone, considered the most effective antiarrhythmic drug, was originally developed in the 1960s as an antiaginal agent. Its cytotoxicity towards a variety of human non cancer cells such as hepatocytes, thyrocytes and endothelial cells has been demonstrated by various studies [1-3].

In the clinical therapy of cancer, resistance to many cytostatic drugs is a major cause of treatment failure. Among other mechanisms, the expression and pumping activity of p-glycoprotein in the membrane of resistant cancer cells is responsible for the reduced uptake of cytostatics. The blockage or inhibition of p-glycoprotein by chemosensitisers seems to be a tenable way to restore sensitivity to antineoplastic drugs.

Moreover, studies on cancer cells have shown that amiodarone increases the efficiency of antineoplastic drugs by restoring sensitization of tumor cells toward them, by suppressing multidrug resistance (MDR) [4,5]. It was demonstrated that amiodarone appears to activate specific intracellular death-related pathways, including possibly the bax-dependent caspase-3 activation pathway and thus induce apoptosis in human lung epithelial cells [6]. We therefore thought it might be of interest to investigate whether amiodarone on its own has also antineoplastic, antileukemic potency.

The purpose of the present study was to investigate the antileukemic effect of amiodarone in leukemia P388 BDF$_1$ bearing mice and its genotoxic and cytostatic effect in cultured normal human lymphocytes.

The potential increase in SCE frequency and the

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decrease in PRIs caused by genotoxic agents are considered as indicators of cytogenic damage (genotoxicity and cytostatic activity, respectively); therefore, SCEs appear to have an application in the clinical prediction of tumor sensitivity to potential chemotherapeutics [7]. Moreover the SCE assay has been proposed as a clinical assay with predictive value, for drugs exhibiting strong correlation between the induction of SCEs and cell killing [8].

Methods

In vivo experiments

Compound for intraperitoneal (i.p.) treatment

Stock solutions of amiodarone used in this study were prepared immediately before use. They were suspended in corn oil in the desired concentration.

Mice

BDF_1 mice weighing 20-23 g, 6-8 weeks old, were used for antitumor testing. Mice were provided by the experimental animal production laboratory of Theagenion Cancer Hospital and were kept under conditions of constant temperature and humidity in sterile cages with water and food *ad libitum*.

The experimental animal production laboratory follows the institutional and national guidelines for the care and use of laboratory animals.

Tumor

In this investigation a transplantable lymphocytic leukemia P388 has been used. This tumor line originated as a lymphocytic leukemia in 1955 in a DBA/2 female mouse after the skin was painted with 3-MCA. Leukemia P388 was maintained in ascitic form in BDF_1 mice by injection of 10^6 cells at 7-day intervals into the peritoneal cavity.

Doses

The treatment dose of 50 μg/g of body weight (b.w.) or 50 mg/kg b.w. (the lowest effective one) is derived by multiplying the concentration by 120, which approximately induces a doubling effect on SCEs. This correlation is indicated on Tables 1 and 2 and has been observed in previous studies as well [9,10].

Antitumor experiments

These were initiated on day 0 by i.p. implantation of 10^6 ascites cells of lymphocytic P388 leukemia into BDF_1 mice, according to the protocol of the NCI, USA [11].

Amiodarone was administered 24 h later either as a single i.p. injection or intermittently on days 1, 4 and 7 after tumor implantation and finally as daily injections on days 1-9 after tumor implantation. The different concentrations tested per treatment schedule used appear on Table 1. The antitumor effect was assessed from the oncostatic parameter T/C (%), which is the percentage increase in median life span of treated animals vs. the untreated controls. Six mice were used per test group and 8 in the control group. According to the NCI, a compound is considered as potential chemotherapeutic when it produces T/C values ≥ 125% [11].

Table 1. Antitumor activity of amiodarone (AM) on leukemia P388-bearing mice

	Treatment schedule	*Dosage (mg/kg)*	*MST (days)*	*T/C%*	*Cures*
Controls	–	Saline	9.5	100	0/6
AM	Day 1	160	8.3	Toxic	0/6
	Day 1	150	14.7	155	0/6
	Day 1	140	14.2	149	0/6
	Day 1	100	12.6	133	0/6
Controls	–	Saline	10.0	100	
AM	Day/ 1,4,7	100	12.2	122	0/6
	Day /1,4,7	75	16.3	163	1/6
	Day /1,4,7	50	14.6	146	0/6
	Day/ 1,4,7	40	11.0	110	0/6
Controls	–	Saline	9.8	100	
AM	Days 1-9	80	9.1	toxic	0/6
	Days 1-9	60	22.5	230	0/6
	Days 1-9	50	14.2	145	0/6
	Days 1-9	25	12.7	130	0/6

MST: mean survival time (days), T/C: mean survival time of drug-treated animals (T) vs. saline-treated controls (C)

Table 2. Enhancement of sister chromatid exchanges and delays in cell cycle kinetics in human lymphocytes induced by amiodarone

Amiodarone concentration μM	*SCE/cell Mean ± SEM (range)*	*PRI*
Control	4.71 ± 0.61 (2-7)	2.83
0.2 (0.135 μg/ml)	7.41 ± 0.70 (3-10)*	2.29**
0.4 (0.27 μg/ml)	9.40 ± 0.69 (6-14)	2.05
0.8 (0.54 μg/ml)	9.92 ± 0.74 (6-16)	1.98
2.0 (1.36 μg/ml)	10.06 ± (6-18)	1.93
4.0 (2.72 μg/ml)	10.30 ± 0.89 (7-19)	1.83
8.0 (5.44 μg/ml)	14.26 ± 1.08 (8-26)	1.65
20.0 (13.62 μg/ml)	17.23 ± 1.13 (11-28)	1.49

PRI: $(1M_1+2M_2+3M_3^+)/N$, where M1 is the number of cells in the first division, M_2 in the 2nd and M_3^+ in the 3rd and subsequent division and N is the total number of metaphases scored for each culture. The sister chromatid exchanges (SCEs) have been correlated with the corresponding PRI values (r= –0.93, t=6.31 and p <0.001). *significant increase (p <0.001) over the control by t-test; **significant decrease (p<0.001) over the control by x^2-test

In vitro SCE assay

Lymphocyte cultures were set up by adding 11 drops of heparinized whole blood from normal subjects, non smokers, to 5 ml of chromosome medium 1A (RPMI 1640, Biochrom, Berlin). For SCE demonstration 5 μg/ml 5-bromodeoxyuridine (BrdUrd) and amiodarone were added at the beginning of the culture period. Throughout, all cultures were maintained in the dark to minimize photolysis of BrdUrd and were incubated for 72 h at 37° C. Metaphases were collected during the last 2 h with colchicine at 0.3 μg/ml. Air-dried preparations were made and stained by the FPG procedure [7]. The preparations were scored for cells in their first mitosis (both chromatids staining dark), second mitosis (one chromatid of each chromosome stained dark) and third and subsequent divisions (a portion of chromosomes with both chromatids staining light).

Thirty suitably spread second division cells from each culture were scored on coded slides for SCEs. For PRIs, at least 100 cells were scored. The PRI was calculated according to the formula $PRI= (M_1+2M_2+3M_3^+)/N$, where M_1, M_2 and M_3^+ indicate the number of metaphases corresponding to first, second and third or subsequent divisions, respectively, and N is the total number of metaphases scored for each culture. For the statistical evaluation of the experimental data the x^2 test was performed for the cell kinetic comparisons. For comparing SCE mean values the Student's t-test was used.

We also calculated the correlation between PRIs and SCE frequencies. The formula for the Pearson product moment correlation coefficient r was applied. Then, a criterion for testing whether r differs significantly from zero was used whose sampling distribution is Student's t-test with n-2 degrees of freedom [12].

Results

Table 1 shows the effect of amiodarone at three different treatment schedules on the survival of leukemia P388 bearing mice. According to the first schedule, with amiodarone given on day 1 once at different doses, a T/C value of 133% appeared at 100 mg/kg b.w. At 150 mg/kg b.w. T/C value increased to at 155%. Higher doses proved lethal.

In the same Table, after applying amiodarone on days 1,4,7 after tumor implantation, a T/C value of 146% was achieved with 50 mg/kg b.w., while after applying at the same treatment schedule, the dose of 75 mg/kg b.w. increased the T/C value to 163% with one cure. Finally, with the daily treatment on days 1-9 schedule, we observed a T/C value of 130% at the dose of 25 mg/kg b.w., while with the same schedule the best T/C value was achieved (230%) at the dose of 60 mg/kg b.w.

In the cytogenetic experiments a statistically significant increase of SCE rates by amiodarone was observed at 0.2 μM (0.136 μg/ml), while at the same concentration a statistically significant suppression of PRI was achieved (Table 2). A doubling effect on SCE levels by amiodarone was observed at 0.4 μM, while the highest concentration tested of 20 μM (13.62 μg/ml) increased the SCE frequency by 4-fold the control level and suppressed the PRI value considerably ($p < 0.001$). Finally, a statistically significant correlation between the magnitude of the SCE induction and the depression of PRI ($r = -093$, $t = 6.31$ and $p < 0.001$) was observed.

Discussion

The antineoplastic effects and the cytogenetic damage observed in the present study by amiodarone (Tables 1,2) may be attributed to the accumulation of free radicals. The possibility of amiodarone's generating O_2^- derived species has been indicated by the fact that amiodarone –mediated injury to red blood cells and lymphocytes can be effectively prevented in a hypoxic environment [13]. Furthermore, the *in vitro* photolysis of amiodarone yields O_2^- derived radicals detected by spin trap studies [13]. It has been also demonstrated that hypoxia, as well as various antioxidants, protect from amiodarone-mediated weight gain of isolated lung tissue. These findings suggest that ambient O_2 concentration may represent an important determinant in the propagation in amiodarone toxicity, a finding previously described with oxidant-generating drugs such as bleomycin or nitrofurantoin [13]. These findings suggest that amiodarone may be capable of inducing antineoplastic and cytogenetic effects by generating toxic oxidants. However, several other possibilities exist: (a) phospholipid inoculation directly induces cell injury; (b) the iodines of amiodarone molecule may be critical to the mediation of cell injury *in vivo* [14]; (c) amiodarone induces modification of cell function and immunological damage [15]. According to the NCI [11] a compound is characterized as potential chemotherapeutic deserving further evaluation in clinical trials when: (a) it is effective against murine P388 leukemia and (b) when it is effective against human xenografts in mice. SCEs on the other hand, have been used in the present study as a visible evidence of possible oxidative DNA damage by amiodarone and subsequent homologous recombination, a condition expected to be more compatible with events dependent upon cell survival e.g. mutagenesis [16].

DNA strand-breaks caused by several genotoxic agents elicit repair processes, whose effects may become apparent as morphologically altered chromosomes or SCEs. SCEs have been often used as sensitive indicators of DNA damage and/or subsequent repair. Since some cancer rodent cells, like normal human cells, are proficient in DNA repair, it would be expected that the DNA repair mechanisms, in both cell types, would be disturbed in a similar manner by the same potential chemotherapeutics [9,10]. Chemically induced cytotoxicity that delays cell turnover times is clearly manifested as a change in the relative proportions of cells in the first, second and subsequent division. Studies in search for a relationship between SCE induction and other expressions of genotoxicity have shown a positive relationship between SCEs and reduced cell survival and alteration in cell cycle delays [9,10]. There are findings indicating that the effectiveness on SCEs induction by antitumor agents in rodent cells *in vitro* and *in vivo* [17] can be positively correlated with the *in vivo* tumor response to these agents and suggest that the SCE assay could be used to predict both the sensitivity of human tumor cells to chemotherapeutics and heterogeneity of drug sensitivity within individual tumors [9,17]. The SCE assay has predictive value as a clinical assay for drugs for which a strong correlation between cell killing and induction of SCEs has been established [9,17]. In the present study a strong correlation ($p<0.001$) between the magnitude of the SCE response and the depression of the PRI caused by amiodarone was observed (Table 2). Further studies are warranted to clarify the structure-biological activity relationship of amiodarone and to establish it as a promising drug for clinical trials. The correlation observed in the present study between SCE enhancement PRI depression and antileukemic activity by amiodarone appears to further substantiate the validity of the SCE assay as a possible method for predicting tumor chemoresponse [9,10,17].

Conclusion

Amiodarone increased the life span of P388 bearing mice at the three different treatment schedules tested. It also statistically enhanced SCE frequency and statistically reduced PRIs in cultured human lymphocytes *in vitro*.

References

1. Vassalo P, Trohman R. Prescribing Amiodarone, JAMA 2007; 298: 1312-1322.
2. Morales Al, Batata JD, Bruges M et al. Acute renal toxic effect of amiodarone in rats. Pharmacol Toxicol 2003; 92: 39-42.
3. Somani P, Bandyopadbycy S, Klanning JE, Gross SA. Amiodarone and desethylamiodarone-induced myelinoid inclusion bodies and toxicity in cultured rat hepatocytes. Hepatology 1990; 11: 81-92.
4. Van der Graaf W, de Vries EG, Uges DR et al. In vitro and in vivo modulation of multi-drug resistance with amiodarone. Int J Cancer 1991; 48: 616-622.
5. Chauffert B, Martin M, Hamman H et al. Amiodarone induced enhancement of doxorubicin and 4-deoxydoxorubicin cytotoxicity to rat colon cancer cells in vitro and in vivo. Cancer Res 1986; 46: 825-830.
6. Chol IS, Kim BS, Cho KS et al. Amiodarone induces apoptosis in L-132 human lung epithelial cell line. Toxicol Lett 2002; 132: 47-55.
7. Karapidaki I, Papageorgiou A, Geromichalos G et al. Cytogenetic and Antineoplastic Effects of Modified Steroidal Alkylators. Gen Test Mol Biomarkers 2010; 14: 93-97.
8. Arsenou ES, Fousteris MA, Koutsourea AI et al. The allylic 7-ketone at the steroidal skeleton is crucial for the antileukemic potency of chlorambucil's active metabolite steroidal esters. Anticancer Drugs 2004; 15: 983-990.
9. Karayianni V, Mioglou E, Iakovidou Z et al. A new approach for evaluating in vivo antileukemic activity using SCE assay. An application on three newly synthesized anti-tumor steroidal esters. Mutation Res 2003; 535: 79-86.
10. Hussein A, Mioglou E, Papageorgiou A et al. Comparison of new nitrosoureas esters with modified steroidal nucleus for cytogenic and antineoplastic activity. In Vivo 2007; 21: 389-396.
11. Goldin A, Sofina Z, Cyrkin A. Experimental evaluation of antitumor drugs in the USA and USSR and clinical correlations. Natl Cancer Inst Monogr 1980; 55: 25-26.
12. Mourelatos D, Dozi-Vassiliades J, Granitsas A. Antitumor alkylating agents act synergistically with methylxanthines on induction of SCE in human lymphocytes. Mutation Res 1982; 104: 243-247.
13. Martin W, Rosenow E. Amiodarone pulmonary toxicity. Recognition and Pathogenesis. Chest 1988; 93: 1242-1248.
14. Athanassiades P, Plassara M, Athanasiadou P et al. Amiodarone cytotoxicity on tumor cell cultures. J Chemother (Suppl) 1989; 4: 1134.
15. Wilson BD, Jaworski AJ, Donner ME et al. Amiodarone –induced pulmonary toxicity in the rat. Toxicol Appl Pharmacol 2009; 240: 327-336.
16. Bakopoulou A, Mourelatos D, Tsiftsoglou A et al. SCE, chromosomal aberrations and delays in cell cycle kinetics in human lymphocytes induced by dental composite resin eluates. Mutation Res 2008; 649: 79-90.
17. Mourelatos D, Dozi-Vassiliades J, Kotsis A et al. Enhancement of cytogenic damage and of antineoplastic effect by caffeine in Erlich ascites tumor cells treated with cyclophosphamide in vivo. Cancer Res 1988; 48: 1129-1131.