Dosimetric Studies of Anti-CD20 Labeled With Therapeutic Radionuclides at IPEN/CNEN-SP

Estudos dosimétricos de Anti-CD20 marcados com radionuclídeos terapêuticas no IPEN / CNEN-SP

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Radioimmunotherapy (RIT) makes use of monoclonal antibodies (MAb) labeled with alpha/beta radionuclides for therapeutical purposes, leading to tumor irradiation and destruction, preserving the normal organs on the radiation excess. The therapeutic activity to be injected in a specific patient is based on information obtained in dosimetric studies. Beta emitting radionuclides such as ^{131}I, ^{188}Re, ^{90}Y, ^{177}Lu and ^{166}Ho are useful for the development of therapeutic radiopharmaceuticals. Anti-CD20 (Rituximab) is a chimeric MAb directed against antigen surface CD20 on B-lymphocytes, used in non-Hodgkin lymphoma treatment (NHL). The association with beta radionuclides have shown greater therapeutic efficacy. Currently, two radiopharmaceuticals with Anti-CD20 for radioimmunotherapy have FDA approval for NHL treatment: ^{131}I-AntiCD20 (Bexar®) and ^{90}Y-AntiCD20 (Zevalin®). Techniques for the radiolabeling of ^{188}Re-antiCD20 have been recently developed by IPEN-CNEN/SP in order to evaluate the clinical use of this radionuclide in particular. The use of ^{188}Re ($T_{1/2}$ 17h) produced by the decay of ^{188}W ($T_{1/2}$ 69d), from an ^{188}W/^{188}Re generator system, has represented an alternative to RIT. Beyond high energy beta emission for therapy, ^{188}Re also emits gamma rays (155keV) suitable for image. The aim of this new project is to compare the labeling of anti-CD20 with ^{188}Re with the same MAb labeled with ^{131}I, ^{177}Lu, ^{90}Y and even ^{99m}Tc. The first step in this project is the review of the published data available concerning the labeling of this MAb with different radionuclides, along with data obtained at IPEN, taking into account labeling procedures, labeling yields, reaction time, level and kind of impurities and biodistribution studies. The pharmacokinetic code will be developed in Visual Studio.NET platform through VB.NET and C^{++} for biodistribution and dosimetric studies of ^{188}Re-AntiCD20 in mice, simulating other compartmental systems and radiopharmaceuticals. After the model definition, a simulation will be performed using the ^{188}Re-AntiCD20, evaluating tumor uptake, critical organs dosimetry and biodistribution study. The total beta radiation dose received by the bone marrow will also be evaluated for all radiopharmaceuticals.

Keywords: Radioimunotherapy; Anti-CD20; Dosimetry; pharmacokinetics

Radioimunoterapia (RIT) faz uso de anticorpos monoclonais (MAb) marcadas com alfa / beta radionuclídeos para fins terapêuticos, levando a irradiação do tumor e destruição, preservar os órgãos normais sobre o excesso de radiação. A atividade terapêutica a ser injetado em um paciente específico é baseado em informações obtidas em estudos dosimétricos. Radionuclídeos emissores beta, tais como 131I, 188Re, 90Y, 177Lu 166Ho e são úteis para o desenvolvimento de radiofármacos para terapia. Anti-CD20 (rituximab) é um anticorpo monoclonal quimérico anti-CD20 de superfície do antigénio em linfócitos B, utilizado no tratamento de linfoma não-Hodgkin (LNH). A associação com radionuclídeos beta mostraram maior eficácia terapêutica. Atualmente, dois radiofármacos com anti-CD20 para radioimunoterapia tem aprovação do FDA para o tratamento de NHL: 131I-AntiCD20 (Bexar ®) e 90Y-AntiCD20 (Zevalin ®). As técnicas para a radiomarcação do 188Re-antiCD20 foram recentemente desenvolvidos por IPEN-CNEN/SP, a fim de avaliar a utilização clínica deste radionuclídeo em particular. O uso de 188Re (T1 / 2 17 h) produzido pela decomposição de 188W (T1 / 2 69d), a partir de um sistema gerador de 188W/188Re, representou uma alternativa para RIT. Além de emissão de alta energia beta para a terapia, 188Re também emite raios gama (155keV) adequados a imagem. O objetivo deste novo projeto é comparar a rotulagem de anti-CD20 com 188Re com o MAb mesmo marcado com 131I, 177Lu, 90Y e até mesmo 99mTc. O primeiro passo nesse projeto é a revisão dos dados publicados disponíveis sobre a rotulagem deste MAb com radionuclídeos diferentes, juntamente com os dados obtidos no IPEN, tendo em conta as regras de rotulagem, etiquetagem rendimentos, tempo de reação, o nível e tipo de impurezas e de biodistribuição estudos. O código de farmacocinética será desenvolvido em plataforma Visual Studio.NET através VB.NET e C + + para biodistribuição e estudos dosimétricos de 188Re-AntiCD20 em ratos, simulando outros sistemas compartimentados e radiofármacos. Após a definição do modelo, uma simulação será realizada utilizando o 188Re-AntiCD20, avaliando captação

tumoral, dosimetria órgãos crítica e estudo de biodistribuição. A dose total de radiação beta recebido pela medula óssea também será avaliada para todos os medicamentos radiofarmacêuticos.
Palavras-chave: Radioimunotherapy; Anti-CD20; Dosimetria; farmacocinética

1. INTRODUCTION

Radioimmunotherapy (RIT) is a new therapeutic approach that makes use of monoclonal antibodies (MAb) labeled with alpha/beta radionuclides for therapeutical purposes (Table 1), leading to tumor irradiation and destruction, preserving the normal organs on the radiation excess. The principle of RIT is to use radiolabeled antibodies as carriers of radionuclides for the selective destruction of a tumor[1]. RIT should be particularly beneficial for the treatment of tumors not easily amenable to surgical control and for the treatment of early recurrence and distant metastases. RIT depends on a high concentration of tissue radioactivity over a long duration. Different to drug and toxin, immunoconjugates of radiolabeled MAbs can kill cells that are at a distance from the targeting site depending on the choice of radionuclide, without the MAb conjugate being internalized. Beta-emitting radionuclides have been complexed with MAbs directed against tumor antigens in an attempt to direct the radiation to tumors, thereby allowing treatment of tumor while minimizing exposure of normal tissue to radiation[2,3].

Table 1: Radionuclides used for imaging and therapy in radioimmunotherapy. Radionuclides with imaging emissions are used for tumor identification and dosimetry (pre-scouting) before the use of a 'matched' radionuclide for therapy[3].

Radionuclides	Emission Energy (MeV)	Physical $T_{1/2}$
Imaging (γ)		
^{99m}Tc	0.142	6.01 h
^{111}In	0.173, 0.247	2.805 days
Imaging (β^+)		
^{64}Cu	1.675	12.701 h
^{72}As	1.17	1.1 days
^{86}Y	1.479	14.7 h
^{89}Zr	0.9	3.27 days
^{124}I	1.53	4.18 days
Therapy (Auger)		
^{111}In	0.86	2.805 days
^{123}I	1.234	13.2 h
^{125}I	0.179	60.1 days
Therapy (α)		
^{211}At	5.980	7.21 h
^{212}Bi	6.051	1.009 h
^{213}Bi	8.537	0.8 h
^{225}Ac	5.3-5.8	10 days
Therapy (β^-)		
^{67}Cu	0.58	2.58 days
^{77}As	0.226	1.6 days
^{90}Y	2.286	2.67 days
^{131}I	0.606	8.04 days
^{177}Lu	0.497	6.75 days
^{186}Re	0.973	3.78 days
^{188}Re	2.118	16.94 days

The therapeutic activity to be injected in a specific patient is based on information obtained in dosimetric studies. Beta emitting radionuclides such as ^{131}I, ^{188}Re, ^{90}Y and ^{177}Lu are useful for the development of therapeutic radiopharmaceuticals.

Anti-CD20 (Rituximab) is a chimeric MAb directed against antigen surface CD20 on B-lymphocytes, used in non-Hodgkin lymphoma treatment (NHL). The association with beta radionuclides have shown greater therapeutic efficacy.

Rituximab was the first anticancer monoclonal antibody to be licensed by the US Food and Drug Administration (FDA), for treatment of recurrent or refractory B-cell lymphoma of low

grade. Rituximab is a chimeric IgG1 antibody that targets CD20 – a cell-surface protein present on healthy B lymphocytes and on 95% of B-cell lymphomas and acts by both complement dependent and by antibody-dependent cell-mediated cytotoxicity, and can also induce apoptosis directly[4].

Two radioimmunotherapy agents have received full FDA clearance for marketing. ^{90}Y ibritumomab tiuxetan (Zevalin®) received FDA clearance in 2002, and ^{131}I-tositumomab (Bexxar®) received its clearance about one year later. Both of these compounds received commercial clearance for the treatment of CD20 indolent B-cell NHL and related conditions. Although both RIT compounds target the CD20 surface antigen, their pattern of use differs significantly, in large part because of the 2 different radioisotopes incorporated into the compounds[5].

^{188}Re ($t_{1/2}$ 16.9h) has a number of characteristics that makes it suitable for targeted therapy, including a β^- emission with a high mean-energy emitted per disintegration of 2.118 MeV (71.6% of emission); a suitable mean energy emitted per unit cumulated activity; a range of beta particles in tissue (R_{tissue}) of 10.15 mm, which permits ^{188}Re to diffuse the radiation effect over a larger distance within tissues; an initial activity that produces 90% cure probability and an optimal radiotoxic range of 23.0 – 32.0 mm. Besides the therapeutic usefulness of ^{188}Re, the emission of gamma photon is an added advantage since the biodistribution of ^{188}Re-labeled antibodies can be evaluated *in vivo* with a gamma camera[3].

Techniques for the radiolabeling of ^{188}Re-antiCD20 have been recently developed by IPEN-CNEN/SP in order to evaluate the clinical use of this radionuclide in particular[6]. The use of ^{188}Re ($T_{1/2}$ 17h) produced by the decay of ^{188}W ($T_{1/2}$ 69d), from an ^{188}W/^{188}Re generator system, has represented an alternative to RIT. Beyond high energy beta emission for therapy, ^{188}Re also emits gamma rays (155keV) suitable for image[7].

The aim of this new project is to compare the labeling of anti-CD20 with ^{188}Re with the same MAb labeled with ^{131}I, ^{177}Lu, ^{90}Y and even ^{99m}Tc. The first step in this project is the review of the published data available concerning the labeling of this MAb with different radionuclides, along with data obtained at IPEN, taking into account labeling procedures, labeling yields, reaction time, level and kind of impurities and biodistribution studies.

2. MATERIALS AND METHODS

2.1. Review of Literature

Literature databases will be used to review the data available for the preparation of anti-CD20 labeled with ^{90}Y, ^{131}I, ^{177}Lu and ^{188}Re. Data obtained from the radiopharmacy group at IPEN/CNEN-SP will also be revised concerning the preparation of ^{188}Re-antiCD20.

2.2. Scope of the work proposed at IPEN-CNEN/SP

An overview of the work plan proposed for the present work will be presented, with the focus on the radiopharmaceutical ^{188}Re-antiCD20.

3. RESULTS

3.1. Review of Literature

Rituximab (MabThera or Rituxan) is an unlabelled monoclonal antibody in routine clinical used for the treatment of lymphomas (Table 2). It's humanized, explaining the fact that can be detected in the blood for up to three months after administration[8]. The humanization also markedly reduces the production of human anti-mouse neutralizing the antibody, allowing patients to receive the treatment on subsequent occasions with clinical efficacy.

Table 2: Pharmacology of the four most widely used monoclonal antibodies [8].

	^{131}I tositumomab	**^{90}Y ibritumomab tiuxetan**	**Rituximab**
Target antigen	CD20	CD20	CD20
Type of antibody	Radiolabelled murine	Radiolabelled murine	Human-mouse chimera
Half-life	8 days	2.6 days	8 days
Serious adverse events	Myelosuppression 2o MDS/AML hypothyroidism infusional toxicity	Myelosuppression infusional toxicity	Infusional toxicity
Common regimens	Dosimetry: small dose of drug a week before main dose	Dosimetry: ^{111}In ibritumomab tiuxetan a week before main dose	375 mg/m^2 weekly for 4 weeks
Cautions	Bone marrow involvement	Bone marrow involvement	Circulating tumor cells

Rituximab is directed against CD20, and causes rapid depletion of both normal and malignant CD20-positive B lymphocytes from the blood, marrow and lymph nodes of the recipient. The depletion of normal B lymphocytes has not been associated with decreased humoral immunity in the trials published to date, and immunoglobulin levels do not decrease significantly

Conjugation of β^- emitting radioisotopes to a monoclonal antibody (radioimmunoconjugate, RIC) endows it with the capability to deliver targeted radiation not only to areas composed exclusively of CD20-positive lymphoma cells, but also to neighboring lymphomatous infiltrates where the antibody finds no access or no antigen to bind to.

When ^{131}I tositumomab is used, individual variations in RIC distribution and clearance and the significant γ activity impose special shielding/isolation requirements. In the case of ^{90}Y ibritumomab tiuxetan, predictable pharmacokinetics and biological half-life are achieved when a schedule of an initial infusion of rituximab is applied. The activity of ^{90}Y that has to be conjugated to Zevalin® can determine the baseline bone marrow involvement (which should not exceed 25% of the marrow area) and platelet count. Bexxar™ seems to be less myelotoxic, overall[9].

3.1.1. ^{90}Y- anti-CD20 labeled MAb (^{90}Y-Zevalin®)

^{90}Y-Zevalin has as an incorporated isotope a pure beta particle emitter with a half-life of approximately 2.7 days. The most energetic beta emission has energy of approximately 2.3MeV, which corresponds to a maximal penetration in tissue of approximately 5mm. Because there are no gamma emissions in the spectrum of this isotope, the radiopharmaceutical is very poorly visualized on routine gamma camera scans. For this reason, a replacement isotope (^{111}In) with an appropriate gamma emission and similar radiometal chemistry is used to allow imaging and biodistribution for the beta-emitting radiopharmaceutical[5,10,11,12]. Its disadvantages include chelate instability, an area under active investigation; ferric ion like post metabolic distribution to radiosensitive marrow space; and absence of imageable gamma emissions helpful for monitoring tumor targeting and developing dosimetry estimates[3]. Based on the physical characteristics of the emission spectra the isotope appear reasonably stable in their antibody linkages. ^{90}Y-Zevalin makes use of an added chemical side arm with a terminal chelation complex providing noncovalent linkage for the radiometal. For ^{90}Y-Zevalin, the chimeric anti-CD20 antibody RTX treatment sequence begins with the preliminary infusion of an excess of a nonradioactive (“cold”) anti-CD20 antibody designed to saturate nonspecific uptake sites and thereby improve the more specific targeting[5].

^{90}Y has the disadvantages of chelate instability, ferric ionlike postmetabolic distribution to radiosensitive marrow space, and absence of imageable gamma emissions helpful for monitoring tumor targeting and developing dosimetry estimates[3].

3.1.2. ^{13}I-anti-CD20 labeled MAb (^{131}I-Bexxar®)

^{131}I-Bexxar is a directly radiohalogenated mixed beta/gamma emitter with a gamma emission spike at 364KeV and a beta emission with an energy of approximately 0.6MeV. This radionuclide can thus be visualized directly on a properly collimated gamma camera though the resolutions of the images obtained with the ^{131}I iodinated compound are typically somewhat blurry[5,12]. Based on the physical characteristics of the emission spectra the isotope appear reasonably stable in their antibody linkages, where ^{131} I-Bexxar involves a covalent bond, For ^{131}I Bexxar, the murine monoclonal antibody tositumomab (TST) is used for the same purpose that ^{90}Y-Zevalin. Although some in vitro studies show increased cytotoxic activity for tositumomab, the mouse-human chimeric antibody rituximab apparently survives longer in the bloodstream and also is able to activate other components of the immune system such as the complement system[5].

^{131}I labeled MAbs have been relatively ineffective owing to limitations of this radionuclide as a therapeutic agent. After uptake of a radioiodinated MAb into a tumor cell there is a degradation of the antibody with release of radioiodine from the tumor cell into the body fluids, resulting in loss of action on tumor cell and delivery of irradiation to normal tissues with consequent development of severe myelosupression[3].

3.1.3. ^{177}Lu-anti-CD20 labeled MAb

^{177}Lu is a radiolanthanide with a β^- emission similar to ^{131}I. The main γ-photons of ^{177}Lu (208keV, 11% abundance) are more suitable for imaging than ^{131}I, and does not need special design of residualizing for labeling with internalizing monoclonal antibodies. The half-life of ^{177}Lu is 6.65days[13]. Its gamma emission is used to obtain in vivo images of biodistribution and dosimetric studies, while beta emission produces the desired therapeutic effect. ^{177}Lu has several advantages for radionuclide therapy: low tissue penetration, scarce damage to normal surrounding tissues, low gamma energy radiation, low abundance and adequate lifetime. Previous reports described the radiolabeling of anti-CD20 with ^{177}Lu by conjugation with DOTA through isotiocyanate-benzyl-DOTA showing good results. In vitro and in vivo studies were carried out in order to demonstrate complex stability and behaviour[14].

3.1.4. ^{188}Re-anti-CD20 labeled MAb

The use of ^{188}Re from a ^{188}W/^{188}Re generator system represents an attractive alternative radionuclide. Rhenium-188 ($t_{1/2}$ 16.9h) is produced from beta decay of the tungsten-188 parent ($t_{1/2}$ = 69d). In addition to the emission of high-energy electrons (E_β 2.118MeV), ^{188}Re also decays with emission of a gamma photon with an energy of 155keV in 15% abundance. Besides the therapeutic usefulness of ^{188}Re, the emission of gamma photon is an added advantage since the biodistribution of ^{188}Re-labeled antibodies can be evaluated *in vivo* with a gamma camera. Also, rhenium is situated below technetium in the periodic table and therefore has chemical properties similar to technetium. Thus, both can be conjugated to antibodies using similar chemistry methods[3,10,15,16].

Labeling methods has been reported showing the advantage that ^{99m}Tc and ^{188}Re represents an attractive pair of radionuclides for biomedical use because of their favorable decay properties for diagnosis. Both can be conjugated to antibodies using similar chemistry methods[17]. The relative high chemical stability of the precursors allows radiolabeling under mild, physiological conditions, suitable for the labeling of temperature-sensitive molecules such as proteins. The study shows for the first time that the use of the tricarbonyl core can be a promising and suitable strategy for the radiolabeling of antibodies with ^{188}Re. The IPEN/CNEN team presented a radiolabeling procedure of the commercial anti-CD20 antibody rituximab with $[^{99m}Tc\ (CO)_3]^+$ and $[^{188}Re(CO)_3]^+$ as a potential alternative to the ^{111}In/^{90}Y-Zevalin system for diagnosis and therapy of NHL[6,18]. The results obtained by Dias et al shows that rituximab could be directly and stably labeled with the matched pair ^{99m}Tc/^{188}Re using the IsoLink technology under retention of the biological activity. The radiolabeling efficiency and kinetics of RTX_{red} was 98%

(^{99m}Tc) after 3h. ^{99m}Tc(CO)$_3$-RTX$_{red}$ was used without purification for in vitro and in vivo studies whereas ^{188}Re(CO)$_3$-RTX$_{red}$ was purified to eliminate free ^{188}Re-precursor. Tumor uptake of ^{188}Re(CO)$_3$-RTX$_{red}$ was 2.5% ID/g and 0.8% ID/g for ^{99m}Tc(CO)$_3$-RTX$_{red}$ 48h after injection. The values for other organs and tissues were similar for both compounds.

^{188}Re is attractive for therapy of solid tumors over expressing CEA, *ior C2*, and EGF-r antigens because it decays with high-energy β^- and also with γ emissions, thus contributing to total radiation. The energy released per unit of activity would yield a significantly higher radiation dose delivered to the tumor. The high energy β emission of ^{188}Re may be of special value for large tumors. The disadvantage of this radionuclide is that can kill non targeted antigen-nonexpressing tumor cells through a crossfire effect[3].

3.2. Pharmacokinetics Studies at IPEN/CNEN-SP

Information about the kinetics and biodistribution upon introduction into living system is essential for *in vivo* applications in nuclear medicine. When a MAb immunoconjugate is injected intravenously, it passes through a number of compartments, including the vascular and extravascular spaces (organs, tissues, and body fluids) as it is metabolized and excreted.

The code that will be used in this work is the Visual Studio.NET and C^{++}. The mathematical routines will be implemented through the *Dynamically Linked Library – DLL*[19]. Figure 1 describes the methodology proposed for this study.

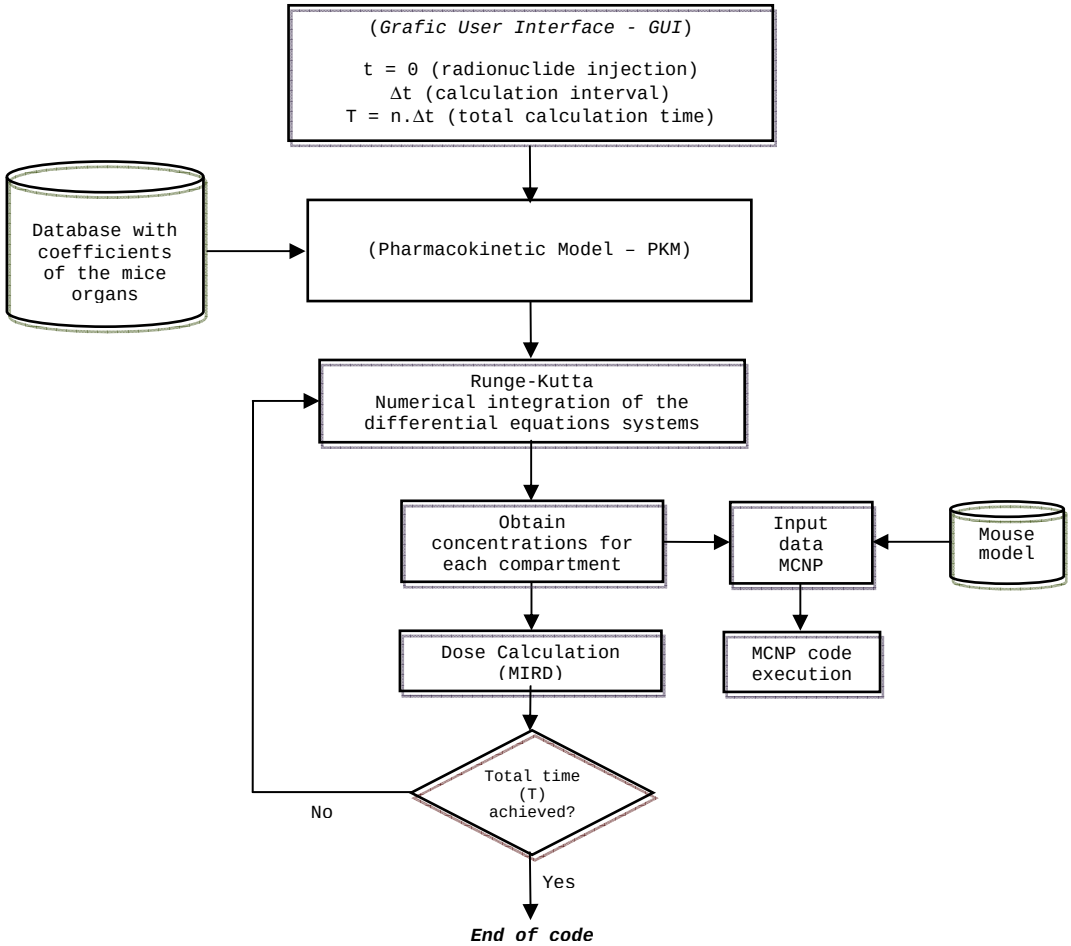

Figure 1: General flowchart, describing the methodology for the development of the code for studies of pharmacokinetics of ^{188}Re-anti-CD20 in the mouse model.

The model will be applied for each study radiopharmaceutical using biodistribution data obtained by DIRF research groups and literature. After the definition of the model, a simulation

will performed using the Anti-CD20 monoclonal antibody labelled with ^{188}Re, ^{90}Y, ^{177}Lu and ^{131}I, evaluating tumour uptake, dosimetry in critical organs and biodistribution studies.

4. CONCLUSION

This work showed a review about the applications of anti-CD20 MAb rituximab labeled with various radionuclides in radioimmunotherapy. An alternative route for the direct radiolabeling of the anti-CD20 MAb rituximab with both ^{99m}Tc and ^{188}Re is using the tricarbonyl technique that has been developed at IPEN/CNEN-SP team, showing the acceptable radiolabeling yields achieved after reduction of the antibody. However, pharmacokinetic studies are necessary through the development of a compartmental model of this antibody for approval by regulators and future use in therapy by the medical purposes.

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