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PHARMACOLOGICAL INTERACTION WITH QUINOID ANTITUMOR DRUGS

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With increasing age, the incidence of neoplastic disease and the likelihood of receiving multiple prescriptions increases. Antineoplastic drugs generally have a narrow therapeutic index and are delivered at doses close to toxic. Thus, a slight increase of the biological activity caused by an interaction with simultaneously delivered drugs could be deleterious for the patient. This article summarizes the known pharmacological interactions with quinoid anticancer drugs of some during antitumor therapy commonly used drugs. The effect of antiemetics (chlorpromazine, dixyrazin, droperidol, metoclopramide), and antimicrobial agents (piperacillin, sulfamethoxazole, benzylpenicillin, amphotericin B), and adrenoceptor antagonists (propranolol, metoprolol, phentolamine) on epirubicin-induced fibroblast toxicity as studied by clonogenic survival and DNA-precipitation assay is described.

KEY WORDS: Epirubicin, interaction, radical.

INTRODUCTION

Drug-drug interactions can result in either augmentation or diminution of pharmacological activity, and probably also account for many drug treatment side effects. Drug interactions are increasingly common due to the flood of new and potent drugs. Simultaneously, the increased life expectancy of the population increases the number of individuals likely to receive multiple prescriptions. Age-related changes, further complicates drug dosage. Other endogenous factors that affect drug response involve genetic predisposition, disease states, and factors that influence absorption, distribution, or elimination of the drug such as hypoalbuminemia and urine pH.

Antineoplastic drugs generally have a narrow therapeutic index, and are delivered at doses close to toxic. As a consequence, a slight increase of the biological activity caused by an interaction with concomitantly delivered drugs could be deletary for the patient. Interactions between different cancer chemotherapeutics,[1] and between radiation and chemotherapeutics are rather well studied.[2] Although, in the last years the problems of tumor antineoplastic drug resistance has drawn attention to the effects of other drugs on tumor growth, considerably less interest has been drawn to interactions between anticancer drugs and other pharmaceuticals.

Bronchodilators are often indicated for patients with airway obstruction or prominent weezing. The main classes of bronchodilators, β-adrenoceptor agonists and methylxanthines, raise the level of 3′5′ cyclic AMP in e.g. mast cells and bronchial smooth muscles, thereby inhibiting mediator production and reducing muscle con-

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tractillity. As cyclic AMP is a second messenger in other cellular events, it is evident that bronchodilators might influence tumor cells and interact with cancer treatment. Indeed, interaction of cAMP on the cytotoxic effect of doxorubicin has been suggested.[3]

Caffeine alone, or combined with chlorpromazine enhances the cytotoxicity of alkylating agents in rodent transplantation tumor systems[4] and increases the cytotoxicity of mitomycin C.[5] Isoproterenol-enhanced mitomycin C uptake of ascites hepatomas increased the life span, and even cured the tumor-bearing rats. The enhanced uptake was blocked by the β-receptor antagonist propranolol.[6] The state of the respiratory system and the subsequent oxygen tension is of importance for the efficiency of radiation and some antineoplastic drugs. Hence, respiratory drugs may indirectly interact with cancer treatment.

A toxicity study on vasoactive drugs on mice showed that when doxorubicin was administered with propranolol, the life span was shortened, whereas nicardipine and dipyridamole increased animal survival. Diltiazem, nifedipine, verapamil, pindolol, disopyramide and clofibrate had no significant effects on doxorubicin-induced toxicity.[7] The heart glycoside digoxin also affected doxorubicin-induced cardiotoxicity.

The reversal of resistance to anthracyclines and vinca alkaloids by the calcium modulator verapamil originally observed in a mouse leukemia cell line[8,9] has now been extended to other animal cell lines and human tumors. Other calcium modifiers (diltiazem, nicardipine and niludipine) enhanced the cytotoxicity for doxorubicin, daunorubicin, mitomycin, actinomycin D, and mitoxanthrone in P388 cells resistant to doxorubicin.[10] The mechanism for the enhancement of calcium modifiers on antineoplastic drugs appears to be related to a blocking effect on the efflux of accumulated drug back into tissue compartments and interference with the DNA repair process.[11,12] Calcium modifier inhibition of cellular oxygen utilization may affect chemotherapy and the agents also caused an increased blood flow to tumors and an increased oxygenation in combination with hyperbaric oxygen.[13]

Dicumarol increased the enzymatic activation of mitomycin C to reactive alkylating metabolites with subsequent increase in cytotoxicity. As the enhancement of mitomycin C cytotoxicity is only seen in hypoxic conditions, it has been suggested that dicumarol and mitomycin C might be useful adjuvants in radiation therapy to increase the effect on radioresistant hypoxic cells.

The tranquilizer diazepam, a lipofolic benzodiazepine, caused enhancement of doxorubicin and mitoxanthrone cytotoxicity to human chronic leukemia cells. The mechanism is unclear but membrane interactions are plausible since anaesthethics are known to alter the fluidity of cell membranes.

The importance of iron and other metal ions for anthracycline antitumor and cardiotoxic effects are well documented. Metal chelators inhibits anthracycline toxicity to cells *in vitro* and also prevents anthracycline cardiotoxicity.[14,15] Amelioration of doxorubicin-induced cardiotoxicity in experimental animals has been noted when the animals were pretreated with selenium,[16] manganese,[17] vitamines A and E, and the calcium modifier prenylamine.[18]

Nausea and vomiting are usual phenomena in cancer treatment and antiemetics are generally used during chemotherapy regimes. Microbial infections remain a serious problem in patients undergoing treatment for different kinds of malignancies. Consequently, antineoplastic drugs are given simultaneously with antimicrobial drugs. Adrenergic antagonists are regularly used in the treatment of cardiovascular diseases. We therefore tested the effect of some commonly used antiemetics, antimicrobials, and adrenergic antagonists on the cytotoxicity of the anthracycline epirubicin *in vitro*.

MATERIALS AND METHODS

Clonogenic survival

A Chinese fibroblast cell line (V79-379A), propagated under standard tissue culture conditions was used. The nutrient medium consisted of Eagle's minimal essential medium in Earles saline (MEM), supplemented with 15% fetal calf serum. For each experiment, cells in exponential growth phase were trypsinized, monodispersed, counted, seeded into plastic Petri dishes and incubated in a CO_2 incubator at 37°C. The number of cells explanted were adjusted according to each epirubicin dose in such a way that 15 to 150 colonies could be expected to survive. In short, the cells were incubated at 37°C in Eagle's MEM without calf serum. Petri dishes were incubated with or without antiemetics, antimicrobials, or adrenoceptor antagonists. After 30 min 1.0 mg/l (final conc.) epirubicin (for antiemetics) or 0.25 mg/l (antimicrobials, or adrenoceptor antagonists) was added. In a separate set of experiments the effect of antiemetics, antimicrobials, or adrenoceptor antagonists alone was compared to untreated controls. The incubation was continued for 1 h, the dishes with the fibroblasts were rinsed twice with Eagle's MEM and then supplemented with MEM containing 15% calf serum. After incubation for 7 days, the surviving clones were fixed and stained *in situ*. The number of surviving clones was defined as the percentage of treated clones that grew into macroscopic colonies as compared to control (untreated cells).

DNA strand break assay

DNA strand breaks were measured with a DNA precipitation assay,[19] modified by the use of double-radiolabelling according to the principles outlined by Rydberg,[20] and Sandström and Johansson,[21] for detection of DNA strand breaks with improved accuracy. Briefly, cells, either labelled with ^{3}H-thymidine or ^{14}C-thymidine, were treated with antiemetics, antimicrobials, or adrenoceptor antagonists. After 30 min epirubicin was added and incubation continued for 1 h. At the end of the drug-treatment period the cells were immediately detached by trypsinization. Drug-treated, ^{3}H-labelled cells were then mixed with ^{14}C-labelled control cells and vice versa. The relative amount of precipitated DNA per sample was determined and the difference of strand breaks between drug-treated and control cells were calculated with a computer program that corrected the number of counts for spillover between the ^{3}H and ^{14}C-channels of the liquid scintillation counter (LKB 1217, Bromma, Sweden). The results were expressed as the mean of the differences of the percentage of precipitated DNA in two differently labelled samples. Thus, any damage caused by the labelling itself will cancel out.[20]

Chemicals

Epirubicin (4′-epidoxorubicin) was obtained as a kind gift from Farmitalia Carlo Erba, Milan, Italy. Eagle's minimal essential medium was from Gibco Ltd, Paisley, Scotland, UK. Fetal calf serum was obtained from Biochrom KG, West Germany. Polystyrene Petri dishes as from Costar, Cambridge, MA, USA. Methyl-^{14}C-thymidine and methyl-^{3}H-thymidine, and was from Amersham International, Amersham, Buckinghamshire, UK. Antiemetics, antimicrobialc, and adrenoceptor antagonists were supplied as purified powders. All other chemicals were of analytical grade.

Statistical analysis

Statistical significance of the differences between surviving clones or number of DNA strand breaks were tested with Wilcoxon's rank-sum test. The level of significance for rejecting the null hypothesis of zero treatment effect was taken to be $P = 0.05$.

RESULTS

Clonogenic survival

Colony survival decreased in a concentration-dependent manner compared to controls when the epirubicin concentration was increased. At an epirubicin concentration of 1.0 mg/l in the incubation medium, the surviving clones were approx. 5% as compared to controls, and at 0.25 mg/l epirubicin approx. 50% survived.

Dixyrazin (1.0 mg/l), metoclopramide (0.5 or 5.0 mg/l), chlorpromazine (0.01 or 0.1 mg/l), or droperidol (0.1 mg/l) significantly enhanced the toxicity of 1.0 mg/l epirubicin. Dixyrazin at 0.1 mg/l, or droperidol at 0.01 mg/l did not affect epirubicin toxicity (Table 1).

Piperacillin (8 mg/l), and sulphamethoxazole (25.6 mg/l) enhanced the toxicity of 0.25 mg/l epirubicin, whereas 3 mg/l amphothericin protected against epirubicin toxicity in the clonogenic survival toxicity assay. Benzylpenicillin (1 mg/l) was without effect on epirubicin toxicity (Table 2).

All tested adrenergic antagonist (propranolol, metoprolol, phentholamine – all at 0.1 mmol/l) enhanced the toxicity of 0.25 mg/l epirubicin (Table 3).

DNA strand break assay

Increasing concentrations of epirubicin decreased the percentage of labelled DNA in the assay. At 0.25 mg/l epirubicin approx. 85% of the labelled DNA precipitated.

TABLE 1

Effect of added antiemetics on the toxicity of 1.0 mg/l epirubicin on fibroblasts as compared to control using a clonogenic survival assay.

Test substance	Surviving clones (% of control) Epirubicin alone	Epirubicin + antiemetic	P-value
Dixyrazin			
(1.0 mg/l) (6)	4.5 ± 0.2	1.4 ± 0.2	< 0.004
(1.0 mg/l) (6)	4.5 ± 0.2	4.7 ± 0.3	NS
Metoclopramide			
(5.0 mg/l) (6)	6.2 ± 0.6	2.9 ± 0.2	< 0.004
(0.5 mg/l) (6)	6.2 ± 0.6	3.8 ± 0.3	< 0.02
Chlorpromazine			
(0.1 mg/l) (6)	4.4 ± 0.7	0.6 ± 0.1	< 0.01
(0.01 mg/l) (6)	4.4 ± 0.7	1.9 ± 0.2	< 0.03
Droperidol			
(0.1 mg/l) (6)	7.2 ± 1.2	2.5 ± 0.4	< 0.03
(0.01 mg/l) (6)	7.2 ± 1.2	6.1 ± 1.3	NS

TABLE 2
Effect of added antimicrobials on the toxicity of 0.25 mg/l epirubicin on fibroblasts as compared to control using a clonogenic survival assay.

Test substance	Surviving clones (% of control) Epirubicin alone	Epirubicin + antiemetic	P-value
Piperacillin (8 mg/l) (9)	53.6 ± 2.8	45.2 ± 2.1	< 0.008
Sulphamethoxazole (25.6 mg/l) (9)	60.4 ± 4.6	46.5 ± 2.7	< 0.011
Benzylpenicillin (1 mg/l) (9)	43.2 ± 1.9	40.8 ± 2.9	< NS
Amphothericin B (3 mg/l) (5)	22.6 ± 0.9	37.8 ± 2.5	< NS

DNA strand breaks induced by 0.25 mg/l epirubicin were enhanced by chlorpromazine (0.1 mg/l), but decreased by sulphamethoxazole (25.6 mg/l), amphothericin B (3 mg/l), and phentholamine (0.1 mmol/l). Induction of DNA breaks was unaffected by simultaneous incubation with propranolol (0.1 mmol/l) (Table 4).

DISCUSSION

Epirubicin inhibition of clonogenic survival of murine fibroblasts was significantly enhanced by the four antiemetics chlorpromazine, dixyrazin, droperidol and metochlorpramide, the antimicrobials piperacillin, and sulphamethoxazole, and the adrenergic antagonists propranolol, metoprolol, and phentolamine. The antifungal amphothericin B inhibited epirubicin toxicity in the assay.

Regarding the antiemetics, metochlopramide has been shown to reduce the emetic effect of cisplatinum without reducing its cytotoxic action,[22] and even to potentiate the effect of cisplatinum on squamous cell carcinoma xenografts without visible toxic effects. However, in a microtiter cytotoxic assay metochlopramide did not interfere with cisplatinum-induced cytotoxicity in murine leukemia L1210 cells.[23] Dom-

TABLE 3
Effect of added adrenergic antagonists on the toxicity of 0.25 mg/l epirubicin on fibroblasts as comparedto control using a clonogenic survival assay.

Test substance	Surviving clones (% of control) Epirubicin alone	Epirubicin + antiemetic antagonist	P-value
Propranolol (0.1 mmol/l) (9)	81.7 ± 4.7	68.8 ± 6.4	< 0.02
Metoprolol (0.1 mmol/l) (15)	46.5 ± 9.3	39.9 ± 8.9	< 0.001
Phentolamine (0.2 mmol/l) (12)	57.5 ± 1.9	34.4 ± 5.7	< 0.002

TABLE 4
The percentage of DNA precipitated after 1 h incubation of fibroblasts with 0.25 mg/l epirubicin, with or without antiemetics, antimicrobials, or adrenergic antagonists.

Test substance	DNA precipitated (%) Epirubicin alone	Epirubicin + test substance	P-value
Chlorpromazine (0.1 mg/l) (20)	83.3 ± 1.7	75.2 ± 1.3	< 0.01
Sulphamethoxazole (25.6 mg/l) (20)	79.8 ± 2.5	87.9 ± 1.6	< 0.03
Amphothericin B (3 mg/l) (30)	89.7 ± 1.1	96.7 ± 1.3	< 0.0002
Propranolol (0.1 mmol/l) (40)	82.4 ± 1.8	82.6 ± 2.2	< NS
Phentolamine (0.1 mmol/l) (20)	86.7 ± 1.6	91.7 ± 0.9	< 0.05

peridone i.v. used as an antiemetic in cisplatinum-containing chemotherapy seemed to aggravate cardiotoxicity with ensuing arrhytmias in humans.[24] The complexity of drug interactions was emphasized by the observation that bleomycin cytotoxicity, in contrast to epirubicin, was reduced by chlorpromazine (Grankvist and Henriksson, submitted). Enhanced cytotoxicity by chlorpromazine has also been seen with nitrosoureas.[25] Interaction of antiemetics with calcium homeostasis has been demonstrated. Thus, as observed for calcium modifiers, inhibition of anticancer drug efflux may be a plausible explanation for at least some of the potentiating effects of antiemetics.

The influence of antineoplastic drugs on antibacterial activity has attracted some interest. On the other hand, interactions between antimicrobial drugs and antineoplastic agents with regard to antineoplastic effect have been considerably less studied. In a case report, penicillin in combination with furosemide impaired methotrexate renal secretion and caused increased toxicity.[26] Penicillin has also been shown to inhibit accumulation of methotrexate in renal slices of rabbits.[27] Injection of penicillin to monkeys, delayed the elimination of methotrexate.[28] In a study on rabbits, therapeutic concentrations of probenecid and piperacillin decreased the elimination of methotrexate by blocking tubular secretion.[29] Decreased antitumor effect of methotrexate has been seen with kanamycin, neomycin and penicillin and is thought to be due to decreased cellular uptake of methotrexate.[30] Doxycycline and the aminoglycoside tobramycin did not interfere with the elimination of methotrexate. However, there is a report on rats in which the aminoglucoside gentamicin, which cause tubular necrosis, aggravated the toxic effects of methotrexate.[31] Furthermore, acute renal failure was observed following cisplatin and gentamicin-cephalothin therapy.[32]

Reports on the interaction of antimicrobials with anthracyclines are scarce. A possible interaction between cefotaxime and doxorubicin has been reported.[33] Amphoterizin B, an antifungal drug, potentiates the cytotoxicity of antineoplastic drugs (doxorubicin, vincristine, CCNA) in leukemia cells of mice.[34] Amphoterizin B has been suggested to potentiate the effect of doxorubicin in human neoplasia.[35] It has also been demonstrated that amphotericin B and other polyene antimicrobials caused

an increased incorporation of daunorubicin into red blood cells.[36] Thus, again, apart from effects on renal elimination of antineoplastic drugs, the only other suggested mechanism for the potentiating effects of some antimicrobial agents is a decreased cellular elimination of antitumoral drugs.

An explanation for the induction of less DNA strand breaks with sulphamethoxazole, and phentholamine in combination with epirubicin is hard to find and needs further investigation. The pharmacological agents could act as scavengers of free radicals, affect antioxidative enzymes of the target cell, or maybe induce DNA crosslinking thereby reducing the percentage of precipitated DNA and hence DNA strand breaks.

Since tumors are mainly the disease of old age, the patients often suffer from other illnesses leading to multiple drug therapy. The specific antineoplastic therapy itself is afflicted with unwanted effects that requires further medication, e.g. antiemetics. The simultaneous medication with multiple drugs may cause an unexpected enhancement or decline in efficacy of the drug in use. Since more than two drugs are often used the problem and complexicity will be even a multidrug interaction. For example, propranolol increase the plasma levels of chlorpromazine,[37] and chlorpromazine may enhance the activity of epirubicin. Pharmacological interactions will never be completely preventable during antineoplastic therapy. The possibility to detect and minimize negative drug interactions, or, on the other hand, the possibility to benefit from advantageous interactions, will demand further studies.

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