Salud Mental 2013;36:219-227
ISSN: 0185-3325
DOI: 10.17711/SM.0185-3325.2013.028

Novel Vaccines against morphine/heroin

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Original article

SUMMARY

Drug addiction is one of the most important health problems worldwide. This pathology results in the death of about 500 000 individuals annually around the globe. Despite this scenario, the development of effective drug therapies against this disease has been slow and not very successful. In recent years, new alternative pharmacological strategies against drug addiction have been designed and validated. Among them are vaccines and their use in immunotherapeutic pharmacological procedures for the treatment of addictive behaviors, both in animal models and in humans.

These new experimental strategies are based on the design and synthesis of various structural formulations of therapeutic vaccines against drugs of abuse. When such vaccines are dosed in active immunization schedules, they induce the production of specific serum antibodies, which recognize and bind these substances in the systemic intravascular space and prevent the drug permeability through the blood-brain barrier, resulting in decreased effects of drugs into the brain.

In 2006, our research group at the National Institute of Psychiatry Ramón de la Fuente Muñiz (INPRFM, in Spanish) achieved and consolidated the design, synthesis, application and validation of immunoprotective therapeutic effects against relapse to morphine/heroin addiction in a rodent animal model, a model vaccine for potential human use against addiction to such substances. This model shows immunogenic capacities (high and sustained titers of highly specific antibodies) and immunoprotection (attenuates the effect up to 15mg/kg sc of morphine) that the structural vaccine models competing have not matched. This makes it the leading vaccine model against the addictive effects of heroin and morphine.

Key Words: Addiction, morphine/heroin, vaccines, immunotherapy, active and passive immunization.

RESUMEN

La adicción a una droga de abuso representa uno de los problemas sanitarios más importantes ya que esta patología genera la muerte de cerca de 500 000 sujetos anualmente en el mundo. A pesar de este panorama, el desarrollo de terapias farmacológicas efectivas contra esta enfermedad es lento y poco exitoso. En los últimos años se han diseñado y validado nuevas estrategias farmacológicas alternativas contra la adicción a drogas de abuso, como las vacunas y su uso en procedimientos farmacológicos inmunoterapéuticos para el tratamiento de esas conductas tanto en modelos de animales como en el humano.

Estas nuevas estrategias experimentales están basadas en el diseño y síntesis de diversas formulaciones estructurales de vacunas terapéuticas contra las sustancias de abuso las cuales, al ser dosificadas en esquemas de inmunización activa, inducen la producción de anticuerpos séricos específicos que reconocen y se unen a estas sustancias en el espacio intravascular sistémico e impiden que crucen la barrera hematoencefálica, con lo cual disminuyen sus efectos en el cerebro.

En el año 2006 nuestro grupo de trabajo en el Instituto Nacional de Psiquiatría Ramón de la Fuente Muñiz (INPRFM) logró y consolidó el diseño, síntesis, aplicación y validación de efectos terapéuticos inmunoprotectores contra recaídas al consumo adictivo de morfina/heroína, en un modelo animal con roedores y su escalamiento potencial para uso humano contra la adicción a esas sustancias. Este modelo muestra capacidades inmunogénicas (títulos altos y sostenidos de anticuerpos altamente específicos) y de inmunoprotección (atenúa el efecto de hasta 15mg/Kg sc de morfina) que los modelos estructurales de vacuna desarrollados por otros grupos de investigadores no han podido igualar. Esto lo convierte en un modelo líder de vacuna contra los efectos adictivos de la heroína y morfina.

Palabras clave: Adicciones, morfina/heroína, vacunas, inmunoterapéutica, inmunización activa y pasiva.

Translation of the original version published in spanish in: Salud Mental 2015, Vol. 36 Issue No. 3.

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INTRODUCTION

The addiction to a drug of abuse stands for one of the most important medical problems worldwide. Every year, the addiction around the globe to nicotine and to alcohol, for example, causes the death of about 440 000 and 80 000 individuals, respectively. In the United States, illicit drug abuse and addiction mean to society an approximate cost of $180.90 billion dollars per year, without considering social and family complications related to this problem.

Despite this difficult outlook and that today the dependent subjects can have an easier access to multiple drugs of abuse, the development of effective pharmacological therapies against this illness has not delivered the expected results. Throughout the three last decades, several research centers and pharmaceutical companies have synthesized and validated a wide variety of medication aimed at detoxifying, decreasing the affective symptoms observed during abstinence, reducing *craving* and/or preventing relapses of addictive use in addicts to opioids and psychostimulants at global level.[1]

However, in spite of the fact that the Food and Drug Administration (FDA) has certified the use of a great variety of such medications, their effect in anti-addictive clinical treatments has not been successful. The initial therapeutic procedure within the treatment of a dependent or addictive subject to any drug of abuse is the use of acute detoxification treatments mainly aimed at reducing and/or blocking the abstinence signs and symptoms. The use of such treatments is easy and economical; nevertheless, so far there is no solid proof of long-term effective results on the maintenance of abstinence in subjects undergoing detoxification treatments.

It is likely that the failure of these detoxification treatments in maintaining abstinence for long periods of time is due to the impossibility of reversing and/or modulating the neuroplastic alterations that chronic use caused over the different neurotransmission systems and pleasant strengthening pathways.[1-3] In addition, most of these therapeutic aspects cause important medium- and long-term toxic collateral effects, which might explain the low attachment the addictive subject shows to these treatments.[1]

Nowadays there is a thought that pharmacological treatments that seek to reduce the abstinence and *craving* symptoms during a chronic addiction, and finally to maintain a withdrawal state during long periods of time, require a very long-term treatment periods (*e.g.*, months or years) and are generally longer than those used in detoxification.[4,5] Furthermore, they are to be applied together with psychotherapy, supportive, expression and motivational development therapies, as well as cognitive-behavioral therapies, among others, in order to extend the abstinence period and maximally prevent long-term relapses.[6]

Today, the neuroadaptive changes in the brain, on a cellular and molecular level, induced by addictive drugs are better known. Thus, also the key role that the dopaminergic system has as a mediator of reward responses has been defined in a more precise manner. This has allowed different laboratories to develop new pharmacological strategies focused on the blocking of the brain pharmacodynamic effects of drugs of abuse.

In the last decade, the National Institute on Drug Abuse (NIDA) has been involved in the development of international programs related to the design of new medications to control and counteract the psychostimulant and opioid addiction on addictive subjects.[1,7,8] Now, there is a high number of medications in process to be approved by the FDA for the treatment of the addiction to such drugs of abuse. Nonetheless, all of these substances of the pharmacopoeia, collectively, have shown a poor therapeutic capability, both in the short and the long term, within the anti-addictive process.[7,8]

In summary, currently, from the therapeutic point of view, most of the classic anti-addictive pharmacological treatments against the addiction to different drugs —such as cocaine or morphine— have not shown significant efficacy. Most of these medications have important collateral toxic effects, so most of the subjects under treatment give up their dosage.[7,8] Due to these disadvantages alternative strategies have been designed. Striking examples of such new strategies are vaccines and their use in immunotherapeutic pharmacological procedures for the treatment of addictive behaviors, both in animal models and in humans.[9]

IMMUNOPHARMACOTHERAPIES

These new experimental strategies are based on the design and synthesis of various structural formulations of therapeutic vaccines against cocaine,[10-13] nicotine[14-16] and heroin/morphine adiction,[17-20] when such vaccines are dosed in active immunization schedules in animal models such as the rodent or the human being, they induce the production of specific serum antibodies, which recognize and bind these substances in the systemic intravascular space. These anti-drug antibodies have the ability to seize the addictive compound circulating in the bloodstream since antibodies are macromolecules (≈150 kD) that normally do not permeate through the blood-brain barrier, thus creating antibody-drug molecular complexes of a high molecular weight, which "seize" and prevent the passing of drug through the blood-brain barrier.[21,22]

Now therefore, in this pharmacokinetic alteration condition of the drug, there is a very significant reduction of the plasmatic "free drug" fraction that spreads to the extracellular space of the nerve and brain tissue and, therefore, which would be available for the functional union and/or blocking of the molecular mechanisms through which they carry out their effect (increase in the dopamine release).[21,22]

Thus, when the synaptic concentration of this neurotransmitter is reduced by the use of the drug, the pleasant strengthening re-consumption will not be anymore developed with an addictive pattern on the actively vaccinated subject and immune to the same substance.[21,22] As a result of this "pharmacological antagonism of the drug's blood-brain permeation" significant alteration, the induced pleasant strengthening value diminishes outstandingly, which reduces the percentage of consumption relapses[21,22] (Figure 1).

This experimental pharmacological strategy of an "immunoprotective" nature has proved to be a treatment with therapeutic efficacy to significantly reduce and/or inhibit the seeking and addictive use behaviors of cocaine, nicotine and morphine/heroin, at a pre-clinical level both in rodents[23-32] and humans.[33,34] Likewise, it is important to emphasize that considering the nature of the aforementioned pharmacokinetic mechanism —in connection with the effects of the drug— when the permeation of the drug towards the brain tissue is significantly diminished the strengthening effects induced by the drug at a central level are blocked, and also there are no collateral toxic side effects often generated with the typical medications commonly used against addiction processes.[33-36]

The immunotherapies designed (both at pre-clinical and clinical levels) that have currently been assessed against the addiction may be divided in a general way, in terms of the paradigms in which they are based into, in "active vaccination" processes (those of the highest rate of reported studies) and, additionally, some other processes of a lower frequency of application and use, focused on "passive vaccination".

Active vaccination is the traditional method of immunization against different diseases. In this case through the administration of formulations of infectious agents (*e.g.*, viruses, bacteria, parasites), chemically attenuated in its virulence or even dead or mechanically fragmented. They confer humoral and/or cellular defense, with long-term memory. This procedure generates antibodies with a great, wide and specific recognition capability of certain antigens or antigenic determinants that invade the body.[21,35-37]

Nevertheless, the immune system also shows the "non-recognition antigenic" capability to numerous low-mass and structural complexity molecules (*v.g.*, ≤ 100-300 daltons), called haptens, which by themselves do not have the capability to generate a activation response (immunogenicity) of the efficient humoral immune response (specific antibodies). Within this context, the drugs of addictive abuse may be included in this group of chemical compounds, given that their nominal molecular mass is of approximately 300 daltons and of a scant molecular complexity.[21,35,36]

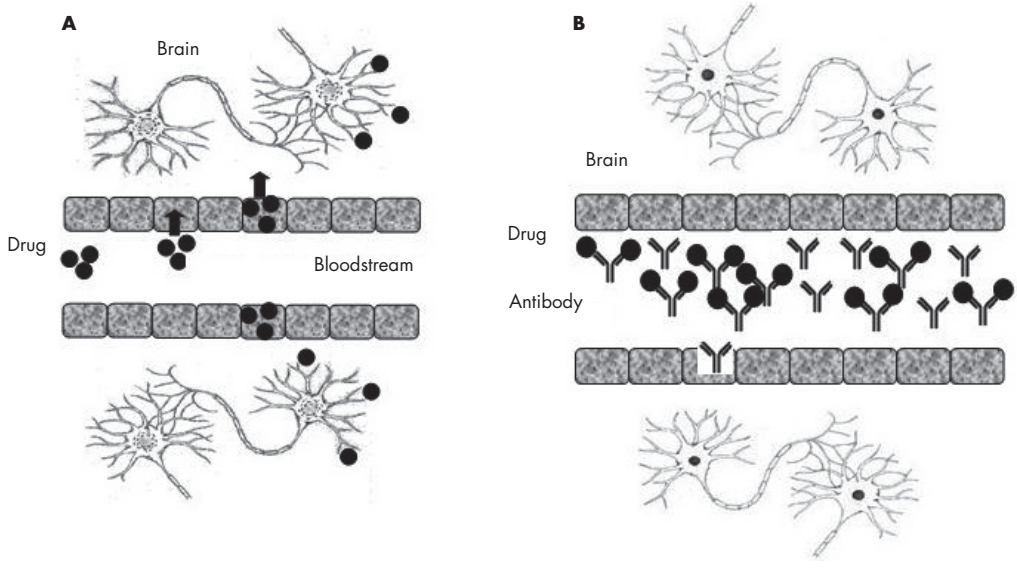

Figure 1. Vaccination Programs. The drugs of abuse are very small molecules having a very simple molecular structure. In addition, physically and chemically these molecules easily cross the blood-brain barrier (A). Together these features allow that the drugs of abuse cannot be recognized by the immune system. On the other hand, when an immunoconjugated is dosed under an active immunization schedule (B), it induces the production of specific serum antibodies, which recognize and bind these substances in the systemic intravascular space. These anti-drug antibodies have the ability to seize the addictive drug circulating in the bloodstream once consumed by the subject, thus creating antibody-drug molecular complexes of a high molecular weight, which "seize" and prevent the permeability of the drug through the blood-brain barrier, thus avoiding the development of the pleasant strengthening induced by a drug of abuse.

Translation of the original version published in spanish in:
Salud Mental 2015, Vol. 36 Issue No. 3.

One of the advantages of active immunization through the dosage of a vaccine or immunogenic preparation is that the average life of generated antibodies is of extended duration (usually between 30 and 90 days), allowing that the immune protection effect mediated by the specific circulating antibodies also spreads for long periods of time (usually between 2 and 6 months).[21,22,35,36] Another advantage is that the pharmacokinetic properties of the drug (such as its metabolism and plasma clearance) are not modified by the procedures of the active immunization against the drug itself.[21,22,35,36] In order to achieve that the immune system is able to efficiently recognize molecules that are strange (antigens) to haptens such as cocaine or morphine, it must be covalently condensed to high-mass and structural complexity protein carrying systems (conjugated-drug-carrying protein =vaccine), which have the capability to structurally present the drug as an antigenic or "strange" molecule to the immune system, and then that it generates an intense response of specific circulating immunoglobulins against the drug.[21,22,35,36]

The pharmacological efficacy degree of a molecular conjugated formulation (vaccine) of addictive drug-carrying protein to stimulate its corresponding humoral immune response is called "immunogenic capacity" of the vaccine.[21,22,35,36] It bears mention that despite different scientific studies have been reported to date proving that active vaccination is an efficient experimental procedure, with capability to generate high concentrations of specific serum antibodies against addictive drugs like morphine/heroine,[17,18,38] nicotine[39,40] and cocaine,[23-28] at a clinical level the results have not been very encouraging. Now, it is known that the success of this immunotherapeutic strategy is mainly based on three parameters: magnitude of antibody concentration, called titer; antibody affinity and specificity towards certain molecular structure (hapten); and the capacity to generate drug-specific antibodies in constitutive manner (long-term maintenance of titer). It is important to mention that the presence of these three parameters depends exclusively on the elements making up the structural model of the immunoconjugated one.

The magnitude of the immunogenic response, measured as antibody concentration, is the result of the type of carrying protein used, to which the hapten is joined, as well as the type of adjuvant used in the final antigenic formulation.[13,35-37,41,42] Another very important characteristic related to the type of carrying protein used is the capability to stimulate TOLL-type receptors, which allow the activation of B cells that produce specific antibodies for the drug in the long term (memory). On the other hand, the antibody affinity and specificity to a hapten are directly linked to a successful immunogenic presentation, which results from a good immunogenic conjugated molecular design (design: Long-hapten and type of space-arm - binding site of hapten to the carrying protein).

VACCINES AGAINST MORPHINE/HEROINE

There is a lack of reports of opioid-addiction immunotherapies. Many of them use vaccination protocols with different structural models and different types of reactions for the hapten obtaining, adapting the hapten in different positions of the molecule. While most of them basically use the morphine for the hapten generation, this is covalently adjusted to different immunogenic carrying proteins[43-47] which, jointly, hinders the analysis of the effects of same.

The first experimental approaches were made 42 years ago, which were mainly focused on the generation of polyclonal antibodies against morphine (Table 1). Nevertheless, none of these reports assessed the amount of antibody generated by the vaccination procedure, nor the pharmacologic antagonism based on antibodies with the purpose of attenuating the behavior effects of opioid drugs. However, they have provided sufficient information for the development of new immunogenic conjugated ones that have the purpose of reducing the effect of different doses of morphine, heroin and its metabolites.

Overall, their data suggest that if the reaction binds the hapten to the position 3´ of morphine, what shall be produced is an immune response able to preferably bind structurally related molecules such as codeine. On the other hand, if the hapten is bound to the position 6´ the antibodies show an equivalent specificity both for heroine and for morphine or codeine. Finally, if the hapten is bound to the position 2´ of morphine then the immune response is generated with greater specificity to morphine.[17-20]

In 2006, our research group, headed by Dr. Benito Antón, achieved in the INPRFM the design and synthesis of a vaccine model for potential human use against morphine/heroin addiction.[17,18] The initial objective was achieving its mechanism was valid and applicable to produce immunoprotective therapeutic effects against relapses to the morphine/heroine addictive use in an animal model with rodents (pre-clinical stage). One of the main advantages of this structural model of the morphine/heroine bivalent (M-TT) vaccine is that its elements are certified by the FDA for human use, which allows it to be quickly used in clinical protocols. The carrying protein used is the tetanus toxoid that is a high-mass and structural complexity protein, which bestows it the capability to be a very antigenic protein. This carrying protein is one of the most used in human active vaccination protocols in order to prevent tetanus with minimal toxic side effects. As the tetanus toxoid is a very antigenic protein it bestows the M-TT vaccine model the capability to strongly stimulate the immune system, generating a large amount of antibodies (very high antibody titers). Another very important characteristic is that it bestows the required structural complexity for stimulating TOLL-type receptors; this

Table 1.

Author	Hapten	Carrying Protein	Antibody specificity
Spector S and Parker CW, 1970.	3-Ocarboxymethyl-morphine-1	BSA	Certain specificity to morphine. Cross-reaction to codeine.
Wainer BH and cols., 1973. Simon EJ and cols., 1972. Koida M and cols., 1974	Morphine-6-hemisuccinate (M6H), adjusted to position 3´ of morphine	BSA	They recognize with the same specificity to heroin, morphine and codeine; but not to naloxone.
Spector S and cols., 1973	Diazotyde-p-aminoacetanilide, adjusted to position 2´ of morphine	BSA	Specific antibodies to morphine, heroin and codeine.
Gross S and col., 1974	Azo-morphine, adjusted to position 2´ of morphine	KLH	Antibodies with a high specificity to morphine, which showed low cross-reactivity to codeine and heroine.
Koida M and cols., 1974	Morphine-3-glucuronide, adjusted to position 6´ of glucuronide	BSA	Antibodies with certain specificity to morphine, codeine and M3G.
Morris B and cols., 1975	N-succinil-normorphine	BSA	Specificity to morphine. Do not show cross-reactivity with codeine and show cross-reactivity with heroine.
Findlay J and cols., 1981	Ncarboxipropyl-morphine	BSA	Specificity to morphine, minimum cross-reactivity with codeine.
Usagawa T and cols., 1993	Naminobutyl	BSA	Specific antibodies to morphine, with low cross-reactivity for codeine, M6G and M3G.
Beike J and cols., 1998	Binding of a nitrogen bridge to the hapten N-aminopropyl	BSA	Generated antibodies were specific to morphine, M3G and M6G with low cross-reactivity to codeine, codeine-6-glucoronide and dihydrocodeine.

is possible because the carrying protein —being an enormous molecular mass molecule made up by a structural pattern of multi-repetition polypeptide subunits— allows the stimulation of these receptors, which induce the release of T-lymphocyte cytokines, which in turn stimulate and activate B cells, producers of specific antibodies for the drug in the long term (memory). These antibodies have the feature that they are not eventually reduced in a significant way, constituting a unique feature in the generation area of addictive vaccines. The hapten of the M-TT vaccine is the Morphine-6-hemisuccinate. The hapten is adjusted to the carrying protein in the position 6´ of the morphine's molecule, which —as had been previously reported— generates antibodies with an equivalent specificity both for heroine and for morphine.

Our group has published several papers showing that a vaccination program in the rat and the mouse causes an intense humoral response. In these preclinical trials the M-TT vaccine was dosed between 50-200 µg/Kg, and aluminium hydroxide was used as adjuvant. The maximum titers, generated by this procedure amounted to 1:250000, producing 0.8 ± 0.2 mg/ml of specific immunoglobulins, in serum, after the fourth immunization. Additionally, the antibody titer was kept practically unaltered by a period of approximately six to eight months. Subsequently, there is a progressive decline of anti-morphine/heroine antibodies, reaching levels not detectable from 10 to 12 months after their last immunization. This suggests that the M-TT vaccine not only is effective in generating large amounts of antibodies but also is capable of activating, in the long term, the humoral immunological memory against these opioids[17,18,48] (Figure 2).

Recently, Li et al., Raleigh et al. and Stowe et al. reported the development of new vaccine models against morphine/heroine,[19,20,49,50] the Morphine-KLH, the M-KLH and the dynamic vaccine, respectively. The immunization with these new vaccine models generated antibody titers of 1:100000 for the Morphine-KLH, 1:100000 for the M-KLH, 1:160000 for morphine, and 1:120000 for heroine, in the case of the dynamic vaccine, after the fourth immunization, respectively.

For the Morphine-KLH vaccine, the antibody titer diminishes rapidly after the last immunization. Between 8-10 days after the last immunization the antibody titers are undetectable. In the case of the M-KLH and the dynamic vaccine the titers for both were of 1:50000 after 21 days of the last immunization.[17,18,49,50]

As mentioned above, the magnitude of the immunogenic response is the result of the type of carrying protein used as well as the type of adjuvant used in the final an-

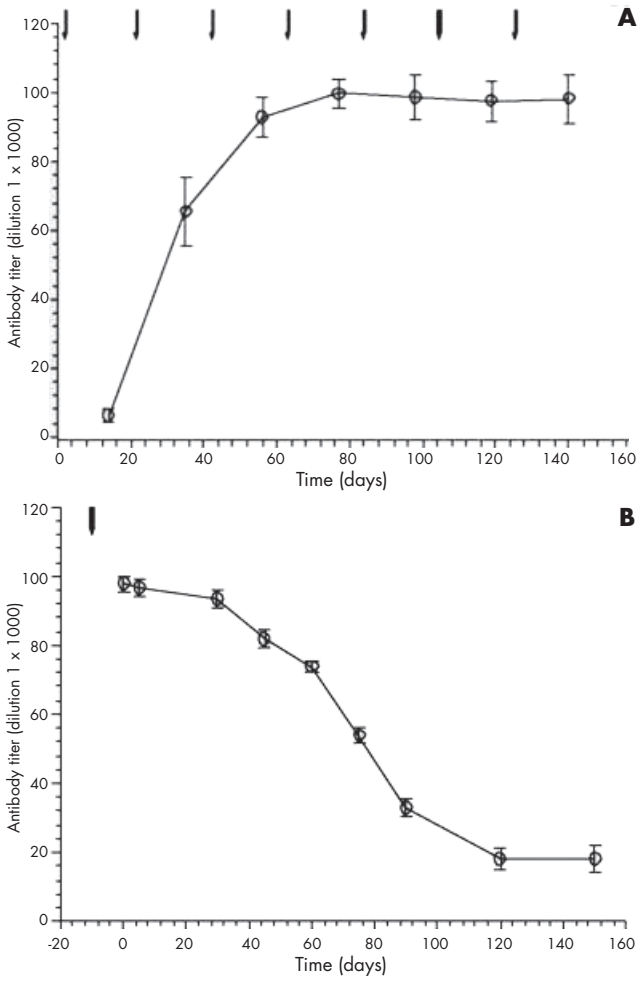

Figure 2. Characterization of the humoral immune response induced by an active vaccination program with the M-TT vaccine in rat. A) Temporal course of the anti-morphine antibody titer in rats immunized with the M-TT vaccine. The arrows indicate the immunizations and 14 days later the antibody titers were in the vaccinated group (empty circles). B) Temporal course of the decline kinetics of the anti-morphine antibody titers. A slow and progressive decline in the serum antibody levels were observed after the last immunization (arrow).

tigenic formulation. If we consider the carrying protein used by each type of vaccine, then the Morphine-KLH, the M-KLH and the dynamic vaccines should generate antibody titers greater than the M-TT vaccine since they use KLH (Keyhole Limpet Hemocyanin) molecules as a carrying protein, which are proteins with a significantly larger structural complexity and mass. Notwithstanding such notable difference in the size and complexity of the carrying protein, the titers are similar among the three vaccine models (Table 2). The foregoing despite the Morphine-KLH and M-KLH vaccines were administered using Freund's adjuvant[19,20,49,50] that is a powerful enhancer of the immune response. It bears mention that a property that outstandingly differentiate the M-TT vaccine from others is the capability to generate antibody titers in a persisting manner. In the case of the models assessed by Li et al., Raleigh et al. and Stowe et al. the titers decreased significantly between 10-20 days after the last immunization, unlike the 6-8 months taken by the titers to diminish in animals immunized with the M-TT vaccine.[19,20,49,50] This immunologic response differentiates outstandingly to the M-TT vaccine of the other vaccine models.

As mentioned before, a very important property to be fulfilled by the new vaccine model is the capability to generate antibodies highly specific for the drug, which depends on the hapten and on its appropriate immunogenic presentation. In the M-TT vaccine, the hapten and the Morphine-6-hemisuccinate in the position 6´ of the morphine's molecule was adjusted to the carrying protein. This allows that the generated antibodies are highly specific for morphine and heroin, with an undetectable cross-reaction with other components such as codeine, hydromorphone, hydrocodone and oxycodone. Therefore, they show null recognition specificity to synthetic analogs of morphine such as methadone, buprenorphine, nalorphine, naloxone and naltrexone. Another very important feature of antibodies generated by this vaccine is that they show an equivalent specificity not only for morphine/heroine but also for their metabolites, which are 10 times more bioactive than both drugs.[17,18]

A very significant difference between the M-TT vaccine and the Morphine-KLH and dynamic vaccines is that the antibodies generated by the latter were not assessed regarding their specificity against the morphine metabolites, which could limit their use.[19,20,49,50]

The four vaccine models share the site in which the hapten is adjusted to the carrying protein (position 6´ of the morphine's molecule). The difference between them lies in the hapten and in the long space-arm that binds the hapten to the carrying protein. In the case of the Morphine-KLH vaccine, the antibodies share the specificity to the morphine, heroin and molecules related to them as shown by the antibodies generated by the M-TT vaccine. Although the hapten is different, both vaccines share a space-arm of similar length. On the other hand, the dynamic vaccine shares the same hapten but the space-arm is much shorter, which reduces the specificity of the antibodies to morphine and heroine. Alternatively, given that the antibodies generated by the M-TT vaccine lack of specificity for the pharmacologic-therapeutic opioid agents, such as naltrexone, methadone and buprenorphine, their application could contribute to abstinence maintenance therapies.[17,18]

At the level of immunoprotection against addictive behaviors induced by morphine/heroine, in 1974 Bonese et al. conducted the first study for assessing the immunoprotection capability of the immunogenic conjugated (BSA-Morphine-6-hemisuccinil [M-6-H]) in the animal model of the primate (*Macacus Rhesus*), previously trained for being

Table 2.

Author	Hapten	Carrying Protein	Adjuvant	Antibody titers	Antibody specificity	Immunoprotection assessment
Bonesse	Morphine-6-hemisuccinil	BSA	————	————	————	Heroin-Self-administration
Anton and col., 2006; 2009.	Morphine-6-hemisuccinil	Tetanus toxoid	Alumina	1:25000	Morphine/heroin and their metabolites	Heroin (0.06 mg/Kg) and morphine (0.6 mg/Kg)-Self-administration. 1-3 mg/kg ip and up to 15 mg/Kg sc of morphine-Tail Flick.
Li and col., 2011.	Morphine-6-glutaryl	KLH	Freund's adjuvant	1:100000	Morphine/heroin	Heroin (0.5 mg/Kg)-Self-administration. 10 mg/Kg sc of morphine-Locomotor activity.
Stowe and cols., 2011.	Morphine-6-hemisuccinil	KLH	Alumina	Morphine 1:160000 and heroin 1:120000	Morphine/heroin	Heroin (0.06 mg/Kg)-Self-administration. 1 mg/Kg sc of heroin-Hot Plate.
Raleigh and cols., 2013.	Morphine	KLH	Freund's adjuvant	1:100000	Morphine/heroin	1 mg/Kg sc of heroin-Hot Plate.

self-administered intravenously, both heroine and cocaine. These authors showed that the active immunization with the BSA-M-6-H immunogenic preparation was able to generate specific anti-morphine/heroine antibodies that in turn could block and extinguish the primate's heroin self-administration behavior but not of cocaine.[38] Subsequently, Antón and Leff, in 2006, reported that the specific antibody titers against morphine and heroine generated by the M-TT (1:250000) were sufficient to block the re-establishment of the seeking and use behavior of heroin (0.06 mg/Kg) and morphine (0.6 mg/Kg) in a self-administration model in the rat (Figure 3). Stowe et al., in 2011, reported —with the dynamic vaccine— antibody titers of 1:160000 for morphine and of 1:120000 for heroine, with the capability to attenuate the acquisition of the self-administration of heroin (0.06 mg/Kg); in turn Li et al. (2011) reported that the Morphine-KLH vaccine generated antibody titers of 1:100000, with the capability to prevent the re-establishment of the seeking behavior of heroin (0.5 mg/Kg) in a self-administration model.[17-20,49,50]

Li et al. (2011) reported the use of morphine doses higher than those used in self-administration protocols to induce strengthening or euphoria. Such authors reported that the antibody titers generated by the Morphine-KLH vaccine were capable to diminish the locomotor activity induced by 10mg/Kg sc of morphine. In turn, Stowe et al. (2011) and Raleigh et al. (2013) reported that the M-KLH vaccine and the dynamic vaccine were able to block the antinociceptive effect of 1 mg/Kg sc of heroine.[19,20,49,50]

Recently, our research group reported that the antibodies generated by the M-TT (1:250000) were sufficient to attenuate, during 60 minutes, the antinociceptive effect of 1 and 3mg/Kg of morphine intraperitoneally administered and up to 15mg/Kg of morphine when the drug was subcutaneously administered, in the *tail-flick* model.[48]

CONCLUSIONS

There can be no doubt that the development field of antiaddictive vaccines is a potentially effective option for controlling this serious global health problem. Efforts from different research groups show the need for seeking new

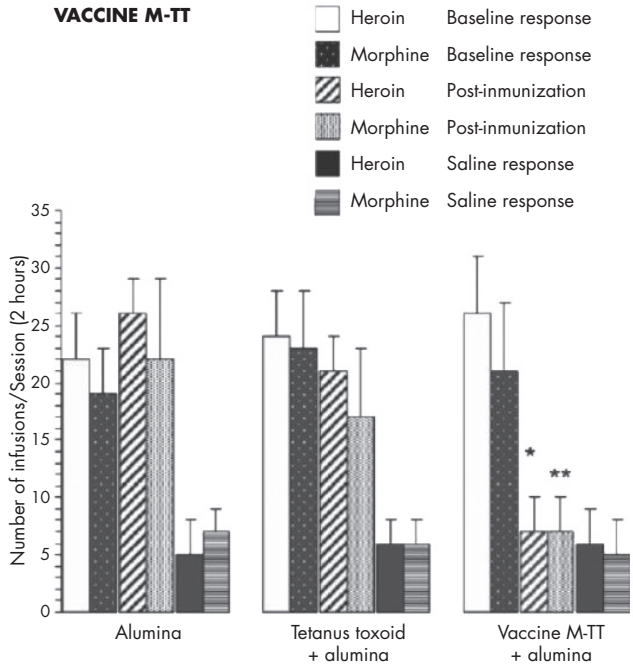

Figure 3. The active vaccination, with the M-TT vaccine, attenuates the re-establishment of the seeking and use behaviors of heroin or morphine in the rat. Rats trained for self-administering reinforcing doses of morphine (600 μg/Kg) or heroin (60 μg/Kg) were vaccinated with the M-TT vaccine. The animals immunized did not show seeking and use behaviors of heroin or morphine.

and better treatment alternatives for the complex challenge addictions represent.

The data exposed in this paper suggest that the structural model of the bivalent vaccine against morphine and heroine shows immunogenic (high and sustained titers of highly specific antibodies) and immunoprotection capabilities (attenuates the effect up to 15mg/kg sc of morphine) that the structural vaccine models have not matched. This makes it the leading vaccine model against the addictive effects of heroin and morphine. However, there is still much progress to be made. Our results shall require to be subjected to toxicological testing and, once overcame, to the first tests in humans.

Translation of the original version published in spanish in: Salud Mental 2015, Vol. 36 Issue No. 3.

ACKNOWLEDGEMENTS

This research was supported by the projects: Fundación Gonzalo Río Arronte, INP-2040, SEP-CONACYT 2009-I0003-106549, NIDA-1R01DA030715-01 and ICyT PINV11-26.

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Declaration of conflict interest: None

Translation of the original version published in spanish in:
Salud Mental 2015, Vol. 36 Issue No. 3.