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(54) Title: ADENOVIRAL-VECTOR-MEDIATED GENE TRANSFER INTO MEDULLARY MOTOR NEURONS

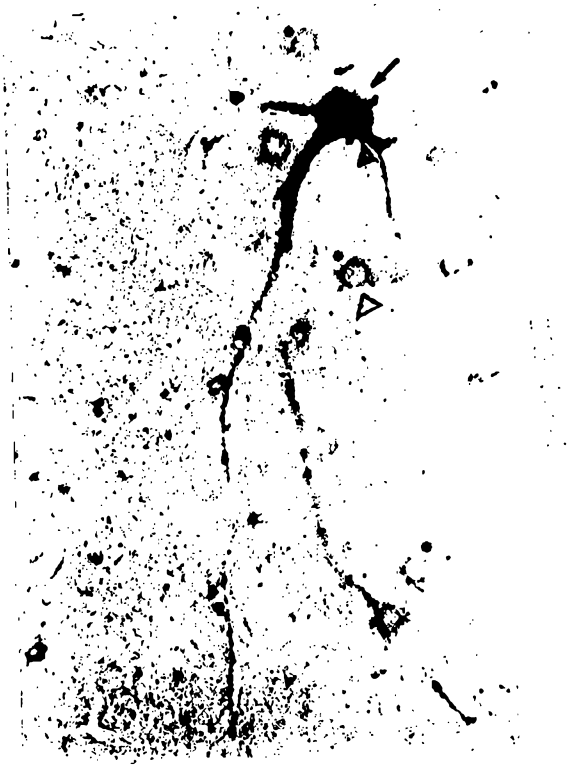
(54) Titre: TRANSFERT DE GENES DANS LES MOTONEURONES MEDULLAIRES AU MOYEN DE VECTEURS ADENOVIRAUX

(57) Abstract

The use of recombinant adenoviruses for transferring nucleic acids into medullary motor neurons is disclosed.

(57) Abrégé

La présente invention concerne l'utilisation d'adénovirus recombinants pour le transfert d'acides nucléiques dans les motoneurones médullaires.



Adenoviral-vector-mediated gene transfer into medullary motor neurons

The present invention relates to the field of gene therapy. It relates more particularly to a new method for treating pathologies of the nervous system by adenoviral-vector-mediated gene transfer into medullary motor neurons.

Gene therapy and the use of modified viruses as vectors for neurodegenerative diseases constitute particularly promising novel therapeutic approaches. Among the viruses used to this end in the prior art, there may be mentioned in particular adenoviruses (Le Gal La Salle et al., Science 259, 988-990), herpes viruses, adeno-associated viruses and retroviruses. The studies described in the prior art show that these vectors, and in particular the adenoviruses, are capable of infecting with a very high efficiency the cells of the central nervous system. These results thus make it possible to develop methods for treating pathologies of the central nervous system by direct injection into the central nervous system (in particular by stereotaxis) of recombinant adenoviruses comprising a therapeutic gene.

As regards the spinal cord, in the context of certain neurodegenerative diseases or of traumas, gene therapy may also make it possible to combat degeneration of the motor neurons (motoneurons) by providing certain genes encoding, for example, growth

factors. However, these applications are still limited by the lack of a simple method making it possible to specifically transfer a gene into the marrow. The present invention makes it possible to overcome this problem.

The present invention indeed describes a particularly efficient method for the selective transfer of genes into the marrow. The present invention stems in particular from the demonstration that it is possible to specifically transfer a gene into the motor neurons by administration, into the muscle, of an adenoviral vector incorporating the said gene. Indeed, the applicant has now shown that, in a particularly advantageous manner, the adenoviruses are absorbed at the level of the neuromuscular junctions (motor endplates), and transported up to the cellular bodies of the motor neurons (ventral horn of the spinal cord) by retrograde transport along the motoneuronal axons. The intramuscular administration of adenoviral vectors according to the invention thus constitutes a new, very specific method for infecting the motor neurons by retrograde transport, which makes it possible to target precisely the medullary stage on which it is desired to act according to the location of the trauma and/or of the degeneration.

The process according to the present invention is most particularly advantageous since it makes it possible, by following the precise map of the

neuromuscular junctions, to specifically and unilaterally infect the motor neurons of the different medullary functional stages. It has been found, furthermore, to be a lot less traumatic than a stereotaxic injection into the medullary parenchyma which would in any case be more diffuse and not restricted to the motor neurons.

As indicated above, the process according to the present invention is very advantageous since it makes it possible to target precisely the motor neurons of each medullary functional stage. Thus, according to the site of the impairment to be treated, the administration is made into a muscle carrying a nerve linkage with the said site. According to the present invention, it is now possible, by a judicious choice of various injections, to infect specifically and unilaterally a large number of medullary motor neurons distributed over the various levels. By way of preferred embodiment, administration into the muscles of the upper limbs (biceps, triceps) makes it possible to transfer a gene into the motor neurons at the cervical level; administration into the muscles of the

thorax (pectoral muscles) makes it possible to transfer a gene into the motor neurons at the thoracic level; or alternatively administration into the muscles of the lower limbs (gastrocnemial muscles) makes it possible to transfer a gene into the motor neurons at the lumbar and sacral levels. Other muscles may of course be used for administration into these motor neurons, and other motor neurons may also be targeted. To this end, it is possible to use precise maps of the neuromuscular junctions in order to determine, depending on the medullar stage targeted, the most appropriate muscle(s) for the administration. Such maps are accessible to persons skilled in the art (see especially Nicholopoulos et al., J. Comp. Neurol. 217, 78-85; Peyronnard et Charon, Exp. Brain Res. 50, 125-132). Depending on the medullar stage which it will prove convenient to infect, one or more muscles known to be innervated by the stage in question can thus be chosen.

The intramuscular administration of adenoviruses can be carried out in various ways. According to a first embodiment, it is performed by injection at several points of the same muscle so as to affect a very large number of motor endplates. This embodiment is particularly efficient when the point of insertion of the nerve into the muscle considered is not identifiable. When the point of insertion of the nerve can be located, the administration is advantageously carried out by one or more injections at

or near the said point. According to this embodiment, the efficiency of the transfer is greater because a high proportion of vector administered is absorbed at the level of the neuromuscular junction.

Preferably the intramuscular administration is carried out by injections at several points of the same muscle.

In another preferred embodiment the intramuscular administration is carried out by injection(s) at or near the point of insertion of the nerve.

The invention provides use of a recombinant adenovirus comprising, in its genome, a nucleic acid of interest for the preparation of a pharmaceutical composition for transferring the said nucleic acid into the cervical medullary motor neurons by administration into the muscles of the upper limbs. Typically the muscles are biceps or triceps. The invention also provides use of a recombinant adenovirus comprising, in its genome, a nucleic acid of interest for the preparation of a pharmaceutical composition for transferring the said nucleic acid into the thoracic medullary motor neurons by administration into the muscles of the thorax. Typically the muscles are pectoral muscles.

The invention provides use of a recombinant adenovirus comprising, in its genome, a nucleic acid of interest for the preparation of a pharmaceutical composition for transferring the said nucleic acid into the lumbar and/or sacral medullary motor neurons by administration into the muscles of the lower limbs. Typically the muscles are gastrocnemial muscles.

The method according to the invention can be carried out using adenoviruses of various origins. Different adenovirus stereotypes, whose structure and properties vary somewhat, have indeed been characterized. Among these stereotypes, the use of the type 2 or 5 human adenoviruses (Ad 2 or Ad 5) or the adenoviruses of animal origin (see application FR 93 05954) is preferred within the framework of the present invention. Among the adenoviruses of animal origin which can be used within the framework of the present invention, there may be mentioned adenoviruses of canine, bovine, murine (example: Mav1, Beard et al., Virology 75 (1990) 81), ovine, porcine, avian or alternatively simian (example: SAV) origin. Preferably, the adenovirus of animal origin is a canine adenovirus, or more preferably a CAV2 adenovirus [Manhattan strain or A26/61 (ATCC VR-800) for example].

According to a specific preferred embodiment of the invention, the adenovirus used is of human origin. According to another advantageous mode, the adenovirus is of animal origin.

The genome of adenoviruses comprises

especially an inverted repeat sequence (ITR) at each end, an encapsidation sequence (Psi), early genes and late genes. The principal early genes are contained in the E1, E2, E3 and E4 regions. Among these, the genes contained in the E1 (especially E1a and E1b) region are necessary for the viral replication. The E4 and L5 regions, for example, are, for their part, involved in the viral propagation. The principal late genes are contained in the E1 to L5 regions. The genome of the Ad5 adenovirus has been completely sequenced and is accessible on data base (see especially Genebank M73260). Likewise, part or even the entire genome of adenoviruses of different serotypes (Ad2, Ad7, Ad12, and the like) have also been sequenced. The adenoviral vectors used for carrying out the present invention comprise the ITRs, a sequence allowing the encapsidation and the nucleic acid of interest.

In a preferred embodiment of the invention, the adenovirus genome used lacks all or part of the E1 region. The E1 region is indeed essential for the viral replication and its inactivation leads to the formation of viruses defective for the replication, that is to say incapable of autonomously replicating in the infected cells. The E1 region, or any other viral region considered, can be made nonfunctional by any technique known to persons skilled in the art, and especially by total suppression, substitution, partial deletion, or addition of one or more bases in the

gene(s) considered. Such modifications can be obtained in vitro (on the isolated DNA) or in situ, for example, by means of genetic engineering techniques, or alternatively by treatment by means of mutagenic agents. Advantageously, the genome of the adenovirus used lacks part of the E1 region corresponding to residues 454 to 3328 (PvuII-Bg1II fragment) or 382 to 3446 (HinfII-Sau3A fragment).

According to a particularly advantageous embodiment, the genome of the adenovirus used also lacks all or part of the E3 and/or E4 region. The applicant has now shown that it is possible to construct vectors carrying these different types of deletions. These additional deletions make it possible to improve the safety of the vector and to increase its capacity. Thus the invention provides use of a recombinant adenovirus whose genome lacks all or part of the E1 region and all or part of the E3 and/or E4 region, and which comprises a nucleic acid of interest for the preparation of a pharmaceutical composition for transferring the said nucleic acid into the medullary motor neurons by intramuscular administration.

The nucleic acid of interest may be inserted into different sites of the adenovirus genome. Advantageously, it is inserted in the E1, E3 or E4 region. However, it is clear that the other sites can be used. In particular, access to the nucleotide sequence of the genome allows persons skilled in the art to identify or to create restriction sites which can be used to this end.

The defective recombinant adenoviruses according to the invention can be prepared by any technique known to persons skilled in the art (Levrero et al., Gene 101 (1991) 195, EP 185 573; Graham, EMBO

J. 3 (1984) 2917). In particular, they can be prepared by homologous recombination between an adenovirus and a plasmid carrying, inter alia, the DNA sequence of interest. The homologous recombination occurs after co-transfection of the said adenoviruses and plasmid into an appropriate cell line. The cell line used should preferably (i) be transformable by the said elements, and (ii) contain the sequences capable of complementing the defective adenovirus genome part, preferably in integrated form in order to avoid risks of recombination. As an example of a line, there may be mentioned the human embryonic kidney line 293 (Graham et al., J. Gen. Virol. 36 (1977) 59) which contains especially, integrated in its genome, the left-hand part of the genome of an Ad5 adenovirus (12 %).

A first method consists in transfecting the DNA of the recombinant (defective) virus prepared in vitro into a competent cell line, that is to say carrying in trans all the functions necessary for the complementation of the defective virus. These functions are preferably integrated into the genome of the cell, which reduces the risks of recombination, and confers an increased stability on the cell line. In the case of adenoviruses in which only the E1 region is deficient, the preferred line is the 293 line.

A second approach consists in co-transfecting into an appropriate cell line the DNA from the defective recombinant virus prepared in vitro and the

DNA from one or more helper plasmids or viruses. According to this method, it is not necessary to have a competent cell line capable of complementing all the defective functions of the recombinant adenovirus. Part of these functions is indeed complemented by the helper virus(es). This or these helper virus(es) are themselves defective.

Strategies for constructing vectors derived from adenoviruses have also been described in Application Nos. FR93/05954 and FR93/08596.

Next, the adenoviruses which have multiplied are recovered and purified according to conventional molecular biology techniques, as illustrated in the examples.

For their use according to the present invention, the adenoviruses are preferably combined with one or more vehicles pharmaceutically acceptable for an injectable formulation. These may be in particular isotonic, sterile, saline solutions (monosodium or disodium phosphate, sodium, potassium, calcium or magnesium chloride and the like or mixtures of such salts), or dry, especially freeze-dried compositions which upon addition, depending on the case, of sterilized water or physiological saline, allow the constitution of injectable solutions. The virus doses used for the administration may be adapted as a function of various parameters, and in particular as a function of the site (muscle) of administration

considered, the number of injections, the gene to be expressed or alternatively the desired duration of treatment. In general, the recombinant adenoviruses according to the invention are formulated and administered in the form of doses of between 10^4 and 10^{14} pfu, and preferably 10^6 to 10^{10} pfu. The term pfu (plaque forming unit) corresponds to the infectivity of a virus solution, and is determined by infecting an appropriate cell culture and measuring, generally after 15 days, the number of plaques of infected cells. The technique for determining the pfu titre of a viral solution are well documented in the literature.

The process according to the present invention is particularly advantageous for the treatment of medullary traumas or of motoneuronal degeneration diseases. Medullary traumas correspond more particularly to sections at the level of the motor neurons which deprive them of their afferences coming from the higher centres and cause their degeneration. The transfer of genes encoding growth factors into the sublesional motor neurons by retrograde transport according to the invention now offers the possibility of reducing or even preventing this degeneration. As regards neuropathies of the motor neuron, there may be mentioned for example amyotrophic lateral sclerosis, spinal amyotrophias type I (Werdnig Hoffman disease), type II or III (Kugelberg-Welander disease), bulbar spinal amyotrophias (such as Kennedy's disease). The

transfer of genes encoding growth factors or other molecules known to exert a neurotrophic effect on the motor neuron undergoing degeneration according to the present invention also offers a new route for the treatment of this type of pathology. The efficacy of the process of the invention can in particular be demonstrated on animal models: model of partial or complete section of the spinal cord, Wobbler mouse (animal model for studying amyotrophic lateral sclerosis (Leestma J.E., Am. J. Pathol., 100, 821-824)); mnd mouse (motor neuron degeneration: animal model for studying amyotrophic lateral sclerosis (Messer et al., 1992, Genomics. 18, 797-802)) or pmn mouse (progressive motor neuron neuropathy: animal model for studying motor neural degeneration during development), as illustrated in the Examples. The incorporation, tolerance and safety for man can be tested on *in vitro* models of culture of human embryonic medullary neurons.

In this regard, the nucleic acid of interest incorporated into the adenoviral vectors according to the invention preferably encodes a neuroactive substance. Typically the substance is capable of exerting a beneficial effect on nerve cells. It may be a substance capable of compensating for a deficiency in or of reducing an excess of an endogenous substance, or also of a substance conferring new properties on the cells. It may be in particular a growth factor, a neurotrophic factor, a cytokine, a neurotransmitter or an enzyme, or a receptor (e.g. a neurotransmitter receptor or a hormone receptor).

Preferably, among the growth factors, there may be mentioned colony stimulating factors (G-CSF, GM-CSF, M-CSF,

CSF, and the like), or fibroblast growth factors (FGFa, FGFb) or vascular cells (VEGF). Among the neurotrophic factors, the preferred factors are especially the ciliary neurotrophic factor (CNTF), the glial cells maturation factors (MGFa, b), GDNF, BDNF, NT3 and NT5. Thus the invention provides use of a recombinant adenovirus comprising, in its genome, a nucleic acid encoding NT3 for the preparation of a pharmaceutical composition for transferring the said nucleic acid into the medullary motor neurons by intramuscular administration.

One embodiment provides use of a recombinant adenovirus comprising, in its genome, a nucleic acid encoding a neurotrophic factor for the preparation of a pharmaceutical composition for the treatment of amyotrophic lateral sclerosis by transferring the said nucleic acid into the medullary motor neurons by intramuscular administration.

The preferred cytokines are the interleukins and the interferons and, among the enzymes, the enzymes for the biosynthesis of neuro transmitters (tyrosine hydroxylase, acetylcholine transferase, glutamic acid decarboxylase), the lysosomal enzymes (hexosaminidases, arylsulphatase, glucocerebrosidase, HGPRT), the enzymes involved in the detoxification of free radicals (super oxide dismutase I, II or III, catalase, glutathione peroxidase) are preferably used. Among the receptors, there may be used, *inter alia*, the androgen receptors (involved in Kennedy's disease).

These different factors may be used in native form, or in the form of a variant or fragment having an activity of the same type.

It is also possible to transfer antisense sequences.

The nucleic acid may be of natural or artificial origin.

It may be especially genomic DNA (gDNA), complementary DNA (cDNA), hybrid sequences or synthetic or semisynthetic sequences. It may be of human, animal, plant, bacterial or viral origin and the like. It may be obtained by any technique known to persons skilled in the art, and especially by screening libraries, by chemical synthesis, or alternatively by mixed methods including chemical or enzymatic modification of sequences obtained by screening libraries. It is preferably cDNA or gDNA.

When the nucleic acid encodes a substance beneficial to nerve cells the nucleic acid may comprise, in addition, signals allowing the expression of the substance in the motor neurons.

Thus generally, the nucleic acid also comprises a promoter region for functional transcription in the motor neurons, as well as a region situated in 3′ of the gene of interest, and which specifies a signal for termination of transcription and a polyadenylation site. All these elements constitute the expression cassette. As regards the promoter region, it may be a promoter region naturally responsible for the expression of the considered gene when the said region is capable of functioning in the infected cell. It may also be regions of different origin (responsible for the expression of other proteins, or even synthetic). In particular, it may be promoter sequences of eucaryotic or viral genes. For example, it may be promoter sequences derived from the genome of the cell which it is desired to infect. Likewise, it may be promoter sequences derived from the genome of a virus, including the adenovirus used. In this regard, there

may be mentioned, for example, the promoters of the E1A, MLP, CMV and RSV genes and the like. In addition, these promoter regions may be modified by addition of activating or regulatory sequences or sequences allowing a tissue-specific or predominant expression (enolase and GFAP promoters and the like). Moreover, when the heterologous nucleic acid does not contain promoter sequences, it may be inserted into the virus genome downstream of such a sequence.

The subject of the present invention is also a method for the transfer of nucleic acids into motor neurons comprising the muscular administration of an adenoviral vector incorporating the said nucleic acid into its genome. Preferably, the method according to the invention is carried out by injection(s) at several points of the same muscle, or when the point of insertion of the nerve can be located, by one or more injections at the level of or close to the said point.

The present invention will be described in greater detail with the aid of the following examples which should be considered as illustrative and nonlimiting.

Legend to the figures

FIGURE 1 (PHOTOGRAPH A):

β-galactosidase labelling of longitudinal sections (50 μm) of rat spinal cord at the lumbar level, after intramuscular injection of adenovirus-β-galactosidase into the gastrocnemial muscle,

- diffuse labelling of numerous motoneuronal cellular bodies (Δ),
- more intense labelling of a few motor neurons at the level of the cellular body and of the neurites,

revealing the typical morphology of the motor neurons (▲).

FIGURE 2 (PHOTOGRAPH B): identical to A, higher magnification.

β-galactosidase labelling of longitudinal sections (50 μm) of rat spinal cord at the lumbar level, after intramuscular injection of adenovirus-β-galactosidase into the gastrocnemial muscle.

FIGURE 3 (PHOTOGRAPH C):

β-galactosidase-immunocytochemical co-labelling (Calcitonin Gene Related Peptide (CGRP) of longitudinal sections (50 μm) of rat spinal cord at the lumbar level, after intramuscular injection of adenovirus-β-galactosidase into the gastrocnemial muscle.

Examples

1. Injection of adenovirus - b-galactosidase into the gastrocnemial muscle in intact rats or in rats having undergone a thoracic hemisection of the spinal cord.

This example describes the transfer of the b-gal gene at the level of the lumbar motor neurons by

administration into the gastrocnemial muscle of an adenovirus incorporating the said gene.

More particularly, the study was carried out on a model of partial or complete section of rat spinal cord performed at the low thoracic level which has the effect of paralysing the animal in one or both of its lower limbs. Such a section deprives the motor neurons of their afferences coming from the upper centres and bring about their degeneration. The administration was carried out so as to infect the sublesional motor neurons by retrograde transport.

The adenoviral vector used in this example is the Ad.RSV.βgal vector. This vector lacks sequences necessary for its replication, but comprises, nevertheless, the sequences necessary for penetrating into the cells infectable by the said vector as well as all the essential sequences necessary for the encapsidation of this adenovirus. It also carries, under the control of the RSV promoter, the E. coli β-galactosidase gene. The construction of the defective recombinant adenovirus Ad.RSVβgal has been described in the literature (Stratford-Perricaudet et al., J. Clin. Invest. 90 (1992) 626). Briefly, the adenovirus Ad.RSVbgal is a defective recombinant adenovirus (from which the E1 and E3 regions have been deleted) obtained by homologous recombination in vivo between the mutant adenovirus Ad-d1324 (Thimmappaya et al., Cell 31 (1982) 543) and the plasmid pAd.RSVbgal (Akli et al., 1993).

The plasmid pAd.RSVbgal contains, in the 5' → 3' orientation,

- the PvuII fragment corresponding to the left end of the Ad5 adenovirus comprising: the ITR sequence, the origin of replication, the encapsidation signals and the enhancer E1A;

- the gene encoding b-galactosidase under the control of the RSV promoter (Rous sarcoma virus),

- a second fragment of the Ad5 adenovirus genome which allows homologous recombination between the plasmid pAd.RSVbgal and the adenovirus dl324.

After linearization with the ClaI enzyme, the plasmid pAd.RSVbgal and the adenovirus dl324 are co-transfected into the line 293 in the presence of calcium phosphate in order to allow homologous recombination. The recombinant adenoviruses thus generated are selected by plaque purification. After isolation, the recombinant adenovirus DNA is amplified in the cell line 293, which leads to a culture supernatant containing the unpurified recombinant defective adenovirus having a titre of about 10^{10} pfu/ml. The viral particles are then purified by centrifugation on a caesium chloride gradient according to known techniques (see especially Graham et al., Virology 52 (1973) 456). The adenovirus was then used in purified form in a phosphate buffered saline (PBS).

Three injections of adenovirus Ad-RSV-b-gal (10^7 pfu per injection) were performed into the

gastrocnemial muscle, just after the animal has (or otherwise) undergone a hemisection of the spinal cord (low thoracic level, which has the effect of paralysing the animal in one of its lower limbs). 9 μl of adenovirus are injected per point of injection with a Hamilton syringe.

The animals were sacrificed (perfusion 4% paraformaldehyde) four days after injection, minimum time for the retrograde transport to occur from the muscle to the spinal cord. Three blocks of spinal cord were cut longitudinally at the cervical, thoracic and lumbar levels, into sections 50 mM thick. The sections were treated for revealing the β-galactosidase which makes it possible to visualize the cells which have been infected by the virus. Some sections were furthermore subjected to an anti-Calcitonin Gene Related Peptide (CGRP) immunocytochemistry which makes it possible to specifically label the motor neurons.

The β-galactosidase was revealed using its substrate, X-gal, and the product of the reaction gives a blue colour.

The Calcitonin Gene Related Peptide, CGRP, is a neurotransmitter, a specific marker for the motor neurons. It is revealed by immunocytochemistry with a secondary antibody coupled to peroxidase and as enzyme substrate diaminobenzidine; the product of the reaction gives a chestnut colour.

The revealing of b-galactosidase made it

possible to visualize the presence of the infected motor neurons, exclusively at the sublesional lumbar level in the case of the hemisectioned rats, and on the side corresponding to the injection.

Two types of labelling were obtained, a diffuse labelling of the cellular body of a large number of motor neurons, and a more intense labelling of the cellular body and of the neurites of a more limited number of motor neurons (photographs A and B). This difference in labelling intensity is probably due to the fact that only a few motor neurons, very close to the site of injection, were able to absorb the virus intensely.

The anti-CGRP immunocytochemistry coupled to the b-galactosidase revealing made it possible to demonstrate, by a double staining, that practically all the CGRP-positive cellular bodies (i.e. motor neurons) were infected by the virus (photograph C).

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. Use of a recombinant adenovirus comprising, in its genome, a nucleic acid of interest for the preparation of a pharmaceutical composition for transferring the said nucleic acid into the cervical medullary motor neurons by administration into the muscles of the upper limbs.

2. Use according to claim 1 wherein the muscles are biceps or triceps.

3. Use of recombinant adenovirus comprising, in its genome, a nucleic acid of interest for the preparation of a pharmaceutical composition for transferring the said nucleic acid into the thoracic medullary motor neurons by administration into the muscles of the thorax.

4. Use according to claim 3 wherein the muscles are pectoral muscles.

5. Use of a recombinant adenovirus comprising, in its genome, a nucleic acid of interest for the preparation of a pharmaceutical composition for transferring the said nucleic acid into the lumbar and/or sacral medullary motor neurons by administration into the muscles of the lower limbs.

6. Use according to claim 5 wherein the muscles are gastrocnemial muscles.

7. Use of a recombinant adenovirus whose genome lacks all or part of the E1 region and all or part of the E3 and/or E4 region, and which comprises a nucleic acid of interest for the preparation of a pharmaceutical composition for transferring the said nucleic acid into the medullary motor neurons by intramuscular administration.

8. Use of a recombinant adenovirus comprising, in its genome, a nucleic acid encoding NT3 for the preparation of a pharmaceutical composition for transferring the said nucleic acid into the medullary motor neurons by intramuscular administration.

9. Use according to any one of Claims 1 to 8, characterized in that the adenovirus is of human origin.

10. Use according to any one of Claims 1 to 8, characterized in that the adenovirus is of animal origin.

11. Use according to any one of the preceding claims, characterized in that the nucleic acid of interest is inserted into the genome of the adenovirus in the E1, E3 or E4 region.

12. Use according to any one of Claims 1 to 7, characterized in that the nucleic acid of interest encodes a substance which is capable of exerting a beneficial effect on nerve cells.

13. Use according to Claim 12, characterized in that the nucleic acid of interest encodes a growth factor, a neurotrophic factor, a cytokine, a neurotransmitter or an enzyme, or a receptor.

14. Use according to Claim 12, characterized in that the nucleic acid of interest comprises, in addition, signals allowing the expression of the substance in the motor neurons.

15. Use according to any one of Claims 1 to 8, characterized in that the intramuscular administration is carried out by injections at several points of the same muscle.

16. Use of a recombinant adenovirus comprising, in its genome, a nucleic acid encoding a neurotrophic factor for the preparation of a pharmaceutical composition for the treatment of amyotrophic lateral sclerosis by transferring the said nucleic acid into the medullary motor neurons by intramuscular administration.

17. Method of transferring a nucleic acid into the cervical medullary motor neurons

comprising administering a recombinant adenovirus which comprises the nucleic acid in its genome, into the muscles of the upper limbs.

18. Method of transferring a nucleic acid of interest into the thoracic medullary motor neurons comprising administering a recombinant adenovirus which comprises the nucleic acid in its genome, into the muscles of the thorax.

19. Method of transferring a nucleic acid of interest into the lumbar and/or sacral medullary motor neurons comprising administering a recombinant adenovirus which comprises the nucleic acid in its genome, into the muscles of the lower limbs.

20. Method of transferring a nucleic acid of interest into the medullary motor neurons comprising administering a recombinant adenovirus whose genome lacks all or part of the E1 region and all or part of the E3 and/or E4 region and which comprises the nucleic acid, into a muscle.

21. Method of transferring a nucleic acid encoding NT3 into the medullary motor neurons comprising administering a recombinant adenovirus which comprises the nucleic acid in its genome, into a muscle.

22. Method of transferring a nucleic acid encoding a neurotrophic factor into the medullary motor neurons for the treatment of amyotrophic lateral sclerosis comprising administering a recombinant adenovirus which comprises the nucleic acid in its genome, into a muscle.

23. Use according to any one of Claims 1, 3, 5, 7, 8 or 16 substantially as hereinbefore described in any one of the Examples.

24. Method according to any one of Claims 17 to 22 substantially as hereinbefore described in any one of the Examples.

DATED this 15th day of September, 1999.

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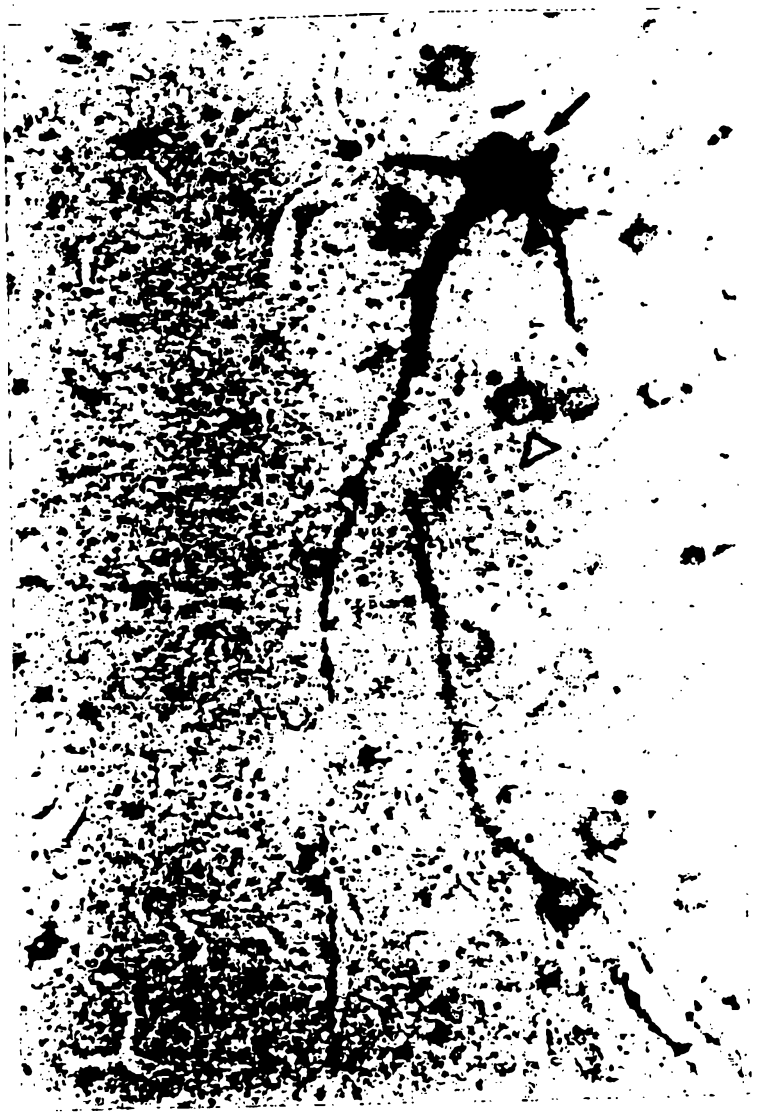

Figure 1

Figure 3

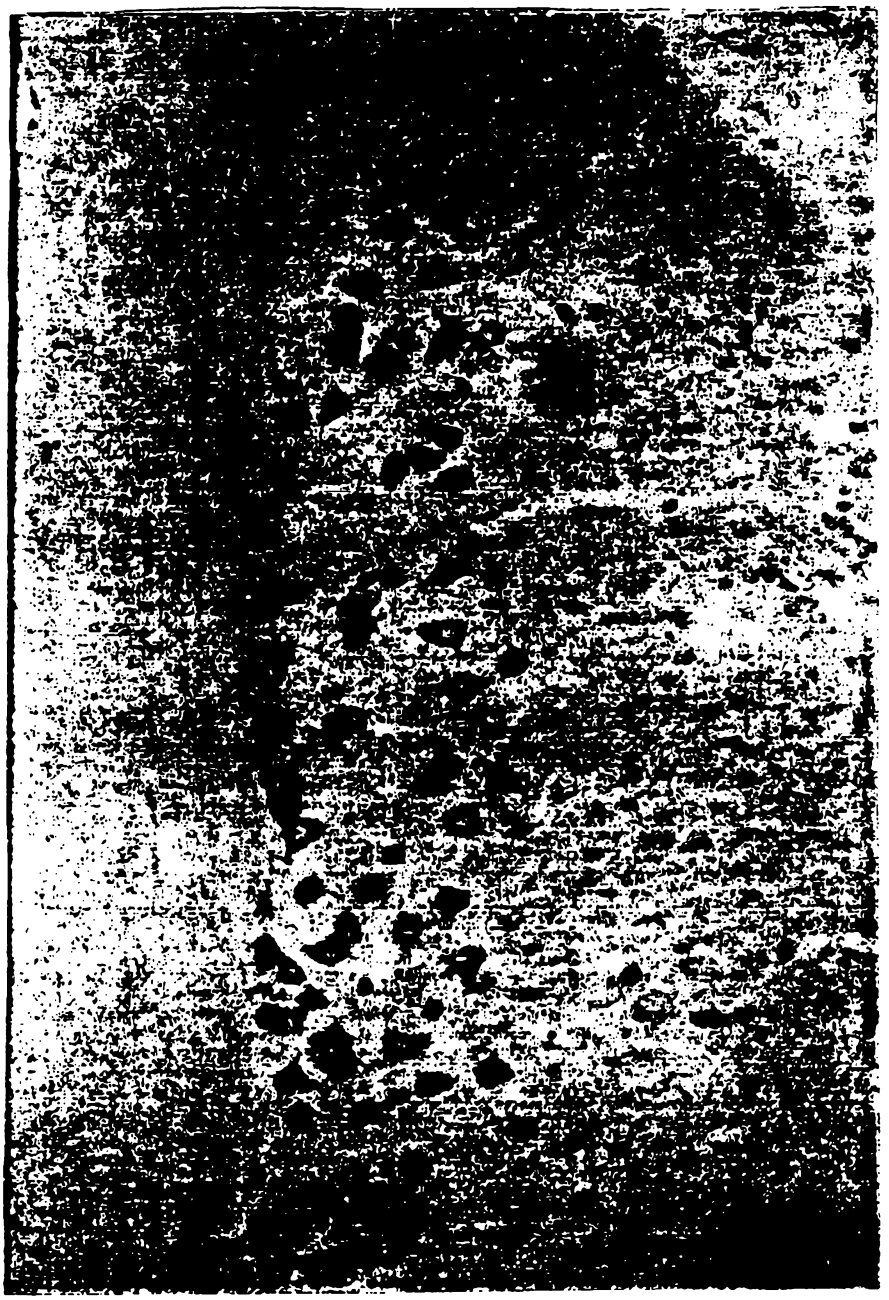

Figure 3