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ABSTRACT

Effect of "*All - trans*" retinoic acid in canine osteosarcoma chemotherapy

R. Ciarcia · C. Pagnini · F. Fiorito · A. Pellicane · S. Montagnaro · R. Russo · S. Florio

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Abbreviations

ATRA	"all-trans" retinoic acid
CIS	CisPlatin
DOXO	Doxorubicin
EPI	Epirubicin

Introduction

Osteosarcoma is the most common bone tumour in humans (Goorin et al., 1985) and, in veterinary medicine, especially in dogs (Harley et al., 1994). This tumor originates from undifferentiated mesenchymal cells during normal osteogenic processes. The role of ATRA in the cell differentiation of normal epithelial tissues has been well demonstrated (Lippman et al., 1987). Currently retinoids are also used in clinical practice as differentiative agents in human leukaemias (Moore, 1967). However, information on the effects of retinoids on solid tumors is limited. Telomerase, a ribonucleoproteic enzyme acting in the terminal site of the

R. Ciarcia · S. Florio
Dipartimento di Strutture, Funzioni e Tecnologie Biologiche, Università di Napoli "Federico II", Napoli, Italy

C. Pagnini · F. Fiorito · S. Montagnaro · R. Russo
Dipartimento di Patologia e Sanità animale, Università di Napoli "Federico II", Napoli, Italy

A. Pellicane
DVM, Veterinary practitioner, Trapani, Italy

R. Ciarcia (✉)
Dipartimento di Strutture, Funzioni e Tecnologie Biologiche, Università di Napoli "Federico II", via Delpino 1, 80137 Napoli, Italy
e-mail: roberto.ciarcia@unina.it

chromosome (TTAGGG)n, plays a key role in the process of "cellular immortalization" and is thus an important target for chemotherapy (Blackburn et al., 1991). Anthracyclines and platinum derivatives are wide spectrum chemotherapics, largely used in therapy for solid tumours but with severe adverse effects (Pacilio et al., 1998). In this study we evaluated the chemosensitizing activity of ATRA on D17 canine osteosarcoma, in relation to chemotherapeutic schemes based on anthracyclines and cisplatin. Specifically, we studied the activity of these chemotherapeutic agents, used alone or in association with ATRA, on telomerase activity, apoptosis and D17 cell viability.

Materials and methods

We used D17 canine osteosarcoma cell lines that were maintained in RPMI-1640 medium, supplemented with 10% fetal bovine serum (FBS), L-glutamine and antibiotics (62.5 mg/ml penicillin and 100 mg/ml streptomycin) and incubated at 37°C in a humidified atmosphere of 5% CO_2. Cytotoxicity assay: Drug Cytotoxicity was assayed by means of the MTT (3–4, 5-dimethylthiazol-2-yl-2,5.diphenyl tetrazolium bromide; Sigma) assay. Apoptosis: In order to detect oligonucleosomal DNA fragmentation, the Cell Death Detection ELISA Plus (Roche Diagnostics, Milan, Italy) was used. Telomerase activity was evaluated by Telomeric Repeat Amplification Protocol (TRAP) assay using the TeloTAGG Telomerase PCR ELISA^{PLUS} (Roche Diagnostics, Milan, Italy): This is a semiquantitative protocol that employs telomere-mimetic oligonucleotide TS (5′ AATCCGTCGAGCAGAGTT) and downstream amplification oligo CX (5′ CCCTTACCTTACCCTTACCCTAA) plays a key role in the process of "cellular immortalization". Statistical Analysis: The results are presented as mean ± SD of three independent experiments. One-way ANOVA with Turkey's post test was performed using GraphPad InStat Version 3.00 for Windows 95 (GraphPad Software, San Diego, California). An error probability with $P<0.05$ was selected as significant.

Results

The cytotoxic activity of chemotherapic drugs on D17 canine osteosarcoma cells was evaluated by means of a concentration effect curve. After 48 hours of incubation at concentrations between 0.1 and 100 μM of DOXO, EPI, CIS and ATRA, D17 cells were found to be relatively insensitive to CIS and ATRA, while DOXO and EPI at a concentration of 50 μM decreased cell viability by 43.5% (DOXO) and 49.2% (EPI). Addition of ATRA at an equimolecular concentration was able to decrease anthracycline and cisplatin induced cell viability. In fact the cell viability, respectively, shifted by a reduction of 43.5% (DOXO) to 61.1% (DOXO+ATRA) and from 49.2% (EPI) to 62.3% (EPI+ATRA) and from 10.1% (CIS) to 43.7% (CIS+ATRA). Chemotherapeutic drugs used alone showed no effect on telomerase activity while ATRA at a 0.5 μM concentration was significantly able to decrease this activity: in fact the absorbance shifted from 1.1 to 0.31. Addition of ATRA, at an equimolecular concentration, to chemotherapeutic drug-treated tumour cells decreased telomerase activity, the absorbance shifted from 1.09 (DOXO) to 0.79 (DOXO+ATRA), from 1.16 (EPI) to 0.74 (EPI+ATRA) and from 0.82 (CIS) to 0.66 (CIS+ATRA). Both chemotherapeutic drugs either used alone or in association with ATRA induced apoptosis. In fact, the percentage of apoptotic cells shifted from 12.2% (DOXO) to 32.1% (DOXO+ATRA); from 11.5% (EPI) to 24.4% (EPI+ATRA) and from 11.8% (CIS) to 34.8% (CIS+ATRA).

Discussion

Is known that Apoptosis is defined by a cascade of biochemical events leading to nuclear fragmentation and cell death. The cytotoxic effect of most chemotherapeutic agents *in vitro* and *in vivo* depends on the induction of apoptosis in susceptible tumour cells (Schmitt and Lowe, 1999). Our data demonstrated that Doxorubicin, Epirubicin and Cisplatin induce apoptosis in D17 canine osteosarcoma cells and that ATRA is probably able to chemosensitise tumor cells to drug-induced apoptosis. Results reported in the present study demonstrate down-regulation of telomerase activity in D17 treated with anthracyclyne, cisplatin and ATRA. Combination drug therapy has emerged as a leading-edge concept for the treatment of cancer and the results reported herein suggest that ATRA could have an important role in this strategy.

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