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INTRAVESICAL ADMINISTRATION OF MISONIDAZOLE IN BLADDER CARCINOMA

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The value of external irradiation of carcinoma of the bladder has been much discussed for several years. The obvious advantage of radiation therapy is that it offers a potentially curable treatment modality while maintaining the possibility of preserving the bladder function. Nevertheless, critical analysis of the published data reveals that the results are not encouraging. For superficial tumours, the results are not superior to those of transurethral resection (FINNEY 1971, MILLER & JOHNSON 1973, RIDER & EVANS 1976). In addition, the technique is ineffective in the case of carcinoma in situ and does not prevent new tumour formation. As the tumour stage increases the rate of success diminishes. The local failure rate following radiation therapy of infiltrating tumours is in the range of 45 to 50 per cent (CRIGLER et coll. 1966, MILLER 1977). The 5-year survival rate does not exceed 25 per cent (EDSMYR et coll. 1971, BIRKHEAD et coll. 1976, FISH & FAYOS 1976).

Tumour cell hypoxia may play a significant role accounting for the poor response to radiation of these tumours. ICHIJO et coll. (1978) found capillary loops in the periphery of papillomatous tumours and in the base of ulcerative papillomatous tumours but not in non-papillomatous tumours. Accordingly, the non-papillomatous solid tumours are expected to have a large amount of hypoxic cell populations. SALK & PROUT (1980) found in a series of patients given radiation therapy that papillary carcinomas were more sensitive to radiation than solid tumours. Following external irradiation, stage reduction evidenced by the absence of residual tumour was observed in 42 per cent of papillary tumours compared with 30 per cent of solid ones. The capillary vascularity of carcinoma of the bilharzial bladder was also examined by OMAR et coll. (1975). The inter-capillary distance was found to be more than 300 μm, indicating a significant degree of vascular inadequacy.

Several methods to overcome the problem of tumour cell hypoxia have been investigated in clinical material. The use of hypoxic cell radiation sensitizers is certainly an attractive approach. Among the currently available nitroimidazole compounds, misonidazole has shown relatively good pharmacodynamic properties. The use of these compounds to promote radiation responsiveness has been the subject of several experimental and clinical trials (GRAY et coll. 1976, URTASUN et coll. 1977, DISCHE & SAUNDERS 1978, THOMLINSON et coll. 1979).

FOSTER et coll. (1975) provided evidence that a peak serum concentration of at least 100 μg/ml must be achieved in man if detectable sensitization is to be anticipated. However, systemic administration of the drug is often associated with serious complications. The neurotoxic side effects of misonidazole have been firmly established. It was demonstrated that neurotixicity usually develops if a total dose exceeding 12 g was administered. The severity and irreversibility of this complication increased significantly with higher doses. It is now clear that it is not

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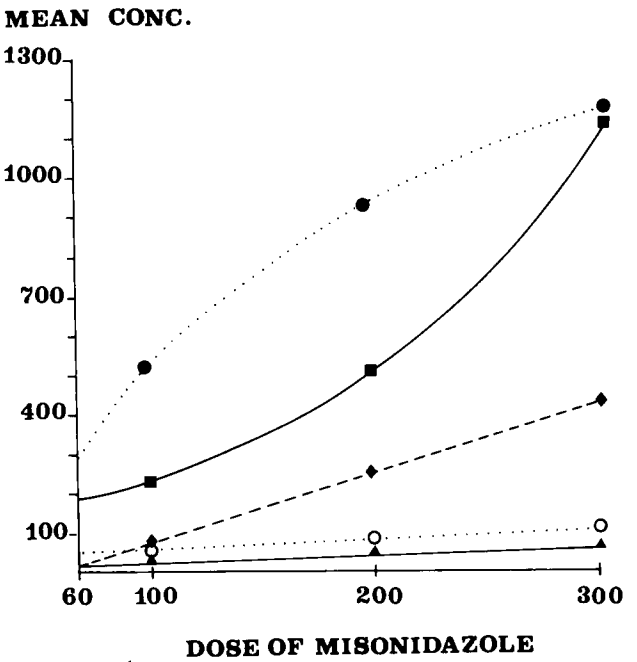
possible to use misonidazole with fractionated irradiation in sufficient doses to obtain a significant degree of tumour sensitization (ADAMS 1981, KIM & CHU 1981). The only possible alternative for the use of systemic misonidazole in promoting radiation responsiveness is to deliver high doses of irradiation in a small number of fractions (DENEKAMP et coll. 1980). In this case the drug could be given before each irradiation in a relatively high concentration that could lead to useful therapeutic enhancement, while the total cumulative dose does not exceed the safety margin that would induce neurotoxicity. Obviously, with such planning, the potential benefits of protracted fractionation are denied.

Taking all these factors into consideration, the possible use of the intravesical route for administration of misonidazole was investigated. The objective was to determine whether significant concentrations of the drug could be achieved in the tumour following intravesical administration without the potential serious toxic side effects of systemic administration.



Regression relation between increasing concentration (μg/g) of intravesical misonidazole (mmol/l) and various tissue concentrations. Normal (■), superficial (●), basal (◆), perivesical (○), lymph nodes (▲).

Material and Methods

^{14}C labelled misonidazole. (1-(2-^{14}C) nitro-1-imidazolyl-3-methoxy-2-propanol) was obtained from The Radiochemical Centre (Amersham, England). It was prepared from (^{14}C) thiourea by the method described by VARGHESE et coll. (1976). The specific activity of the labelled substance was 140.6 kBq/mg (3.8 μCi/mg; 760 μCi/mmol/l). The purity of the product as tested by thin layer chromatography was 98 per cent. The infrared spectrum of the synthetic sample was identical to that of authentic misonidazole.

Other chemicals used. Hyamine hydroxide (Sigma), Polysorbate 80 (Tween 80, Sigma), Methylbenzene (Toluene, Sigma), 2,5-diphenyl oxazole (P.P.O., Sigma), P-bis-2-(5-phenyl oxazolyl)-benzene (P.O.P.O.P., New England Nuclear).

Preparation of solutions for intravesical installation. In 1 ml Tween 80 11.76 g of cold misonidazole were emulsified and then dissolved with distilled water to 200 ml. Of the labelled material 240 mg were added and the solution well mixed. A solution containing 300 mmol/l of misonidazole and 1.2 mg/ml labelled material (4.56 μCi/ml) was thus obtained. Through serial dilution of this solution, three further concentrations were prepared, containing 200, 100 and 60 mmol/l, respectively.

Clinical experiment. Since patients with bladder tumours cannot tolerate large volumes of fluid in their bladders, nor retain it for prolonged periods, dose response curves were determined utilizing increasing concentration gradients of misonidazole only. The time of contact and the volume were kept constant as far as possible. The clinical experiment was carried out in 19 patients for whom radical cystectomy was indicated. A concentration of 60 mmol/l was instilled intravesically in 4 patients, 100 mmol/l in 4 patients, 100 mmol/l in 6 patients, 200 mmol/l in 5 patients and 300 mmol/l in 4 patients.

Immediately after anaesthesia, a catheter was introduced into the bladder. Irrigation with a solution of saline was used to wash out any debris. Twenty ml of the assigned solution were injected and the catheter was clamped. Ten ml of the given solution were saved for determination of the specific gravity. The patient was immediately explored, and if the tumour was operable, the ureters were ligated to prevent further dilution of the drug in the bladder. Following the removal of the surgical specimen (usually within 2 h), the bladder was emptied of the solution. A heparinized blood sample was also collected and saved. About 1 g pieces were cut from the following sites: superficial part of the tumour, deep part of the tumour, perivesical pelvic cellular tissue and fat, a representative lymph node from the

Table

Serum concentrations of misonidazole following intravesical administration

Concentration of solution (mmol/l)	Mean serum concentration (μg/ml)	Standard deviation
100	0.8	–
200	12.25	±2.093
300	27.60	±2.449

iliac group, and apparently normal bladder mucosa at a distance from the tumour. These pieces were preserved in accurately weighed glass tubes, tightly sealed and labelled.

Determination of misonidazole concentration in tissues. The pieces of tissues obtained were fragmented with sharp scissors, the fragments were accurately weighed and 9 ml ethanol were added for each gram of tissue. The samples were stored for at least 48 h with occasional shaking. They were then centrifuged at 2500 rpm for 15 min and 2 ml of the supernatant solution was transferred into a counting vial. The ethanol was evaporated to dryness using a hot plate and a stream of nitrogen gas. One ml hyamine hydroxide was added to the residue and the samples were incubated in a water bath (50°C) until complete solution. One ml methanol and 14 ml phosphor liquid (4.5 P.P.O.+50 mg P.O.P.O.P./1 toluene) were added and the activity (x) was counted. This was followed by addition of a known count of the standard radioactive solution to each vial (s) and the activity was recounted ($\bar{x}$). The activity of each gram of tissue sample was calculated according to this equation: $\text{cpm/g}=5\ (x \cdot s)/(\bar{x}-x)$.

The specific activity (SA) of the original solution (cpm/μg) was determined.

The tissue concentration of misonidazole was calculated according to the following equation: $\mu\text{g/g}=(\text{cpm/g})/(SA)$.

Statistical analysis. The data were entered into a Hewlett Packard desk top computer model 9845 B. The mean concentration in a given tissue was correlated with the various concentrations of misonidazole instilled intravesically. Regression analysis was carried out by 3 models: linear, logarithmic and exponential. The f-ratio was determined for each model to ascertain the best fit. The f-ratio was defined as regression mean square/residual mean square.

Results

The mean content of the drug in various parts of the cystectomy specimen was correlated with the concentration of misonidazole in the bladder. The regression analysis of this correlation appears in the Figure. For each given tissue, the regression curve with the best f value was chosen. It was found that (a) In the superficial parts of the tumour substantial amounts were obtainable at low concentrations of the drug. These concentrations were even higher than those detected in the normal mucosal lining. (b) In the deeper parts of the tumour significant values were obtainable only with high concentrations of the drug (200 mmol/l and more). (c) In the perivesical tissues and draining lymph nodes, only modest amounts were detected even with high drug concentrations.

The corresponding serum values of misonidazole were determined and are given in the Table. It may be noted that with even the highest drug concentrations (300 mmol/l), the serum levels were only minimal (30 μg/ml).

Discussion

Infiltrating bladder carcinoma would appear to have many advantages for testing the efficiency of misonidazole (GRAY 1978). Misonidazole had a broad spectrum of activity in different experimental bladder tumours including the FANFT-induced transitional cell carcinoma of the bladder in mice (HAMPEL & PERSKY 1981).

Sensitization to radiation is a function of misonidazole concentration (ASQUITH et coll. 1974). It was concluded by FOSTER et coll., that a peak serum concentration of at least 100 μg/ml would have to be achieved in man if detecable sensitization is to be anticipated.

The distribution of misonidazole in bladder carcinoma after a 1 g oral dose was reported by ASH et coll. (1979). With this dose, the serum level 4 h after administration of the drug was 27 μg/ml. The concentration of the drug in the tumour was 45 per cent, in the normal mucosa and muscle 43, and in the perivesical tissue 15 per cent of that of the serum level. Thus, following such a dose, a significant degree of sensitization cannot be anticipated. The tissue distribution of the drug 3.5 h after an oral dose of 3 g/m^2 was analyzed by AWWAD et coll. (1970). A fairly uniform distribution within the bladder tumour could be obtained. In 3 patients, the

drug concentration in the tumour was 58 to 95 per cent of the serum level (84–115 μg/ml). It was also noted that the pelvic lymph nodes had a somewhat higher concentration than the tumour and serum (117–130%). While such a dose can produce adequate tissue concentrations, it is so high that neurotoxicity may be expected if more than 4 treatments are utilized.

Critical analysis of the present results reveals that significant concentrations of misonidazole (more than 100 μg/g) are obtainable in the superficial and basal parts of the tumour with a concentration of 200 mmol/l. On the other hand, only modest amounts were detected in the perivesical tissues and draining lymph nodes even when higher drug concentrations were used. These concentrations were lower than those obtained by AWWAD et coll., when an oral dose of 3 g/m^2 was given.

At this point, it may be postulated that intravesical instillation of misonidazole may be tried in the following situations:

(1) Curative irradiation of superficial bladder tumours (T1, T2) particularly for those with a tendency for multiplicity, or frequent and repeated recurrences. Adjuvant intravesical instillation of misonidazole before external irradiation can enhance the radiation responsiveness with subsequent improvement in the end result of treatment.

(2) Treatment of carcinoma in situ (Tis), which currently presents a serious challenge from the therapeutic as well as prognostic points of view. Its superficial position will ensure that high concentrations of the drug will be readily available and a significant enhancement ratio may be anticipated if misonidazole is administered intravesically as an adjuvant to external irradiation.

(3) Intravesical and oral misonidazole in combination may be tried as an adjuvant to curative irradiation of infiltrating bladder tumours. The intravesical administration will lead to therapeutic enhancement in the superficial and basal parts of the tumour while oral therapy can lead, as well, to promotion of the radiation responsiveness in the perivesical tissue and draining lymph nodes.

SUMMARY

Using ^{11}C labelled material, the diffusion of the hypoxic cell sensitizer misonidazole was tested in 19 patients with carcinoma of the bladder following its intravesical administration. Increasing concentration gradients were tested. Evidence was provided that a 20 ml solution containing 200 mmol/l of misonidazole is followed by high concentrations of the drug in the superficial as well as in deep parts of the tumour. The corresponding serum concentration was extremely low. It was concluded that this route of administration provides high local concentrations of the drug that may result in a significant therapeutic enhancement if utilized as an adjuvant to external irradiation.

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