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Conference Paper

Report of the Pilot Study Done for the Proposed Investigation on the Possible Synergic Effect between High-Dose Ascorbic Acid Application and Oncothermia Treatment

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According to recent investigations, the parenteral application of ascorbic acid (vitamin C) at high doses has significant antitumor activity in *in vitro* assays. The goal of our experiment was to determine the possible potentiating effect of application of high dose pH-neutralized ascorbic acid to the normal oncothermia treatment method. The NMRI mice were inoculated with C26 murine colon carcinoma cell line subcutaneously at both of their femoral regions and were kept till the tumors reached symmetrically 10 mm in diameter. We created four experimental groups, containing 5 male and 5 female animals in each. Both vitamin-C and oncothermia treatments were applied once; ascorbic acid was applied *intra-peritoneally*. Oncothermia treatment was applied only to the right limb tumor; the other side will be used as internal control. After the treatment, the animals were sacrificed, and all tumors were removed and analyzed histopathologically. Our main question centers on the comparison of the cell destruction ratio of the various applied treatment regimes, and studies the possible synergy or additive cross-potentiating of the methods. The results of this experiment turned out to be controversial, since the ascorbic acid did not change the remission rate of the allografts and showed no synergy with oncothermia.

1. Introduction

According to recent investigations, the parenteral application of ascorbic acid (vitamin C) at high doses has significant antitumor activity in *in vitro* assays. This fact is very important using ascorbic acid as complementary drug with standard antitumoral therapy or in cases where currently no other potent treatment is possible. Although the beneficial effect of the ascorbic acid on antineoplastic therapy has some controversial reports in the literature [1–3] and the specific method of action is still unclear: high concentration of ascorbic acid produces oxidative shock by H_2O_2 lethal for tumor cells beyond a certain level, healthy cells can survive the same stress effect [4]. As for the application, it was reported that intravenous ascorbic acid treatment is much more efficient, since this way more than 70-fold higher plasma concentration is elucidative than in case of oral application [5]. To achieve proper effect, high plasma level of ascorbic acid is required; so in human cases intravenous dosages are considered between 0,15 and 1,5 g/kg doses [6, 7].

2. Objective

The goal of our experiment was to determine the possible potentiating effect of application of high-dose pH-neutralized ascorbic acid to the normal oncothermia treatment method. The dose we used was considered to be 2 g/kg

according to human trials [7] and our intraperitoneal acute toxicity test (not reported). We choose the *intraperitoneal* application because the absorption from abdominal cavity is very fast and complete, nearly equal to *intravenous* application and it is much easier to perform in mouse than IV application. However, in this case not only irritative materials can be applied, but also neutralization of ascorbic acid by sodium hydroxide is necessary.

3. Method

The NMRI mice were intended to inoculate with C26 murine colon carcinoma cell line subcutaneously at both of their femoral regions and were kept till the tumors reached symmetrically 10 mm in diameter. C26 cells were cultivated in RPMI 1640 Glutamax medium (Invitrogen, Carlsbad, CA, USA). Inoculation was be done by 7500000 cell/mL concentration liquid cell suspension, using 0,1 mL subcutaneously at each side. Incubation time for tumor growth is expected to be around two weeks.

Vitamin C solution of 1 M in concentration was created by using dry ascorbic acid (Sigma Aldrich, St. Louis, MO, USA) and sterilized purified water and neutralized by 10 M sodium hydroxide solution (Sigma Aldrich, St. Louis, MO, USA).

We created four experimental groups, containing 4 female animals in each. The formed groups of animals were

Gr1: no treatment (control);

Gr2: only ascorbic acid treatment;

Gr3: only oncothermia (OTM) treatment;

Gr4: both ascorbic acid and oncothermia treatments.

Both vitamin C and oncothermia treatments were applied once ("single-shut" treatment regime); ascorbic acid was pH-neutralized and applied intraperitoneally in a dose of 2 g/kg bodyweight. Oncothermia treatment was applied only to the right limb tumor, the other side was used as internal control, and the incubation period between vitamin C application and OTM treatment was 30 minutes. The animals were sacrificed 24 hours after the treatment; all tumors were removed and analyzed histopathologically. The other organs were routinely checked as well.

4. Results

In our pilot study, we discovered that vitamin C as monotherapy did not do any change in tumor remission compared to the control samples. As for OTM as monotherapy, the treated side tumor showed greater dead tissue area than both of untreated side and control animals, same level as experienced in earlier studies. The combinational therapy showed controversial result, as the dead tissue amount in the tumors was the same in both treated and untreated sides, and it was smaller than in case of OTM monotherapy at the treated side.

5. Conclusion

In this pilot study, results are showing that because of some unknown reasons, high-dose ascorbic acid application showed no synergic or adjuvant effect with OTM therapy, even the combination decreased the effectivity of the OTM compared to the results of monotherapy.

When planing the proposed, large-number animal experiment, the results of this current pilot study should be taken into consideration. It is highly probable that "single-shut" ascorbic acid application will not be appropriate to achieve any synergic effect. Also, incubation time between vitamin C application and OTM treatment should be greater than the applied 30 minutes.

According to the results we have got in our experiment, we should turn our interest from ascorbic acid to other possible materials as potentiating agent for oncothermia treatment in the future.

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