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ORIGINAL ARTICLE

Nanoparticles as drug carrier system of 5-fluorouracil in local treatment of patients with superficial basal cell carcinoma

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Summary

Purpose: *To develop an alternative nonsurgical treatment for basal cell carcinoma using colloidal systems as drug carriers. We investigated the possibility of polybutylcyanoacrylate nanoparticles loaded with 5-fluorouracil (5-FU) to be applied in local treatment of patients with basal cell carcinoma.*

Patients and methods: *32 patients (mean age 74 years, range 56-90) with histologically confirmed superficial basal cell carcinoma were treated with 5-FU-loaded polybutylcyanoacrylate nanoparticles. The nanoparticles were prepared by anionic polymerization of butyl-2-cyanoacrylate monomer for use as drug delivery system. The preparation of 5-FU-loaded polybutylcyanoacrylate nanoparticles was carried out by adsorption of the drug on the surface of previously prepared nanoparticles. This preparation was applied once a day for 35-40 days. The effect of treatment on the immunological parameters, measured by phytohaemagglutinin (PHA)-induced DNA synthesis of T lymphocytes and also their number in the peripheral blood were analyzed in 28 of 32 treated patients and compared against a group of 24 healthy individuals (controls).*

Results: *31 of 32 patients achieved histologically confirmed complete tumor resolution. Treatment did not cause significant changes both in the number of T lymphocytes and PHA-induced DNA synthesis of T lymphocytes of the treated patients.*

Conclusion: *Local treatment with 5-FU-loaded nanoparticles provides a nonsurgical treatment alternative in patients with superficial basal cell carcinoma. This effective and well tolerated method is preferred by patients who are not surgical candidates or who prefer nonsurgical treatment.*

Key words: basal cell carcinoma, 5-fluorouracil, local treatment, nanoparticles

Introduction

Nanoparticles exhibit extraordinary properties due to their small size. The colloidal polymer nanoparticles are well tolerated *in vivo* due to their biocompatibility, biodegradability, and low toxicity [1,2].

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Nanoparticles, originally developed for industry, revolutionized modern medicine. Until recently, nanotechnology was preliminary based on manufacturing super computers, whereas the first major breakthrough in the field of nanoparticles was in biomedical applications, such as early disease detection, imaging and drug delivery [3-6]. The fundamental idea behind targeted drug delivery is the ability to deliver a drug to a precise location in the body where it will be more effective. There is evidence that nanoparticles can be programmed to carry a designed drug that targets cancer cells without affecting healthy cells. Recently many works on research and development of novel technology utilizing polymers for biotechnology and medical application have been published [7-11].

5-FU is an antineoplastic antimetabolite. Rich evidence has appeared about 5-FU in the past few years

concerning its chemical nature, mode of action, indications, contraindications and side effects.

It has been shown that parenteral 5-FU inhibits the growth of human neoplasms and that its effects are greatest on bone marrow, the intestinal mucosa and certain tumors like breast, rectal and colon cancer. In 1962, Schnelderman reported the use of 5-FU in patients with malignancies of internal organs [12]. The possibility of 5-FU and other chemotherapeutic drugs to be applied topically or injected in malignant tumors were reported by Klein et al. [13,14]. Dillaha et al. [15] reported application of 20% 5-FU on the skin of patients with extensive actinic keratosis. Relatively recently 5-FU has been used in the topical treatment of epidermal dysplasia or it has been injected intralesionally [16,17].

The precise mechanism of action of 5-FU has not been determined, but the drug is considered to function as an antimetabolite in at least 3 different ways. 5-FU is a fluorinated pyrimidine which is structurally similar to uracil. The deoxyribonucleotide of the drug, 5-fluoro-2'-deoxyuridine-5'- phosphate, inhibits thymidylate synthetase, thus inhibiting methylation of deoxyuridylic acid to thymidylic acid and thereby interfering with the synthesis of DNA. In addition, 5-FU becomes incorporated to a small extend into RNA, producing an unnatural RNA. It inhibits utilization of preformed uracil in RNA synthesis by blocking uracil phosphatase. Since DNA and RNA are essential for cell division and growth, 5-FU may provoke unbalanced growth and death of the cell.

Based on these findings, we attempted to enhance the local therapeutic activity and establish dosage levels of 5-FU in patients with superficial basal cell carcinoma using polybutylcyanoacrylate colloidal nanoparticle systems as carriers, which are biodegradable and biocompatible.

The use of new methods of treatment and immunomodulating drugs in cancer patients requires monitoring of the immune system. Because of that the effect of this method on some immunological parameters was analyzed.

Patients and methods

Thirty-two patients with histologically confirmed superficially spreading basal cell carcinoma (T1N0M0) entered this study.

The patients' mean age was 74 years (range 56-90). Most of them belonged to the 70-85 years age group.

Only one of all patients had been previously subjected to several operations for local recurrences. The remaining 31 patients had not undergone any form of local therapy (surgery, radiotherapy) or systemic chemotherapy.

Preparation of 5-FU - loaded polybutylcyanoacrylate nanopatricles

The biodegradable polybutylcyanoacrylate nanoparticles were prepared by anionic polymerization of butyl-2-cyanoacrylate monomer for use as targeted drug delivery system.

The preparation of 3% 5-FU-loaded polybutylcyanoacrylate nanoparticles was carried out by adsorption of the drug on the surface of previously prepared nanoparticles. The resulting suspension was used to prepare 3% 5-FU cream based on 1: 1 lanoline/vaseline.

Treatment

The cream of 5-FU-loaded nanoparticles was applied topically once a day for 35-40 days. Patients were first examined on the 20th day from the beginning of treatment and at the end of therapy. Follow-up was done after 6 and 12 months. The results were photographically documented before and after treatment.

Immunological parameters

The proliferative response of human peripheral T lymphocytes to T-cell mitogen PHA and the number of T lymphocytes were measured before and on the last day of treatment. Also, the same procedure was carried out in 24 healthy individuals (mean age 41 years, range 32-53).

Human peripheral blood T lymphocytes were isolated by Ficoll-Hypaque gradient centrifugation. The cells were resuspended in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 30 μg/ml gentamycin, and then were stimulated with different concentrations of PHA. The cells were cultured for 72 h in a humidified atmosphere at 37° C with 5% CO_2. The DNA synthesis rate was measured by using ^{3}H-thymidine incorporation. Eighteen hours prior to harvest, cultures were pulsed with 1μCi ^{3}H-thymidine. The incorporated radioactivity was precipitated with 5% cold trichloracetic acid onto membrane filters. The filters were placed in scintillation vials containing 5 ml of scintillation fluid (toluene, PPO, POPOP) and counted in liquid scintillation counter (Packard). The tests were carried out in triplicate. The ^{3}H-thymidine incorporation was measured as counts per minute (cpm) and the respective stimulation indices (SI) were calculated. The SI was expressed as the ratio of ^{3}H-thy-

midine incorporation in stimulated and unstimulated cell cultures.

The T lymphocytes were identified by their ability to form E-rosettes (at 4^{o} C and 20^{o} C). The treatment-related changes were analyzed by Student's *t-test*.

Results

Thirty-one out of 32 (96.9%) patients achieved histologically confirmed complete tumor resolution. In all 31 patients complete response lasted for the whole period of follow-up (1 year). The non-responding patient underwent surgical treatment.

After 5-10 days of treatment, the treated skin became red and irritated. Sores and crusts appeared in some cases in the course of application, which healed afterwards. The application, of 5-FU-loaded nanoparticles resulted in selective inflammatory erosion and disappearance of sores without significant alteration of the normal skin. Figures 1, 2, 3, and 4 present two patients before and after treatment with 5-FU-loaded nanoparticles.

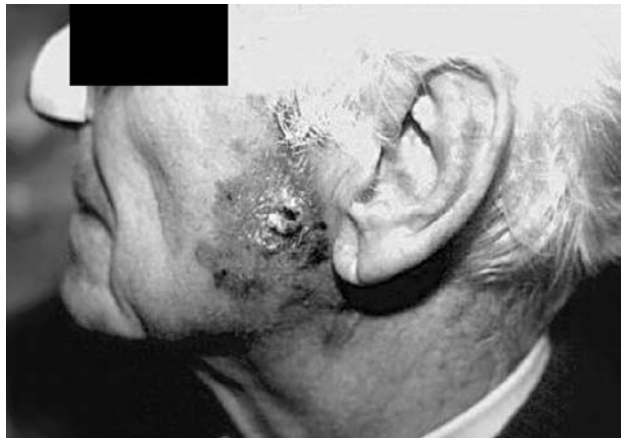

Figure 1. Patient A with basal cell carcinoma before treatment.

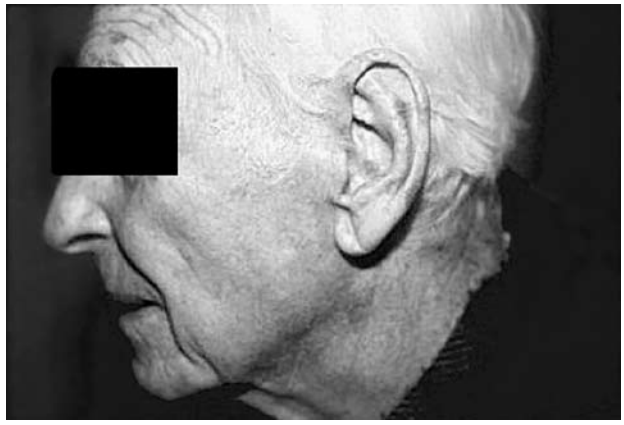

Figure 2. Patient A after treatment with 5-FU nanoparticles.

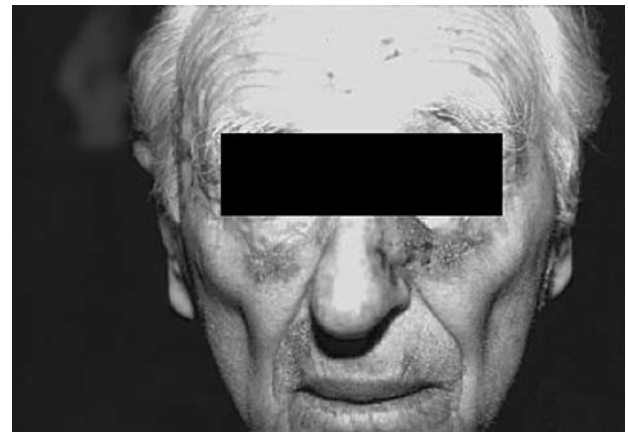

Figure 3. Patient B with basal cell carcinoma before treatment.

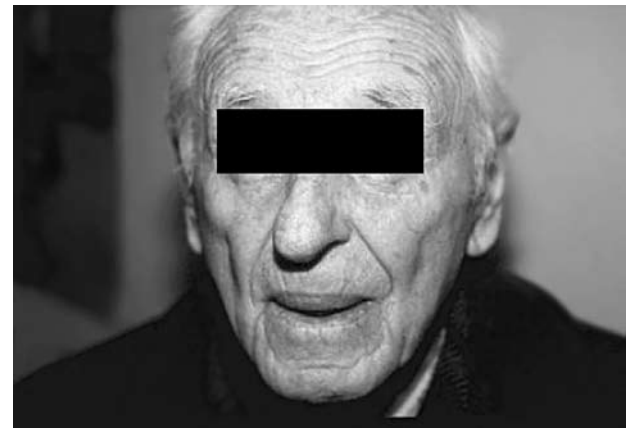

Figure 4. Patient B after treatment with 5-FU nanoparticles.

The patient with local recurrence of basal cell carcinoma who had undergone several surgical interventions for local relapses before being treated with nanoparticles had tumor regression for a year. The intervals between surgical resections of recurrent tumor were few months only. The interval of apparent tumor recurrence after local 5-FU treatment was about one year. Now this patient preferred local treatment again, because of its easy application, beneficial effect and longer interval of recurrent tumor appearance.

The effect of treatment on the immunological parameters measured by PHA-induced DNA synthesis of T lymphocytes and their number in the peripheral blood was analyzed in 28 of 32 treated patients.

The results of response of T lymphocytes to PHA and their number are summarized in Table 1 and demonstrate no significant difference between mean values before and after treatment. However, the response of T lymphocytes to PHA and their number before treatment

Table 1. Effect of local treatment of 5-FU-loaded nanoparticles on immunological parameters in patients with basal cell carcinoma (M ± m)

Immunological parameter	*No. of patients*	*Before treatment*	*After treatment*	*p-value*	*No. of controls*	*Results in controls*
Lymphoproliferative response to PHA (SI)	28	3.8±0.9	4.3±1.1	NS	24	86.6±1.8
T lymphocytes (4° C, %)	28	24.2±3.2	22.6±2.3	NS	24	58.5±2.6
T lymphocytes (20° C, %)	28	33.3±3.4	40.1±2.9	NS	24	36.3±1.1

NS: non significant

were lower when compared with those of the controls (SI 82.6±18.8; T lymphocytes at 4° C– 58.5±2.6; T lymphocytes at 20° C 36.3±1.2).

The number of T lymphocytes (measured at 4°C and 20°C) showed individual changes after treatment, but overall, this treatment did not cause further immune suppression of T lymphocytes compared to initial pretreatment values.

Similarly, we found no significant differences in PHA-induced lymphocyte DNA - synthesis (SI) before and after treatment.

Discussion

This work provides an alternative nonsurgical treatment for patients with superficial basal cell carcinoma. Local treatment with 5-FU-loaded polybutylcyanoacrylate nanoparticles is an effective therapeutic method for these patients. This treatment is preferred by elderly individuals from surgical or radiation therapy.

Despite the lower pretreatment immunological parameters (compared with healthy controls), especially the ability of patients' lymphocytes to respond to mitogen stimulation, all studied patients have responded to therapy with 5-FU-loaded polybutylcyanoacrylate nanoparticles. Hence the evaluation of the immune system in cancer patients is based on the assumption that improved immunity is associated either with a better prognosis and clinical course or a better response to antineoplastic therapy [18,19]. As a rule, a growing tumor burden has been associated with decreasing immunocompetence [20]. However, different results have been reported in the literaturee, depending on different neoplastic diseases, age of patients, disease stage etc. [21-23].

The diminished immunological parameters in our patients could be attributed to the patients' age.

Many published studies showed that the peripheral blood lymphocytes in cancer patients had an impaired capacity to proliferate in response to a mitogenic stimulation. Wanebo et al. [24] showed that peripheral blood lymphocytes in cancer patients generally had normal capacity to generate interleukin-2 (IL-2), but this capacity was not related to the proliferative response to PHA which was frequently suppressed in these patients.

Diminished ability of T lymphocytes from elderly individuals to proliferate *in vitro* is well documented as is the fact that *in vitro* IL-2 secretion by lymphocytes from elderly humans is defective. High affinity IL-2 receptors on mitogen-stimulated T cells have been shown to efficiently bind IL-2 and to transduce the activation signals that produce proliferation. Nagel et al. [25] determined that there were no differences in the number of these receptors in young and in elderly individuals. However, their results demonstrated that a significantly larger percentage of lymphocytes from elderly individuals do not respond to mitogenic stimulation and, probably due to stimulus stress, die in culture.

Hence, the suppressed immunological parameters in patients with basal cell carcinoma are likely to relate with impairment of the defense to tumor development due to their age.

Conclusion

Local treatment with 5-FU-loaded nanoparticles is an effective and well tolerated method to treat superficial basal cell carcinoma in patients who do not prefer surgical intervention. Despite the low immunological parameters in these elderly patients, they showed excellent response to this treatment.

We believe that this novel method of treatment in such patients can be easily applied in clinical practice.

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