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Systemic Influence of Intravesical Chemotherapy with Verapamil

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Summary. The influence of the calcium blocker verapamil (VR) on systemic toxicity resulting from the intravesical instillation of Adriamycin (ADM) and thiotepa (THT) was assessed in mice. Eighty per cent of the animals receiving THT + VR developed a generalized alopecia. Data gathered at necroscopy failed to reveal any trauma to the major organs or the presence of a drug-induced myelosuppression. Combination of ADM and VR did not produce an enhancement of systemic toxicity, manifest as myelosuppression. The drug combination did not produce a cardiomyopathy as assessed by histologic examination. The use of VR in combination with antineoplastic agents posed no more of a threat to the animals than did the use of cytotoxin alone.

Key words: Verapamil, Thiotepa, Adriamycin, Intravesical toxicity.

Introduction

Verapamil (VR) blocks the transmembrane flux of calcium ions [3] and has been used to manage cardiac arrhythmias and angina [2, 8]. Although not cytotoxic itself, verapamil has been used recently to overcome vincristine, vinblastine [14, 15] and Adriamycin resistance [15] in P388 leukemia both in vitro and in vivo.

Chemosensitivity was also restored to a daunorubicin-resistant line of Ehrlich ascites carcinoma by verapamil [13]. The results achieved by the intravesical administration of an antineoplastic agent may be improved should the calcium blocker elicit a similar response in chemoresistant bladder cancer. In an attempt to evaluate the possible role of verapamil in the clinical management of bladder carcinoma, the toxicity resulting from its intravesical instillation in combination with thiotepa (THT) and Adriamycin (ADM) was assessed using C57BL mice.

Materials and Methods

Adult female C57BL mice were maintained on a 12 h light-dark cycle and received food and water ad lib. Animals were randomized into groups of five and received 0.5 ml of saline, drug (THT at 10^{-4} M or ADM at 10^{-5}), or drug in combination with VR (10^{-5} M) via an Intermedic PE10 catheter under pentobarbital anesthesia. Anesthesia was maintained for at least 1 h to prevent premature voiding. Treatments were repeated weekly for three weeks and the animals were sacrificed on day 30. Body weight and wet weights of the heart, lungs, liver, kidneys, spleen, bladder, and adrenal glands were determined at necroscopy. Forty microliter samples of whole blood were drawn and diluted. The number of erythrocytes (RBC) and leukocytes (WBC) per mm^3 was determined using a Coulter counter. Tissues were fixed in 10% neutral buffered formalin and processed for light microscopy.

Results

Thiotepa

All animals receiving THT (10^{-4} M), alone or in combination with VR (10^{-5} M), survived the experimental period. Of those receiving the drug combination 80% (4/5) displayed varying degress of alopecia; those animals receiving THT alone exhibited no evidence of hair loss. At sacrifice no differences in body weight or organ wet weights were found among the three groups (Table 1). This was also true when adjusted to a per gram body weight basis (Table 2). Specifically, there was no evidence of splenomegaly or adrenal hypertrophy.

Blood analysis revealed no severe myelosuppression resulting from THT alone or administered in combination with VR (Table 3). A mild leukopenia was found in animals receiving THT alone but was not evident in animals given a combination of THT and VR.

Table 1. Organ wet weights determined at sacrifice in animals treated intravesically with thiotepa (10^{-4} M) alone and in combination with verapamil (10^{-5} M) versus controls

Organ	Controls	THT	THT + VR
Body (g)	21.54 ± 0.82	20.97 ± 0.53	21.34 ± 0.41
Liver (g)	1.04 ± 0.05	1.03 ± 0.09	1.13 ± 0.06
Heart (mg)	126.28 ± 12.55	122.46 ± 42.96	133.83 ± 17.19
Lung (mg)	151.03 ± 31.24	195.48 ± 77.54	177.41 ± 16.75
Kidney (mg)	246.23 ± 5.51	240.16 ± 5.71	256.70 ± 11.99
Spleen (mg)	76.03 ± 9.18	64.37 ± 7.16	78.72 ± 7.78
Adrenal Gland (mg)	9.10 ± 1.06	10.42 ± 0.48	8.70 ± 2.25
Bladder (mg)	92.98 ± 13.96	98.91 ± 28.17	98.51 ± 32.27

Stastical analysis: No significant differences ($p > 0.05$)

Table 2. Adjusted organ weight determined at sacrifice in animals treated intravesically with thiotepa (10^{-4} M) alone and in combination with verapamil (10^{-5} M) versus controls

Organ	Controls	THT	THT + VR
Liver (g/g)	0.048 ± 0.001	0.049 ± 0.004	0.053 ± 0.003
Heart (mg/g)	5.87 ± 0.68	5.85 ± 0.63	6.50 ± 0.76
Lung (mg/g)	7.01 ± 1.41	9.25 ± 3.42	8.32 ± 0.72
Kidney (mg/g)	11.45 ± 0.43	11.45 ± 0.28	12.03 ± 0.51
Spleen (mg/g)	3.54 ± 1.08	3.07 ± 0.32	3.69 ± 0.41
Adrenal Gland (mg/g)	0.42 ± 0.04	0.50 ± 0.03	0.41 ± 0.10
Bladder (mg/g)	4.31 ± 0.61	4.74 ± 1.43	4.61 ± 1.49

Statistical analysis: No significant differences ($p > 0.05$)

Table 3. Cellular analysis of blood samples drawn at sacrifice from animals intravesically treated with thiotepa (10^{-4} M) alone and in combination with verapamil (10^{-5} M) versus controls

	RBC/mm^3	WBC/mm^3
Controls	828,000 ± 9.03	9,659 ± 0.97
THT	870,887 ± 12.62	7,865 ± 0.34
THT + VR	851,800 ± 9.60	9,814 ± 0.24

Note: Standard deviations x 10^4
Statical analysis: No significant differences ($p > 0.05$)

Adriamycin

All animals receiving ADM (10^{-5} M) alone or in combination with VR (10^{-5} M) survived the experimental period. No animal receiving either ADM or ADM in combination with VR developed any notable alopecia. At autopsy neither drug-treated group displayed any weight loss as compared to controls (Table 4), nor was there significant alteration in organ wet weights. Animals receiving ADM plus VR did yield a lower kidney weight per gram body weight, but this difference was not significant (Table 5). Although a slight

Table 4. Organ wet weights determined at sacrifice in animals treated intravesically with Adriamyicin (10^{-5} M) alone and in combination with verapamil (10^{-5} M) versus controls

Organ	Controls	ADM	ADM + VR
Body (g)	20.66 ± 1.15	21.74 ± 1.57	21.77 ± 1.15
Liver (g)	1.31 ± 0.30	1.19 ± 0.15	1.15 ± 0.32
Heart (mg)	169.39 ± 41.73	178.18 ± 56.51	156.23 ± 35.38
Lung (mg)	197.88 ± 75.56	181.97 ± 49.64	193.80 ± 54.49
Kidney (mg)	273.44 ± 42.10	273.76 ± 57.06	212.55 ± 48.92
Spleen (mg)	76.02 ± 9.17	71.94 ± 3.64	61.77 ± 8.57
Adrenal Gland (mg)	9.96 ± 2.63	8.39 ± 2.77	10.10 ± 2.22
Bladder (mg)	79.41 ± 23.02	62.57 ± 27.61	80.33 ± 14.89

Statistical analysis: No significant differences ($p > 0.05$)

Table 5. Adjusted organ weights determined at sacrifice in animals treated intravesically with Adriamycin (10^{-5} M) alone and in combination with verapamil (10^{-5} M) versus controls

Organ	Controls	ADM	ADM + VR
Liver (g/g)	0.059 ± 0.012	0.054 ± 0.003	0.053 ± 0.005
Heart (mg/g)	7.49 ± 1.84	8.13 ± 2.34	7.20 ± 1.69
Lung (mg/g)	8.76 ± 3.25	8.37 ± 2.15	8.94 ± 2.63
Kidney (mg/g)	12.05 ± 1.60	12.59 ± 2.36	9.83 ± 2.47
Spleen (mg/g)	3.53 ± 0.38	3.57 ± 0.16	2.93 ± 0.43
Adrenal Gland (mg/g)	0.42 ± 0.10	0.39 ± 0.12	0.46 ± 0.09
Bladder (mg/g)	3.66 ± 1.11	3.15 ± 1.46	3.81 ± 0.72

Statistical analysis: No significant differences ($p > 0.05$)

Table 6. Cellular analysis of blood samples drawn at sacrifice from animals treated intravesically with Adriamycin (10^{-5} M) alone and in combination with verapamil (10^{-5} M) versus controls

	RBC/mm^3	WBC/mm^3
Controls	750,544 ± 16.31	14,493 ± 1.31
ADM	759,700 ± 19.37	19,561 ± 1.63
ADM + VR	828,057 ± 20.19	14,473 ± 1.38

Note: Standard deviation x 10^4
Statistical analysis: No significant differences ($p > 0.05$)

decrease in cardiac mass seemed to occur after combination therapy, this was eliminated when adjusted for body size.

The intravesical administration of ADM, with or without VR, failed to have an impact on hematopoesis (Table 6). A slight elevation in the number of erythrocytes per mm^3 was present after combination therapy while the number of leukocytes per mm^3 was somewhat elevated after administration of ADM alone. Generally, however, there was no evidence of a drug-induced myelosuppression.

Histologic examination of hearts removed from animals treated with ADM, either alone or in combination with VR, revealed no evidence of drug-induced cardiac toxicity.

Discussion

We have found that verapamil, a calcium influx blocker, enhances the in vitro efficacy of thiotepa and Adriamycin against bladder cancer without being cytotoxic or antimitotic itself [12]. This study was designed to assess the possible systemic influence of verapamil on intravesical chemotherapy. Verapamil, a potent vasodilator, may increase blood flow within the bladder wall, thereby enhancing the uptake of drug from the lumen.

The permeability barrier established by the urothelium is most effective against compounds of a molecular weight greater than 200 daltons [7]. Any potentiation of systemic toxicity from the use of verapamil should, therefore, be apparent when administered in combination with antineoplastic compounds of low molecular weight. Thiotepa crosses the urothelium and enters the circulation [11]. The resultant myelosuppression may be life threatening [1, 2]. Simultaneous administration of thiotepa and verapamil, on a schedule mimicking that used clinically, produced alopecia in a high percentage of the animals. Data gathered at autopsy, however, revealed no other complications resulting from drug treatment. There was no alteration in either raw wet weights or relative mass of either livers or spleens of drug treated animals as compared to controls. Thrombocytopenia, a common manifestation of thiotepa toxicity [2], did not occur. Although thiotepa alone did produce a mild leukopenia this was not evidenced following combination therapy. The absence of adrenal hypertrophy also indicated that drug treated animals were not stressed any more than those receiving sham instillations.

Due to the size of the Adriamycin molecule little of the drug crosses the urothelium [11]. The presence of Adriamycin in the systemic circulation would produce myelosuppression [16, 17] and a diffuse cardiomyopathy [5, 6]. Samples collected at sacrifice failed to reveal any form of myelosuppression among either the drug treated group when compared to controls, nor was there any significant alteration in the wet weight or relative mass of either liver, spleen or adrenal glands. Heart size remained constant among all three groups suggesting the absence of a drug-induced cardiomyopathy. Histologic examination did not reveal the characteristic vacuolization and loss of contractile elements [10] in animals treated with Adriamycin, alone or in combination with verapamil. Adriamycin-induced renal toxicity has been demonstrated in some experimental animals [9]. Although there was a decrease in kidney mass in animals receiving Adriamycin plus verapamil, the discrepancy was not significant. Data presented here indicate that verapamil combination therapy was of no greater threat to the animals than the administration of the cytotoxic agent alone.

The vasodilator verapamil did not potentiate the systemic influence of the antineoplastic agents Adriamycin and thiotepa when administered intravesically. This calcium blocker, when combined with chemotherapeutic agents may provide a means for the elimination of chemoresistance without posing a greater risk to the bladder cancer patient.

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