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PLASMA AMYLASE ACTIVITY AS A BIOCHEMICAL INDICATOR OF RADIATION INJURY TO SALIVARY GLANDS

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Abstract

Irradiation of the salivary glands produces a rapid increase of salivary amylase in serum, released by the highly radiation sensitive serous cells of the glands. Serial assays of salivary amylase in serum were performed in patients treated by radiation to the upper neck region. The changes observed were compared with the amount of salivary gland mass irradiated and with the dose fractionation modality used. The irradiated volume included either the entire salivary gland mass or less than 50 per cent of the gland. Two fractionation modalities were used: a conventional fractionation of 2 Gy per day, 5 times a week, or a multiple daily fractionation of 2 Gy, 3 times a day in two series of 4 days with a 4-day interval. Both parameters (salivary gland mass irradiated and fractionation modality used) significantly influenced the shape of the amylase curve in the serum. Serum amylase may therefore be considered a reliable biologic indicator of early injury to the salivary glands.

The main factor limiting the tumor dose in radiation therapy is the tolerance of normal tissues. The aim of the different treatment modalities attempted in irradiation of tumors has in fact been to achieve maximum tumor cell killing with minimum damage of normal tissues.

The comparison of different irradiation schedules from this aspect has usually been based on the severity of the acute and late effects in normal tissues in relation to the therapeutic results obtained. Clinically observable conditions, such as desquamation, mucositis, fibrosis, and necrosis, have been commonly used as parameters of radiation damage. This evaluation is however highly subjective, needs a long period of observation and allows only a rough quantification of the degree of damage.

In the past few years, a research program on biochemical indicators able to give early quantitative information on radiation damage of normal tissues has been developed at the radiation biology laboratory of this Institute. In order to have reliable application in clinical use, a biochemical indicator of radiation damage should present a significant quantitative correlation with the radiation dose, with the fractionation modality, and with the dimensions of the irradiated volume; a specific correlation of the different indicators with specific tissues is also desirable.

Changes in the serum salivary amylase following irradiation of the salivary glands in man have been observed by several authors (3, 6, 11). A rapid increase in the salivary amylase within a few hours after irradiation, and a return to normal levels in the subsequent days, has been demonstrated after a single dose and during fractionated irradiation. The release of zymogen grains stored in the serous salivary cells, due to variation of cellular permeability or to cellular lysis, has been reported to be responsible for the described phenomenon. The salivary origin of the increased enzyme in the serum has been demonstrated by electrophoretic separation (9, 12). The release of intracellular amylase seems to be a specific response of the salivary gland tissue to

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irradiation, as no variant of the enzyme was observed in the serum after irradiation of other tissues able to produce amylase (5, 7, 8). This phenomenon also appears to be specific to man, and has not been observed in animals after total body irradiation or localized high dose irradiation of the parotid glands (1, 2).

The value of serum amylase for quantitative assessment of early radiation damage has been studied in the present paper. The behaviour of this enzyme in the serum was explored in patients receiving therapeutic irradiation of the upper neck region, with varying dose fractionation and with fields including different portions of the salivary glands.

The main chemical components of the salivary secretion were also analysed during an initial phase of the investigation. An increase of amylase, maltase, and LAP was observed and also a significant decrease of the pH value. The quantitative correlation of these variables to the degree of radiation damage was, however, unreliable. The collection of comparable samples of saliva was very difficult due to mucositis, reduction of salivary flow, and increased viscosity of the saliva following the predominant damage of the serous secretion. The present investigation was therefore limited to the serum level of salivary amylase.

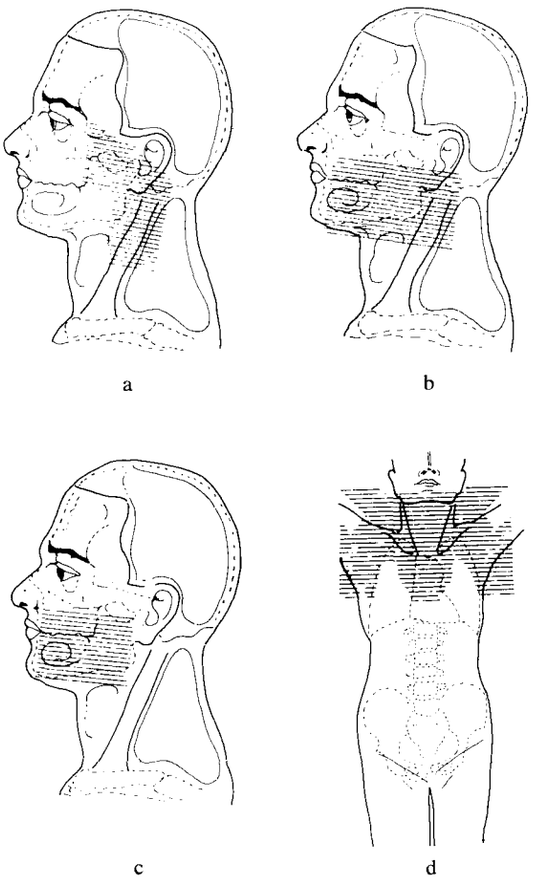

Fig. 1. Treatment fields. In a) and b) 100 per cent and in c) and d) less than 50 per cent of the salivary glands were included in the irradiated volume.

Materials and Methods

Repeated serum salivary amylase assays were performed in 50 patients (33 males, 17 females) before and during radiation therapy of regions which included the salivary glands (Fig. 1). The age of the patients ranged between 12 and 81 years (mean age 52 years). The neoplastic condition affecting these patients was lymphoma in 15 and epithelial tumor of the ear, nose and throat (ENT) region in the remaining 35.

Two different fractionation modalities were used. Forty patients received their treatment with a conventional fractionation schedule (CF): 2.0 Gy per fraction, 5 fractions per week. This group included 15 lymphomas and 25 ENT tumors. A second group of 10 patients, all with ENT tumors, were treated with an unconventional fractionation regimen (MDF) consisting of 3 daily fractions of 2.0 Gy, separated by an interval of 2 to 3 hours, and given in two series on 4 consecutive days with 4 days of rest in between.

In patients with lymphoma, irradiation to the supradiaphragmatic nodes was given through an anterior field (mantle). The upper limit of this field usually included less than 50 per cent of the mass of the parotid and submaxillary glands. The given dose along the central axis (usually located 3 cm below the jugular notch) of the anterior field was estimated. The absorbed dose in the parotid glands was estimated to be about 18 Gy, for a total dose of 36 to 40 Gy at the target. Patients with tumors of the ENT region were mainly treated through two lateral opposed fields, including the entire volume of the parotid glands and a large portion of the submaxillary glands. The dose given was in these patients calculated at the mid-point between the centers of the fields. The total dose was 60 to 70 Gy with the CF regimen and 48 to 52 Gy with the MDF regimen.

Small variations of the treatment were made in some patients and will be taken into account in the discussion of results. In 4 patients with ENT tumors

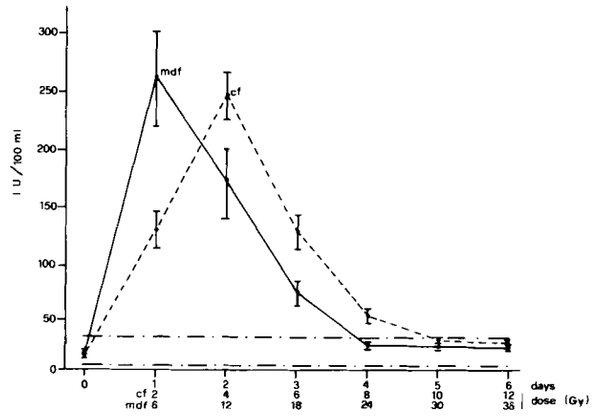

Fig. 2. Mean changes of plasma amylase level during the first week of irradiation according to fractionation modality in patients treated against the entire salivary gland mass. The 'time' on the abscissa means days of effective treatment. The peak value and the return to normal level occurred earlier in patients treated with hyperfractionation (MDF) than with conventional fractionation (CF).

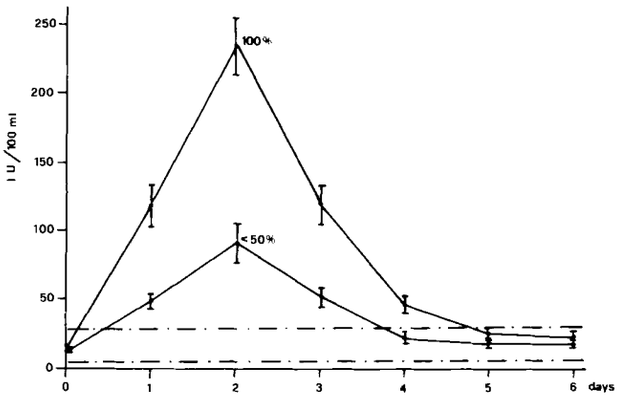

Fig. 3. Mean changes of the plasma amylase level during the first irradiation week according to the amount of salivary gland mass irradiated in patients treated with conventional fractionation. The peak level was higher when 100 per cent of the salivary gland mass was irradiated.

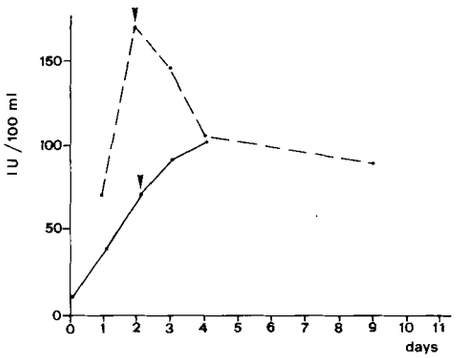

Fig. 4. Individual changes of plasma amylase level in 2 patients treated with conventional fractionation and in whom the irradiated volume was enlarged to include the entire salivary gland mass after a dose of 4 Gy.

treated with conventional fractionation, the treatment was briefly interrupted after the first few days because of problems not related to the condition of the patients. In 2 patients with lymphoma, the irradiated volume was changed after the first few fractions from the standard mantle field to include the Waldeyer ring and consequently the entire salivary gland mass.

The salivary amylase level in the serum was measured by the STREET & CLOSE method (10) and expressed in international units (4). The normal range was determined in a control group of 35 patients affected by different conditions not impairing the salivary function; the minimum and maximum values were 5.4 and 30.8 IU per 100 ml, with a mean ± SEM of 17.73±1.66 IU/100 ml.

Samples were taken daily during the first week and weekly thereafter, up to the end of the treatment course.

To assess the specificity of the serum amylase level as an indicator of the salivary damage, a number of determinations were also carried out in 10 patients with gynecologic tumors irradiated to the abdominopelvic region. In these patients the mean value of the serum amylase level was 14.4±1.51 IU/100 ml before treatment; during the irradiation a slight increase of the amylase level occurred on the second treatment day with a maximum mean value of 21.9±2.41, which was well within the normal range.

The statistical significance of the observed changes in the irradiated patients was determined with Student's t-test and the Wilcoxon rank sum test.

Results

All the patients included in the investigation had initial values within the normal range.

The general type of the curve describing the changes in serum amylase during the irradiation was similar, irrespective of the fractionation regimen and the amount of the salivary gland mass irradiated. In all patients a sharp rise of the serum amylase level was observed soon after the first irradiation day. After a peak, the amylase level rapidly decreased to the normal range within the fifth day of treatment. No further elevation was observed thereafter, although the radiation treatment was continued.

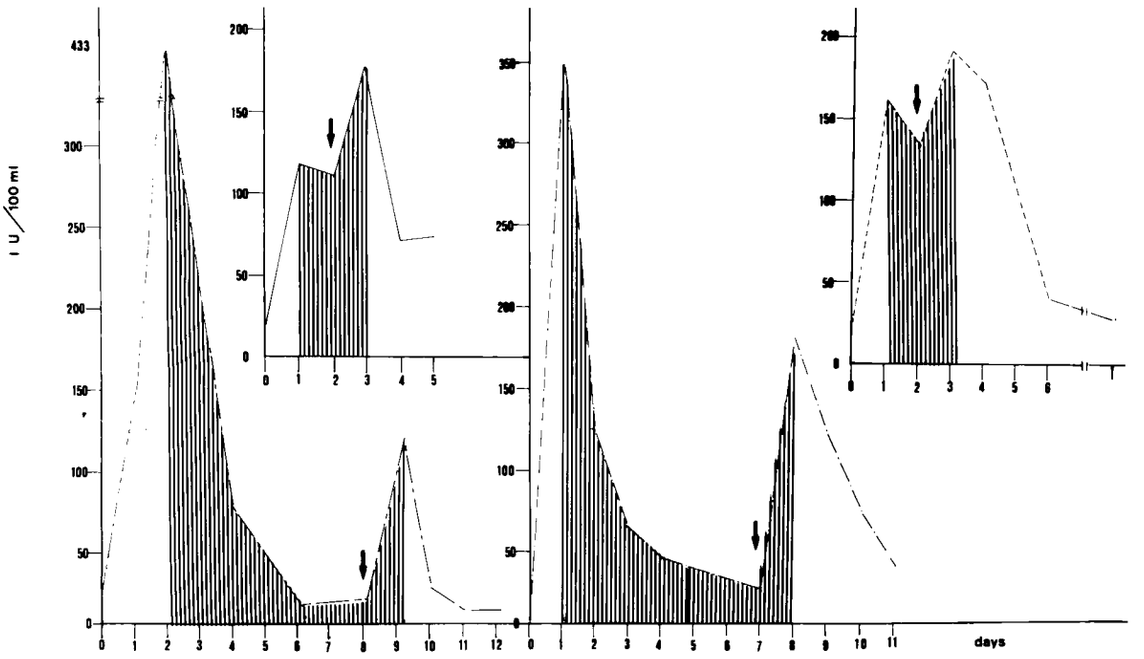

Fig. 5. Individual changes of plasma amylase activity in 4 patients treated with conventional fractionation and in whom treatment was interrupted for one or more days.

The influence of the fractionation regimen and salivary gland mass irradiated was evaluated by comparing the mean values of the amylase peak level and the time of its occurrence.

The effect of the fractionation modality is shown in Fig. 2, which compares serum amylase levels in patients irradiated to the entire salivary gland mass, either with the CF regimen or the MDF regimen. A clear-cut difference was evident between the two curves. In patients treated with the MDF regimen the serum amylase reached the peak level more rapidly after the first day of irradiation and receded to the normal range within the fourth day of treatment. In patients treated with the CF regimen, the peak value was reached after the second day of treatment and the level returned to the normal range on the fifth day of treatment. Statistical significance, tested by the Wilcoxon rank sum test, was p (Z) <0.02 on the first, third and fourth days; only on the second day was the difference not apparently statistically significant (p (Z) >0.1). No significant difference was observed between the mean values of the peak.

The effect of the amount of salivary gland mass irradiated is demonstrated in Fig. 3. Serum amylase levels in patients treated with the same fractionation regimen (CF) but including different proportions of the salivary glands are compared.

Patients in whom the irradiated volume included less than 50 per cent of the salivary gland mass had a significantly lower peak level than patients with the whole salivary gland mass irradiated. In the first group the amylase values also returned to the normal level more rapidly than in the second group. The difference between the two curves was significant (p (Z) <0.005) only on the initial three days.

A special type of behaviour was observed in the few patients with small variations in their irradiation course. In the 2 patients with lymphoma, where the irradiated volume was enlarged on the third day of treatment to include the entire salivary gland volume, the serum amylase presented a further increase in one, and a delay of the decline to normal level in the other patient (Fig. 4). In the 4 patients treated with the CF regimen to the entire salivary gland volume, whose treatment was interrupted after the first or second irradiation fraction, the restart of irradiation was followed by a new increase in the amylase (Fig. 5). The rise depended on the duration of the interruption; the second peak was higher

when the interruption lasted about 24 hours and lower if it lasted for several days.

Discussion

The changes in the salivary amylase in serum observed in these patients were similar to those already described by KASHIMA et coll. (6) after single doses of 10 to 20 Gy and by VAN DEN BRENK et coll. (11) after a hypofractionation regimen devised for irradiation in hyperbaric oxygen (weekly fractions of 6.0 to 7.25 Gy).

In the present analysis the effect on the serum amylase changes was influenced by the fractionation regimen (one conventional and one hyperfractionation schedule) and by the amount of salivary gland mass irradiated.

Treatment during 4 to 5 days with conventional fractionation or hyperfractionation seemed to completely abolish the production of the enzyme in the salivary gland tissue included in the irradiated volume. The following fractions did not produce any further change in the salivary amylase level.

The MDF regimen appeared to give a faster increase of the serum salivary amylase and an earlier return to a normal level. This could be explained by the higher dose accumulated in these patients with respect to time, and by the consequently more massive and rapid effect on the cells producing enzymes. The final effect of the two fractionation regimens was however similar, probably because the irradiated salivary gland mass had the same size and both treatment schedules completely abolished the function of the enzyme producing cells.

The influence of the amount of the salivary gland tissue irradiated on the amylasemia has been described previously (6, 11) and it was confirmed by the present series. The peak level and duration of the increase seem to measure the total enzyme released into the blood and to depend on the number of enzyme producing cells destroyed or functionally suppressed. Further support for this interpretation was obtained in the patients in whom the irradiated volume was increased after the initial 4 Gy to include the entire salivary gland mass. The peak level was lower in these patients than in patients who received the first irradiation against the entire salivary gland mass.

The observations in patients with an interruption of treatment after the first 2 or 4 Gy can probably be explained by a dose-dependence of the salivary amylase changes in the serum. The absorbed dose before the interruption was probably insufficient to completely destroy or suppress the enzyme producing cells. The new rise observed after restarting the irradiation was then a consequence of the destruction of cells that survived the first fraction.

The investigation suggests that serum amylase changes can serve as a sensitive and quantitative measure of radiation damage in the serous part of salivary gland tissue. The sensitivity of the test is however limited to the very early damage. No information can be gained on the effect of the overall treatment, as the dose inducing complete release of the enzyme from the damaged serous cells is largely below the therapeutic dose.

ACKNOWLEDGEMENT

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